Bioenerg. Res. (2014) 7:540–550
DOI 10.1007/s12155-014-9417-9

Soil Microbial Community Response to Corn Stover Harvesting Under Rain-Fed, No-Till Conditions at Multiple US Locations

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Published online: 7 February 2014

Abstract Harvesting of corn stover (plant residues) for cellulosic ethanol production must be balanced with the requirement for returning plant residues to agricultural fields to maintain soil structure, fertility, crop protection, and other ecosystem services. High rates of corn stover removal can be associated with decreased soil organic matter (SOM) quantity and quality and increased highly erodible soil aggregate fractions. Limited data are available on the impact of stover harvesting on soil microbial communities which are critical because of their fundamental relationships with C and N cycles, soil fertility, crop protection, and stresses that might be imposed by climate change. Using fatty acid and DNA analyses, we evaluated relative changes in soil fungal and bacterial densities and fungal-to-bacterial (F:B) ratios in response to corn stover removal under no-till, rain-fed management. These studies were performed at four different US locations with contrasting soil-climatic conditions. At one location, residue removal significantly decreased F:B ratios. At this location, cover cropping significantly increased F:B ratios at the highest level of residue removal and thus may be an important practice to minimize changes in soil microbial communities where corn stover is harvested. We also found that in these no-till systems, the 0- to 5-cm depth interval is most likely to experience changes, and detectable effects of stover removal on soil microbial community structure will depend on the duration of stover removal, sampling time, soil type, and annual weather patterns. No-till practices may have limited the rate of change in soil properties associated with stover removal compared to more extensive changes reported at a limited number of tilled sites. Documenting changes in soil microbial communities with stover removal under differing soil-climatic and management conditions will guide threshold levels of stover removal and identify practices (e.g., no-till, cover cropping) that may mitigate undesirable changes in soil properties.

Keywords Corn stover · Maize · Microbial community · Residue management · Soil microorganisms · Soil quality

Electronic supplementary material The online version of this article (doi:10.1007/s12155-014-9417-9) contains supplementary material, which is available to authorized users.

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Introduction

Crop residues, particularly corn (*Zea mays* L.) stover, are viewed as candidate feedstocks for cellulosic ethanol production. Crop residues provide valuable services in terms of soil organic matter (SOM), fertility, water dynamics, and erosion control and therefore are central to the sustainability of agricultural systems [1, 2]. Accordingly, the removal of crop residues for cellulosic ethanol production must be balanced against the negative effects residue removal may have on current and future agricultural productivity [3]. Several studies have proposed practical limits to harvesting crop residues, based on measures of soil erosion [4] or soil organic carbon (SOC) [5], a key indicator of soil quality. A number of studies have examined changes in soil quality parameters due to corn residue removal [6–11], but there are limited data on changes in soil biology in these systems. Modest decreases in soil extracellular enzyme activities were observed in plots (0- to 5-cm depth interval) where corn residue had been removed during two of three preceding years [11]. After 35 years of corn stover removal, a significant reduction in decomposition activity was recorded in soils (1 to 6-cm depth interval) under no-till conditions [10]. Data on changes in soil microbial community structure with corn residue removal are nearly non-existent, although several studies have reported microbial biomass dynamics at a single site with variable findings depending on year and duration of stover removal [12–14]. Shifts in soil microbial community composition have been linked to changes in soil function and affect system sustainability, including long-term soil fertility [15, 16], crop protection [15, 16], C and N cycling [17–19], and climate change mitigation potential [20, 21]. Identifying significant changes in soil functional parameters is required to provide guidance for corn stover removal, but also to document mitigating practices such as no-till, cover cropping, or crop rotation.

In order to detect fundamental changes in soil microbial communities associated with corn residue removal, we measured total fungal and bacterial densities and calculated fungal-to-bacterial (F:B) ratios in soils collected from experimental plots at four geographic locations. Soil F:B ratios reflect land use practices and are a potential indicator of soil quality [22–24]. In a critical assessment of microbiological methods for assessing soil quality, F:B ratios were considered to be most promising [25]. In general, higher F:B ratios are thought to reflect a more positive state of the soil community where organisms that specialize in the depolymerization of complex organic macromolecules from plant residues contribute substantially to overall soil biodiversity. Fungal-dominated soils have been found to retain more C and N [26] and provide more ecological services such as nutrient conservation [27] compared to bacterial-dominated soils. Higher F:B ratios have been linked to agricultural management practices such as no-till, cover cropping, and organic fertilizer use [24]. Corn stover removal decreases the amount of high C:N plant residue and is expected to select against some soil fungi and result in lower F:B ratios.

The four locations range considerably in soil-climatic factors where long-term, replicated plots have been established to examine the effects of corn stover removal on agronomic and soil properties. With exception of one location where soil compaction was an issue, these field plots were managed under no-till conditions which has been shown to minimize changes to some soil properties when stover is removed [10]. All field plots were rain-fed row cropping systems, which is an important consideration since cellulosic ethanol production from irrigated lands would incur additional costs.

Materials and Methods

Field Site 1. Brookings, SD Site location details, climate, soil texture, and nominal values for SOM and pH are provided (Fig. 1; Table 1); additional site information is available in prior publications [8, 9, 28] and other reports in the current issue. Experimental research plots were established in the spring of 2000 at the USDA-ARS North Central Agricultural Research Laboratory in a randomized complete block with three replications. The crops were grown under rain-fed conditions in a no-tillage corn/soybean (*Glycine max*) rotation with each crop planted each year. Experimental treatments initially included three levels of corn residue removal: low, medium, and high residue removal corresponding to reported residue removal rates of 37, 55, and 98 % of above ground biomass, respectively [8]. Low residue removal (LRR) consisted of harvesting grain with all stalks, leaves, and cobs remaining on the soil surface. Under medium residue removal (MRR), grain was combined and stalks chopped, windrowed using a Loftness Windrow crop shredder, and immediately baled. High residue removal (HRR) consisted of cutting stalks 0.15 m from the ground and removing. Individual plot size was 30×30 m. In the fall of 2005, the residue removal treatments were split and a cover crop treatment (with or without cover crop) was integrated into the overall design, thus adjusting the experimental design from a randomized complete block design to a split-plot design. Cover crop consisted of winter legume broadcast into soybeans at the end of R6 and winter grass broadcast into corn at tasseling [8]. Composite soil samples were collected in the fall of 2009 and 2010 from all subplots (n=18) in the corn phase following harvest, but prior to the ground freezing (3 December 2009; 1 November 2010). Composite soil samples were composed of nine cores (3.2-cm diameter, 0- to 15-cm depth) collected in an "X"-shaped pattern covering each plot. Soils were immediately frozen at −80 °C until further processing.

Soil microbial communities at site 1 (Table 1) were evaluated by measuring F:B ratios using quantitative PCR (qPCR)

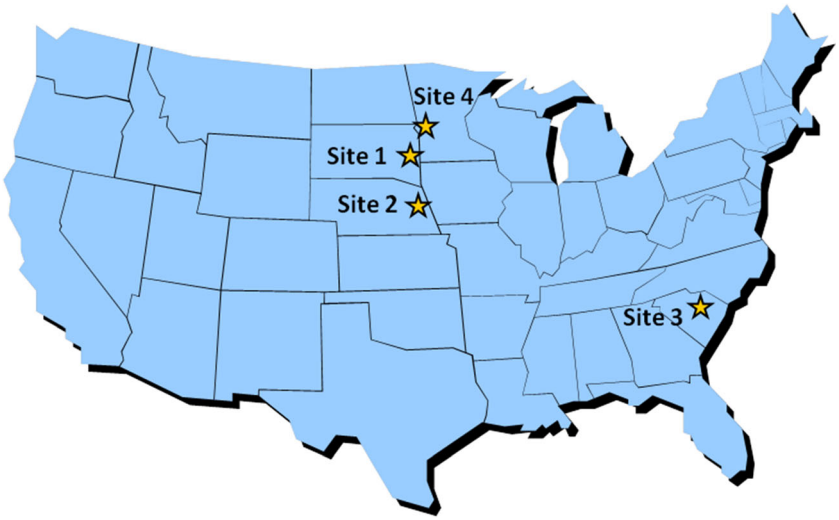

Fig. 1 Location of field research sites in the USA. *Site 1* Brookings, SD; *Site 2* Lincoln, NE; *Site 3* Florence, SC; *Site 4* Morris, MN

[29]. Thawed soils were sieved (4.25 mm) at field moisture, thoroughly mixed, and sub-sampled three times. DNA was extracted from each soil subsample with the Powersoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA) per manufacturers' instructions, screened on a 0.7 % agarose gel (100 V, 25 min), and then combined into a single DNA extract to represent each plot. PCR reactions were conducted in triplicate for each DNA extract using an MX3000P qPCR instrument (Stratagene, La Jolla, CA), SYBR Green I, and Quantitect SYBR Green PCR Master Mix (Qiagen, the Netherlands). A 180-bp segment of eubacterial 16S rRNA gene was amplified using the primers Eub338F (5′-ACTCCTAC GGGAGGCAGCAG) and Eub518R (5′-ATTACCGCGGCT GCTGG) and an approximately 300 bp fungal rRNA gene segment (ITS region) was amplified using 5.8S (5′-CGCTGC GTTCTTCATCG) and ITS1f (5′-TCCGTAGGTGAACCTG CGG). Concentrations of forward and reverse primers for both targets were 300 nM which was determined as optimal in preliminary experiments using a range of concentrations (50–500 nM) for each primer. Annealing temperatures (53 °C) for both reactions, inclusion of bovine serum albumin (0.4 mg mL^{-1}), and other thermocycling conditions were as used by Fierer et al. [29]. Data were reported as rRNA gene copies per gram soil using standard curves constructed via serial dilution of target DNA amplified from type organisms (eubacteria: *Escherichia coli*; fungi: *Saccharomyces cerevisiae*) and cloned into a plasmid via *E. coli* JM109 using the (pGEM-T Vector System II (Promega, Madison, WI). Plasmids were purified with Wizard Plus Minipreps (Promega) and quantified using Picogreen (Molecular Probes, Eugene, OR), Lambda DNA standards, and a fluorescent microplate reader (Hidex, Turku, Finland). Triplicate standard curves, positive (type organism DNA), and negative (no template) controls were included on each qPCR microplate. Assay detection limits were $<10^3$ rRNA copies per reaction and no sample measurements fell outside of the standard curve range. For each year, significant effects of residue removal and cover crop treatments on rRNA copy numbers and F:B ratios (square root-transformed) were determined at the $P<0.10$ level using analysis of variance (ANOVA) procedures with

Table 1 Site characteristics

Field site location	Elevation (m)	Lat. (°N)	Long. (°W)	MAT (°C)	MAP (mm)	Years no-till[a]	Crop rotation	Years with residue removal[b]	Soil texture	SOM (g kg^{-1}) (0–15 cm)	pH (1:1 soil to water) (0–15 cm)
1. Brookings, SD	500	44.20	96.47	6.2	579	14–15	CS	9–10	Silty clay loam	>50	6.3–7.3
2. Ithaca, NE	363	41.15	96.40	10.5	766	12	CC	10	Silt loam	30–50	5.2–6.9
3. Florence, SC	140	34.17	79.44	17.3	1300	2–3[c]	CC	2–3	Loamy sand	<20	5.5–6.5
4. Morris, MN	370	45.41	95.48	5.8	645	13	CS	8	Clay loam	~50	6.0–6.3

MAT mean annual temperature, *MAP* mean annual precipitation, *CS* corn-soybean, *CC* continuous corn

[a] Number of years plots were in no-till prior to soil sampling event(s)

[b] Number of years plots had residue removal treatments prior to soil sampling event(s); corn rotated with soybean has half the number of residue removal occurrences

[c] Planter with in-row subsoiler used

block as a random factor. Pairwise comparisons were performed using Fisher's least significant difference (LSD) at the $P<0.10$ alpha level.

Field Site 2. Ithaca, NE Experimental research plots are located at the University of Nebraska Agricultural Research and Development Center near Ithaca, NE (Fig. 1). Field site information is provided in Table 1, prior publications [30, 31], and other reports in the current issue. Continuous corn was grown under rain-fed, no-till conditions as one of the primary treatments established in 1999 in a split-split plot, randomized complete block design with three replications. These plots were split by three levels of nitrogen application (low, medium, high, or 60, 120, and 180 kg N ha^{-1}, respectively). In 2001, stover removal treatments were applied to corn sub-subplots as no (low) residue removal (LRR) or 50 % (medium) residue removal (MRR) remaining after grain harvest. Removed stover yields were determined from two non-border rows of each sub-subplot harvested for its entire 30 m length with a plot-flail harvester. Stover from remaining rows was harvested with a field-scale flail harvester set at the same 10-cm height as the plot harvester. Complete descriptions of the full experimental design were reported in Varvel et al. [30] and Follett et al. [31]. Soil samples were collected on 1 November 2011 from the center of each sub-subplot ($n=18$) using a flat-bladed shovel, undercutting and removing the soil from the 0- to 5- and 5- to 10-cm depth intervals as previously described [31]. Fresh subsamples were stored in plastic bags, refrigerated at 5 °C within 12 h of field sampling, and then stored at −20 °C within 48 h of sampling until extraction for microbial ester-linked fatty acid methyl esters (EL-FAMEs).

Soil microbial EL-FAMEs were quantified and identified by the methods detailed in [32]. Peak areas were used to quantify concentrations (nanomoles per gram soil) or relative abundances (nanomole percent) of microbial EL-FAME biomarkers. EL-FAMEs were categorized by functional group using fatty acid biomarkers previously described [33–35]. Following omega fatty acid nomenclature: (1) Gram-negative bacteria (cy17:0, cy19:0); (2) Gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, a17:0, 17:1ω8c); (3) actinomycetes (10Me16:0, 10Me17:0, 10Me18:0); (4) saprophytic fungi (18:1ω9c, 18:2ω6c). Biomarkers not fully identified or present in low concentrations (<0.05 nmol g^{-1}) were excluded from the analysis. Total bacteria were the sum of Gram-negative, Gram-positive, and actinomycetes biomarkers. Fungal-to-bacterial ratios were calculated by dividing saprophytic fungi by total bacteria. Soil concentrations of total EL-FAME were used as a proxy for soil microbial biomass. Significant effects of residue removal and nitrogen treatments on biomass, EL-FAME biomarkers, and F:B ratios (square root-transformed) were determined at the $P<0.10$ level for each sample depth using ANOVA procedures with block as a random factor. Pairwise comparisons were performed as previously described.

Field Site 3. Florence, SC Experimental research plots are located at the Clemson University Pee Dee Research and Education Center (Fig. 1). Field site information is provided in Table 1, prior publications [7, 36, 37], and other reports in the current issue. In the spring of 2008, continuous corn with three levels of corn residue removal treatments (0 % or low, LRR; 50 % or medium, MRR; and 100 % or high, HRR) was established within a randomized block design (four blocks) that contained additional treatments. The corn planter was equipped with an in-row subsoiler to break up the hard pan at 40 cm. Corn was harvested with a combine equipped with a soybean head and canvas tarp "diaper" attached to the rear to capture corn stover. Residue removal rates for the treatments were imposed by returning to each plot the appropriate weight of residue (adjusted for residue moisture content) captured in the diaper during grain harvesting.

Composite soil samples (0- to 5 cm) for each plot ($n=12$) were collected in March of each year (2010, 2011) by hand probe at 20 randomly selected locations within each experimental plot. Soil samples were frozen at −80 °C within an hour of being pulled from the field and shipped on dry ice for offsite analysis of phospholipid fatty acid methyl esters (PL-FAME) profiles by the Stable Isotope Research Unit at Oregon State University (Corvallis, OR). Lipids were extracted from the soils according to the modified Bligh/Dyer method [38], and the phospholipids were separated from neutral lipids and glycolipids using solid-phase extraction columns with methanol extractant. Phospholipids were converted to FAMEs through mild alkaline methanolysis and analyzed by capillary gas chromatography with flame ionization detector [33]. Assignment of fatty acid biomarkers was done as described above for site 2 with the following exceptions: (1) Gram-negative bacteria included the additional biomarkers 16:1ω7, 18:1ω7, and 17:1ω9; (2) Gram-positive bacteria did not include 17:1ω8c; (3) fungi did not include 18:1ω9c due to potential artifacts associated with this biomarker in sandy soils [39]. For each sampling year, significant effects of residue removal on biomass, PL-FAME biomarkers, and F:B ratios (square root-transformed) were determined at the $P<0.10$ level using ANOVA procedures with block as a random factor. Pairwise comparisons performed as previously described.

Field Site 4. Morris, MN Experimental treatments were established in 2005 at the Swan Lake Research Farm (Fig. 1) with low, medium, and high corn residue removal rates (LRR, MRR, and HRR, respectively). A corn-soybean rotation was grown under no-till, rain-fed conditions with each crop phase present each year within randomized blocks replicated four times. Field site information is provided in

Table 1, prior publications [7, 11, 36, 37], and other reports in the current issue. Through the 2008 growing season, corn stover removal treatments were imposed by cutting and removing half (MRR) or all (HRR) of the stalks following grain harvest with a two-row flail-knife forage harvester. Starting in 2009, a prototype one-pass combine with variable cutting height was used at 70 cm (above soil) for MRR and 10 cm for HRR.

Composite soil samples (0- to 5- and 5 to 10-cm depth intervals) for each plot (n=12) consisted of soil cores (3.2-cm diameter) collected in the spring of 2013 at two predetermined locations in each plot that had completed the corn phase in the prior growing season. Field moist samples were immediately passed through a 5-mm sieve, and shipped overnight to Lubbock, TX where they were frozen at −80 °C. Fatty acids were extracted from the soil samples, methylated, and analyzed as EL-FAMEs by gas chromatography using the procedure described for the Ithaca, NE site (2). Assignment of fatty acid biomarkers was done as described above for site 2 with the following exception: Gram-positive bacteria did not include 17:1ω8c. Significant effects of residue removal and sample depth on biomass, EL-FAME biomarkers, and F:B ratios (square root-transformed) were determined at the P<0.10 level using ANOVA procedures with block as a random factor.

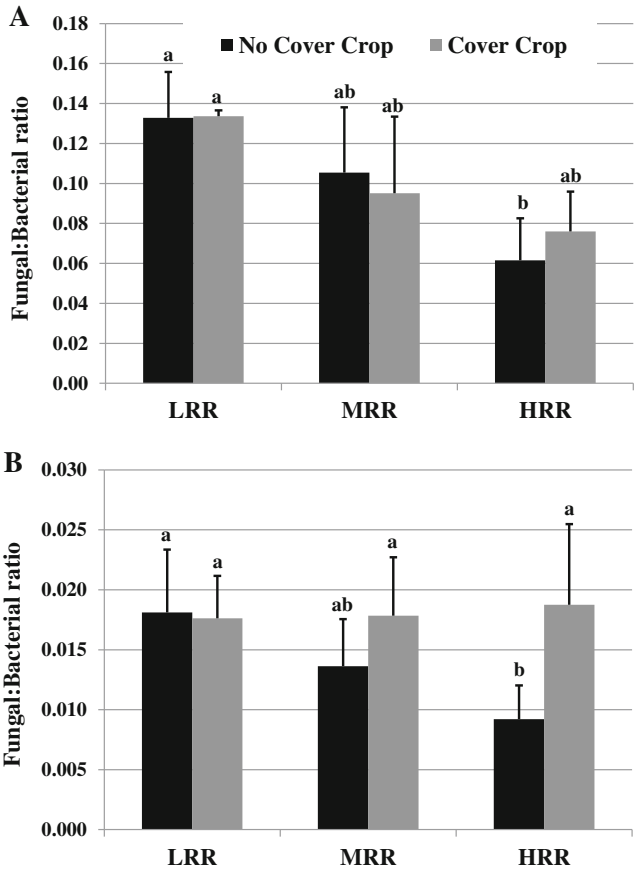

Fig. 2 Fungal-to-bacterial ratios for soils (0–15 cm) collected from all residue removal and cover crop treatments following the corn phase at the South Dakota location (1). *LRR* low residue removal, *MRR* medium residue removal, *HRR* high residue removal. *Error bars* represent one standard error, n=3. *Bars* with different *letters* were statistically different at the P<0.10 alpha level following pairwise comparisons (Fisher's LSD). **a** Fall 2009. **b** Fall 2010

Results

Field Site 1 (SD) In both years, average F:B ratios decreased with escalating rates of residue removal in plots without cover crops (Fig. 2). In 2009, the F:B ratio for HRR without cover crops was significantly lower than LRR with and without cover crops (P<0.10). No significant effect of cover crop treatment on F:B ratios was observed in 2009; however, cover crops significantly (P<0.05) increased F:B ratios in plots with residue removal in 2010. The 2010 HRR treatment without cover crops had significantly (P<0.05) lower F:B ratios than all cover cropped treatments (LRR, MRR, HRR) and LRR without cover crops. In 2009, average F:B ratios from all treatments ranged from 0.06 to 0.14. In 2010, the F:B ratios (0.01–0.02) were much lower than observed in 2009. In 2009, fungal and bacterial rRNA gene copies were about 3×10^8 g^{-1} and 3×10^9 g^{-1}, respectively, across all treatments (Tables 2 and 3). In 2010, fungal and bacterial numbers were much lower than in 2009, averaging about 6×10^6 and 4×10^8 rRNA genes g^{-1}, respectively, across all treatments. There were no significant treatment effects on fungal numbers in 2009 or 2010 (Table 2). However, both residue and cover crop treatments affected bacterial numbers in 2009; LRR without cover crops had significantly (P<0.05) fewer bacteria than MRR with cover crops and both HRR treatments (Table 3). In 2010, the cover crop treatment had a significant effect (P<0.01) on bacterial numbers with the cover crop treatments having fewer bacteria than MRR and HRR without cover crops.

Field Site 2 (NE) Stover removal treatment did not significantly affect F:B ratios for either 0–5-cm or 5–10-cm soils (Fig. 3). Nitrogen treatment did have a significant (P<0.05) effect on F:B ratios from 0- to 5-cm soils with the highest values observed in the 120 kg N ha^{-1} treatment and the lowest in the 180 kg N ha^{-1}. Nitrogen treatment had no significant effect on F:B ratios in the 5–10-cm soils. While not explicitly tested, F:B ratios were higher in 0–5-cm soils compared to 5–10-cm soils.

The relative abundances of all fungal or bacterial biomarker categories were not affected by residue removal at either soil depth. Nitrogen treatment significantly affected (P<0.05) fungal abundance in the 0–5-cm soils with the highest values observed at 120 kg N ha^{-1} (Table 4). Nitrogen treatment significantly affected total bacterial abundances in the 0–5-cm soils (P<0.001) and 5–10-cm soils (P<0.10) with bacteria increasing in relation to the amount of nitrogen. Gram-

Table 2 Fungal rRNA gene copy numbers for soils collected at the South Dakota location (1)

	2009		2010	
Residue treatment	No cover crops	Plus cover crops	No cover crops	Plus cover crops
LRR	1.57E+08 (4.25E+06)	3.78E+08 (3.96E+07)	6.96E+06 (4.87E+05)	6.30E+06 (1.19E+06)
MRR	3.12E+08 (1.35E+08)	3.57E+08 (1.11E+08)	6.50E+06 (1.43E+06)	5.92E+06 (1.04E+06)
HRR	2.62E+08 (1.32E+08)	2.38E+08 (1.86E+07)	4.56E+06 (1.18E+06)	6.54E+06 (1.80E+06)

Data are the means (±1 SE) of the numbers of gene copies per gram soil, n=3

LRR low residue removal, *MRR* medium residue removal, *HRR* high residue removal

positive (P<0.005) and Gram-negative (P<0.005) biomarkers were also significantly affected by nitrogen treatment in the 0–5-cm soils, again increasing in relation to nitrogen amendment (Table S1). There were no significant treatment effects on actinomycetes biomarkers or total biomass on 0–5-cm soils. For the 5–10-cm soils, only Gram-negative bacteria were affected by nitrogen treatment with the highest values associated with180 kg N ha^{-1} (Table S2). Lower amounts of fungi and total biomass could be observed in the 5–10-cm soils compared to 0–5-cm soils, although the effect of depth was not statistically tested.

Field Site 3 (SC) In the spring of 2010 after two seasons of residue treatment, F:B ratios were significantly (P<0.05) elevated with increasing rates of stover removal (Fig. 4a). Increased F:B ratios were driven by a significantly (P<0.05) elevated levels of fungal biomass in plots with stover removed (Table 5). There were no significant effects of residue treatment on total bacterial biomarkers (Table 5), bacterial group biomarkers (Gram-positive, Gram-negative, or actinomycetes), or total biomass which averaged 82.9 nmol (±6.1) PL-FAME g^{-1} soil across all three treatments (Table S3).

In 2011, there was no significant effect of residue treatment on F:B ratios, although the lowest ratio was observed in the HRR treatment (Fig. 4b). There was no effect of treatment on total bacterial or fungal biomarkers (Table 5). The only significant effect of residue treatment on bacterial subdivisions was for the Gram-negatives (P<0.10) which were proportionally higher in the HRR treatment (Table S4). Total biomass trended upwards with residue removal (no significant effect, P=0.431), and biomass averaged about 75.0 nmol (±6.7) PL-FAME g^{-1} soil across the three treatments (Table S4).

Field Site 4 (MN) There was no significant effect of residue removal treatment at either soil depth on F:B ratios (Fig. 5), total fungi or bacteria (Table 6), biomass, and other biomarker categories (Tables S5 and S6). Soil microbial biomass ranged from 66 to 162 nmol EL-FAME g^{-1}. Microbial biomass, total fungi, and total bacteria were lower in the 5–10-cm soils compared to the 0–5-cm soils; however, this was not statistically tested for this study.

Discussion

Field Site 1 (SD) In both years, residue removal without cover cropping was accompanied by decreasing F:B ratios indicating there may be gross changes in the soil microbial community associated with removal of corn residue. In 2010 (but not 2009), cover crop treatments significantly increased F:B ratios when residue was removed. The residue removal treatment has been in place since 2000 so four to five cycles of the 2-year rotation have occurred. Previous work at this site has demonstrated four cycles were sufficient to detect statistically significant changes in soil aggregation and soil organic matter parameters for the 0- to 5-cm depth interval [8, 9]. On the other hand, the cover crop treatments had only been in place

Table 3 Bacterial rRNA gene copy numbers for soils collected at the South Dakota location (1)

	2009		2010	
Residue treatment	No cover crops	Plus cover crops	No cover crops	Plus cover crops
LRR	1.25E+09 b (2.48E+08)	2.84E+09 ab (3.60E+08)	4.30E+08 bc (7.83E+07)	3.60E+08 cd (4.94E+06)
MRR	2.61E+09 ab (7.94E+08)	4.02E+09 a (3.46E+08)	4.95E+08 ab (5.73E+07)	3.62E+08 cd (6.17E+07)
HRR	3.84E+09 a (6.35E+08)	3.41E+09 a (5.37E+08)	5.13E+08 a (2.98E+07)	3.74E+08 cd (4.33E+07)

Data are the means (±1 SE) of the numbers of gene copies per soil gram, n=3. Within a single year, means with different letters were statistically different at the P<0.10 alpha level following pairwise comparisons (Fisher's LSD)

LRR low residue removal, *MRR* medium residue removal, *HRR* high residue removal

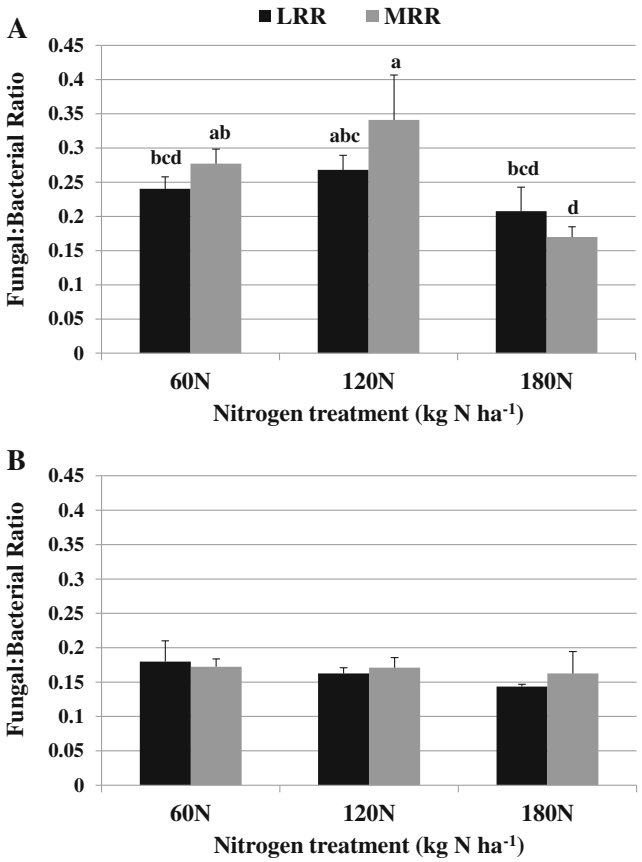

Fig. 3 Fungal-to-bacterial ratios for soils collected at two levels of residue removal (*LRR* low residue removal, *MRR* medium residue removal) across the three levels of nitrogen (*N*) treatment in the fall of 2011 at the Nebraska location (2). *Error bars* represent one standard error, $n=3$. *Bars* with different *letters* were statistically different at the $P<0.10$ alpha level following pairwise comparisons (Fisher's LSD). **a** Soils collected from 0- to 5-cm depth. **b** Soils collected from 5 to 10-cm depth

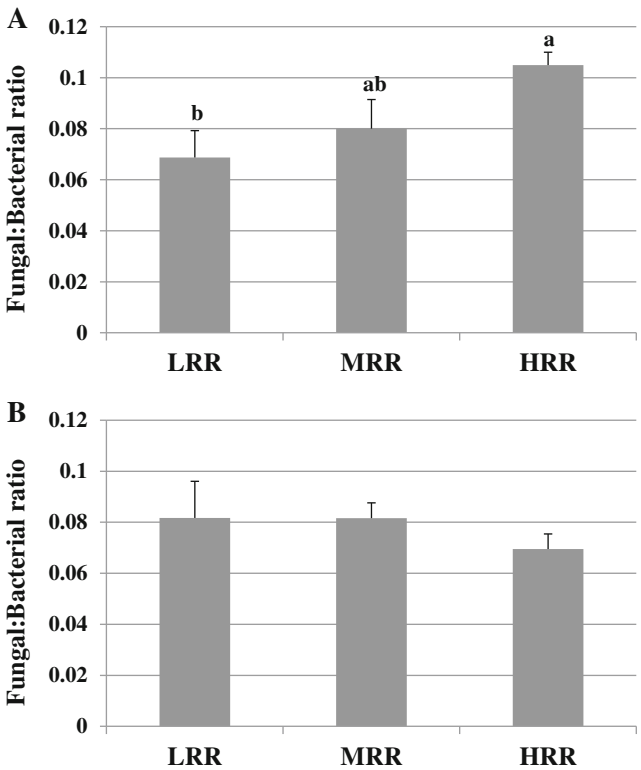

Fig. 4 Fungal-to-bacterial ratios for soils (0–5 cm) collected from all residue removal treatments following the corn phase at the South Carolina location (3). *LRR* low residue removal, *MRR* medium residue removal, *HRR* high residue removal. *Error bars* represent one standard error, $n=4$. *Bars* with different *letters* were statistically different at the $P<0.10$ alpha level following pairwise comparisons (Fisher's LSD). **a** Spring, 2010. **b** Spring, 2011

since fall 2005 (about two cycles) and no statistically significant effects on soil aggregation (0- to 5 cm) were found after this time frame [8, 9]. Our F:B ratios were measured on soils from the 0- to 15-cm depth which may exhibit a slower treatment response compared to the top 5 cm.

In 2009, both fungal and bacterial numbers were lowest in the LRR plots with no cover crops. We speculate that not only the quantity of residue, but also the residue particle size and

Table 4 Total soil fungal and bacterial fatty acid biomarkers at the Nebraska location (2)

		Fungi		Bacteria	
Residue treatment	Nitrogen treatment[a]	0–5 cm	5–10 cm	0–5 cm	5–10 cm
LRR	60	5.6 (0.4) b	4.2 (0.5)	23.5 (0.6) cd	24.2 (1.3) bc
MRR	60	6.1 (0.4) b	3.9 (0.2)	22.0 (0.8) d	22.5 (0.8) d
LRR	120	6.4 (0.2) b	3.9 (0.3)	24.2 (1.1) c	24.1 (1.5) bcd
MRR	120	8.3 (1.4) a	4.3 (0.3)	24.8 (0.8) bc	25.3 (0.7) ab
LRR	180	5.7 (0.9) b	3.6 (0.2)	27.5 (0.7) ab	25.3 (1.0) b
MRR	180	4.8 (0.4) b	4.4 (0.9)	28.2 (0.3) a	27.1 (0.5) a

Data are the means (±1 SE, $n=3$) of the mole percentages of total FAME. Within a single depth, means with different letters were statistically different at the $P<0.10$ alpha level following pairwise comparisons (Fisher's LSD)

LRR low residue removal, *MRR* high residue removal

[a] Annually applied inorganic N, in kilograms of nitrogen per hectare

Table 5 Total soil fungal and bacterial fatty acid biomarkers at the South Carolina location (3)

	Fungi		Bacteria	
Residue treatment	2010	2011	2010	2011
LRR	3.2 (0.4) b	3.9 (0.6)	47.3 (0.7)	48.8 (2.0)
MRR	3.7 (0.5) ab	4.0 (0.2)	47.2 (1.3)	49.1 (0.8)
HRR	4.7 (0.3) a	3.5 (0.3)	44.7 (0.3)	50.7 (0.8)

Data are means (±1 SE, n=4) of the mole percentages of total PLFA. Within a single year, means with different letters were statistically different at the P<0.10 alpha level following pairwise comparisons (Fisher's LSD)

LRR low residue removal, *MRR* medium residue removal, *HRR* high residue removal

Table 6 Total soil fungal and bacterial fatty acid biomarkers at the Minnesota location (4)

	Fungi		Bacteria	
Residue treatment	0–5 cm	5–10 cm	0–5 cm	5–10 cm
LRR	24.4 (4.5)	15.5 (3.1)	33.1 (3.1)	23.1 (4.0)
MRR	21.0 (2.9)	18.2 (1.1)	31.3 (3.1)	23.7 (1.3)
HRR	22.2 (1.4)	18.2 (2.8)	31.2 (3.5)	30.2 (2.9)

Data are means (±one standard error, n=4) of the mole percentages of total FAME

LRR low residue removal, *MRR* medium residue removal, *HRR* high residue removal

contact with the soil may influence soil microbial community structure. Smaller crop residue particle size can promote decomposition activities due to surface area considerations [40] and physical disruption of plant structural polymers [41]. Residue contact with soil increases the exposure to soil microorganisms and provides access to N which is required to decompose high C:N ratio residues like corn [42]. In the MRR and HRR (with no cover crops) where fungal and bacterial numbers were higher than LRR, the process of removing corn stover or cutting silage produces small residue particles that are distributed directly to the soil surface. This phenomenon may be particularly relevant to no-till systems. In 2010, fungal and bacterial numbers were about 50- and 10-fold lower than in 2009, respectively. The presence of cover crops seemed to further depress bacterial numbers. This location experienced the wettest year on record in 2010 with 899 mm recorded precipitation leading to frequently saturated soil conditions [43] which may depressed soil microbial numbers.

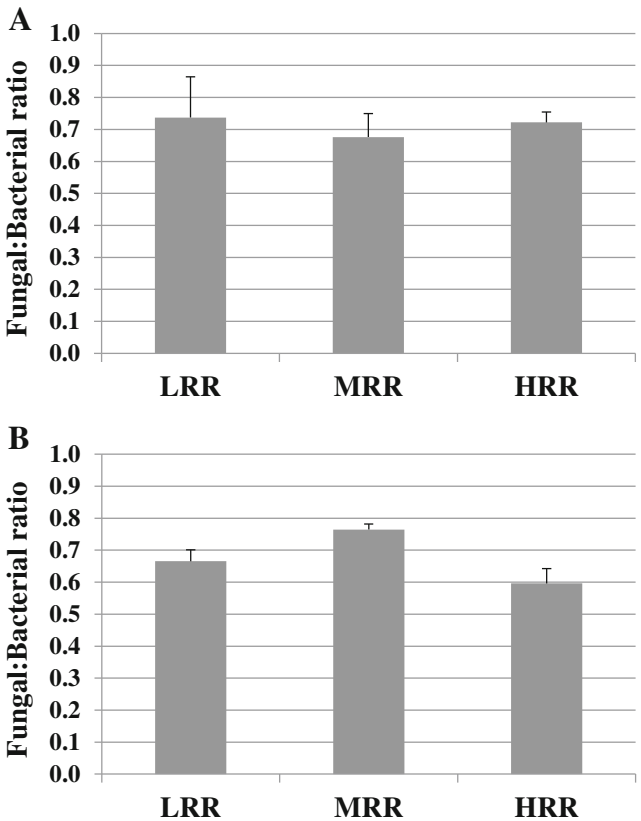

Fig. 5 Fungal-to-bacterial ratios for soils collected in the spring of 2013 from residue removal treatments following the corn phase at the Minnesota location (4). *LRR* low residue removal, *MRR* medium residue removal, *HRR* high residue removal. *Error bars* represent one standard error, n=4. **a** 0–5-cm soils. **b** 5–10-cm soils

Field Site 2 (NE) After 11 years of treatment, corn stover removal (MRR) did not result in a decrease in either F:B ratio or microbial biomass at either soil depth examined. At this site, these measurements were not made on the HRR treatment. Fungal-to-bacterial ratios and fungal biomass were responsive to N rate in 0- to 5-cm soils with the highest values in the 120-kg N ha^{-1} treatment and lowest in the 180-kg N ha^{-1} treatment. In other managed agroecosystems, soil fungal abundance has responded negatively to increasing N fertilizer applications [44, 45]. Bacterial biomarkers at both soil depths were significantly increased with N treatment. Thus, relative decreases in fungi and increases in bacteria with increasing nitrogen amendment indicate an altered soil microbial community with potential functional implications. Previous studies at this site have found long-term corn grain and stover yields increased from 60 to 120 kg N ha^{-1} $year^{-1}$ but no further yield gains occurred at 180 kg N ha^{-1} $year^{-1}$ [30, 31]. Most of the significant effects on biomarkers by N treatment were observed in the 0–5-cm soils suggesting that no-till practices limit the vertical extent of soil microbial responses to surface management practices.

Field Site 3 (SC) After only 2 years of residue removal, there were a higher proportion of fungal biomarkers, and consequently higher F:B ratios, observed in the soils where the most residue was removed (HRR). This was contrary to expectations, and potentially the result of fungal populations adjusting

to changes in management over the initial 2 years of the study. After 3 years, the reverse was observed, and followed conventional thought, with the HRR treatment having the lowest F:B ratio, but it was not significantly different from both LRR or MRR. Fungal biomarkers in the HRR decreased approximately 10 % compared to LRR, and significantly more Gram-negative bacteria were detected in the HRR compared to the LRR. Given the limited differences in soil microbial community structure observed after 3 years of residue removal, we conclude that the duration of this experimental treatment may be insufficient to impose major changes in these sandy soils.

Field Site 4 (MN) After 8 years of treatment (four stover removal episodes), F:B ratios were not significantly affected by residue removal. This finding is consistent with a previous report where soil enzymatic activities (acid phosphatase, ß-glucosidase, ß-glucosaminidase) were not significantly different with stover removal (two stover removal episodes) from these same plots [11]. The previous study demonstrated that the highest level of stover removal (HRR) resulted in decreased particulate organic matter and increased highly erodible aggregates in 0–5-cm soil depths during same time frame [11]. Since these soils have relatively high SOM, are often wet, and experience a short growing season, soil microbial communities may be slow to respond to biennial stover removal.

Summary of Microbial Community Response Across All Locations All of the data was collected following corn from field plots where no-till practices were used and crops were grown under rain-fed conditions. The four locations cover a range of soil-climatic conditions and vary widely with respect to key soil characteristics, pH [46, 47], and SOM [48, 49] that have been shown to influence microbial communities. There were differences in the sampling time (fall, sites 1, 2; spring, sites 3, 4) and rotation, either continuous corn (sites 2, 3) or corn-soybean (sites 1, 4). At site 1, we observed a significant decline in F:B ratios which would be expected with decreased crop residues possessing a high C:N ratio. At site 2, the lowest F:B ratios were in the MMR treatment receiving the highest level on N amendment. Unfortunately, no data were available for the HRR plots at this location. At site 3, the variable response of F:B ratios over the 2 years of measurement may be related to the recent establishment of these treatments. However at site 4, despite four cycles of residue removal, no significant effects on F:B ratios were found.

Broad patterns in soil microbial community structure have been established by linking F:B ratios measured with FAME [24, 26, 27] or rRNA genes [49, 50] with key soil parameters (pH, C:N), and land use. Our methods varied per site and as such, the F:B ratios are not comparable across sites, but are limited to evaluating treatment effects at a single site. Quantitative extraction of fatty acids is subject to sample matrix effects and biomarker assignments are limited by a lack of 1:1 correspondence between fatty acid(s) and taxon(s) [51]. Quantitative extraction and amplification of rRNA genes are subject to sample matrix effects, PCR biases, multiple RNA operons per organism, and the limitation of primer specificity [29]. There is no basis to expect that F:B ratios measured by fatty acid and DNA methods would of equivalent value. However, changes in F:B ratios with either method can reasonably be expected to reflect gross changes in the soil microbial community which could have significant effects on C and nutrient cycles as well as other ecological roles soil microbes occupy.

These data also point to specific environmental and technical issues with detecting changes in soil microbial communities in response to crop residue removal. Cross-site differences in historical and current agricultural practices as well as soil type likely contributed to variable findings. Key factors will be the duration of the residue removal treatment and the number of cycles of residue removal (depending on rotation) experienced. In no-till systems, changes may be slower than in tilled systems and more likely to be observed in the upper soil layers; however, there are scarce data for comparison with ours. Johnson et al. [11] found the arbuscular mycorrhizal fungal biomarker 16:1ω5c was significantly reduced with stover removal in a tilled system while we did not observe any changes in this biomarker with no-till at sites 3 and 4 (data not shown). Halpern et al. [12] found after 16 years of continuous corn with stover removal at an eastern Canadian site, soil organic carbon (SOC) was reduced along with microbial biomass C in 0- to 5-cm soils in tilled treatments, but not in no-till treatments. At this same site, an earlier study reported 9 years of residue removal negatively affected microbial biomass C regardless of tillage, but there was no effect of either treatment factor on total PL-FAME or microbial community structure [13].

A potentially important factor in assessing interactions between soil microbes and residue harvesting is the method of stover removal, its effect on particle size of the remaining residue, and its contact to the ground in comparison to standing stalks. Based on observations at sites 1 and 2, it is likely the distribution of residue particles of small size to the soil surface may influence the soil microbial community in the upper soil depths, especially in no-till systems. Thus, the overall effect of stover removal on soil microbial communities over long durations may be masked by temporary changes associated with the distribution of small residue particles during residue harvesting. Soil parameters that covary with residue removal such as soil temperature, pH, and moisture may also strongly influence soil microbial communities. Short growing seasons and high SOM may also contribute to buffering soil microbial communities from responding to reduced residue inputs. Lastly, annual weather patterns that produce strong effects like flooding or drought may influence the soil

microbial community to such a degree that responses to management are not detectable. We observed >10× lower fungal and bacterial numbers in 2010 at the South Dakota site, probably due to prolonged saturated conditions in the experimental plots. The response of F:B ratios to cover crops in this site-year suggests the addition of cover crops to reduce seasonal fallow may be a tactic to minimize effects of stover harvesting on soil microbial communities. It is likely the effect of cover crops will interact with soil moisture conditions and thus vary according to precipitation received in a given year.

Conclusions

Based on the data collected at these four locations, soil microbial communities may be restructured by continued residue removal, but changes are slow in no-till systems and other site-specific factors may provide resistance to soil microbial responses. Our ability to detect these changes depends on some key parameters such as soil-climatic conditions, duration and method of stover removal, tillage, annual weather patterns, and soil sampling depth. The data reported in this manuscript represent an intermediate time-point in these long-term experiments which continue to provide data broadly based in time and space. Data on the responses of microbial community structure to long-term corn stover removal in tilled systems would be useful for comparison.

Acknowledgments This research was funded by the USDA-Agricultural Research Service (ARS) as part of the USDA-ARS-REAP (Resilient Economic Agricultural Practices) project, with additional funding provided by the North Central Regional Sun Grant Center at South Dakota State University through a grant provided by the US Department of Energy (DOE)—Office of Biomass Programs [now Bioenergy Technology Office (BETO)] under award number DE-FC36-05GO85041. Technical assistance in the field and/or lab is acknowledged from Kayla Miller, Meggan Kowalski, Kurt Dagel, Amy Christie, Don Watts, Joe Million, Jerry Martin, Ray Winans, Anthony Shriner, Warren Busscher, Chris Bauer, Aaron Bereuter, Dennis Francis, Paul Koerner, Susan Siragusa-Ortman, Jamie Pesek, Steve Swanson, David Walla, Chad Rollofson, Gary Amundson, and Nancy Barbour.

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