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Soil Bacterial Community Diversity and Composition as Affected by Tillage Intensity Treatments in Corn-Soybean Production Systems

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Abstract: Our understanding on the effects of tillage intensity on the soil microbial community structure and composition in crop production systems are limited. This study evaluated the soil microbial community composition and diversity under different tillage management systems in an effort to identify management practices that effectively support sustainable agriculture. We report results from a three-year study to determine the effects on changes in soil microbial diversity and composition from four tillage intensity treatments and two residue management treatments in a corn-soybean production system using Illumina high-throughput sequencing of 16S rRNA genes. Soil samples were collected from tillage treatments at locations in the Southern Coastal Plain (Verona, MS, USA) and Southern Mississippi River Alluvium (Stoneville, MS, USA) for soil analysis and bacterial community characterization. Our results indicated that different tillage intensity treatments differentially changed the relative abundances of bacterial phyla. The Mantel test of correlations indicated that differences among bacterial community composition were significantly influenced by tillage regime (rM = 0.39, $p \leq 0.0001$). Simpson's reciprocal diversity index indicated greater bacterial diversity with reduction in tillage intensity for each year and study location. For both study sites, differences in tillage intensity had significant influence on the abundance of *Proteobacteria*. The shift in the soil bacterial community composition under different tillage systems was strongly correlated to changes in labile carbon pool in the system and how it affected the microbial metabolism. This study indicates that soil management through tillage intensity regime had a profound influence on diversity and composition of soil bacterial communities in a corn-soybean production system.

Keywords: soil microbial ecology; bacterial diversity; conservation tillage; high throughput sequencing

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1. Introduction

Soil disturbing agricultural practices, such as tillage and residue management operations have a direct influence on soil quality [1–3]. Soil quality captures an overall picture of soil functionality that includes chemical, physical and biological features necessary for long-term, sustainable productivity. The role and importance of soil quality for sustainable crop production is receiving increasing attention from both farmers and researchers [4,5]. Healthy soil maintains diverse microbial communities that have been shown to control plant diseases, effectively recycle plant nutrients, improve soil structure, and improve overall crop production [6,7]. Soils are highly heterogeneous both physico-chemically and biologically, thereby encompassing a wide range of niches available to sustain microbial

diversity [8–10]. Numerous studies have shown the influence of both abiotic and biotic factors on microbial community structure and function [2,6]. Since many soil factors influence the composition of soil microbial communities, changes in these factors may cause major differences in the distribution of microbial species [11]. Studying biologically driven soil properties in agricultural production may provide a model system for ecological agriculture. Among the different options, management of microbial diversity seems promising since soil microorganisms play a vital role in agroecosystems due to their participation in numerous soil functions.

Studies have shown that tillage has profound and complex influence on soil physical, chemical and biological properties [12–14]. Tillage causes physical disturbance in soil and results in relocation of crop residues which eventually affects soil moisture, soil temperature, aeration, and labile carbon availability [15]. These physical disturbances caused by tillage affect the organisms that inhabit these environments and several studies have reported increase in soil microbial biodiversity due to conservation tillage, particularly no-tillage systems [16–18]. This increase in microbial diversity also improves the ability of microorganisms to effectively use the carbon sources. Additionally, increased microbial diversity and abundance usually accompanies increase in soil organic matter level. Thus, besides reducing cost of production, conservation tillage practices have also shown potential for improving organic matter and reducing farm inputs in the long-term [19].

Reduced tillage practices, especially No-till, are getting worldwide popularity in farming systems with an aim to reduce machinery and energy inputs [18,20]. Despite numerous potential benefits, many associated problems of continuous No-till faming systems are recognized [20]. Build-up of herbicide-resistant weeds, increased incidence of stubble-borne diseases, and surface immobilization of plant nutrients including carbon are some of the biggest concerns for farmers adopting these conservation tillage practices [1]. Strategic tillage is the new tillage concept that offers growers a flexible approach to manage some of the shortcomings of the No-till farming systems [1,21]. Strategic tillage involves occasional and carefully timed tillage operations based on customized tillage implements to suit the agro-climatic conditions of the crop production systems [22]. As tillage is known to influence soil biological properties, there are concerns that the strategic tillage operations might disrupt soil microbial communities and their associated benefits obtained by No-till practices [13]. Factors like the type of tillage implement utilized plays an important role in determining the effects of occasional, low-intensity tillage on the soil microbial community and the information concerning the effects of this approach on soil microbial communities is limited.

The aim of this study is to characterize differences in soil microbial community dynamics under different tillage and crop residue management practices. This study is a part of a long-term crop production study established in Southern Coastal Plain (SCP) and Southern Mississippi River Alluvium (SMRA) regions of Mississippi. Bacterial community diversity and composition were determined using Illumina-based high-throughput sequencing. Our hypothesis is that different tillage and crop residue management practices will have a significant influence on structural and compositional diversity of soil bacterial communities. The specific objectives were to (1) characterize the influence of four different tillage intensity treatments under two residue management regimes on community distribution and phylogenic composition of the soil bacterial communities, and (2) study the variation and changes in the abundance and composition of soil microbiomes in relation to soil properties under different tillage intensity treatments.

2. Materials and Methods

2.1. Site Description and Experimental Design

This study is part of a long-term trial that began in 2011 which was established at two locations in Mississippi. One study was established at North Mississippi Research and Extension Center, Verona, MS on a non-irrigated Fluvaquentic Eutrudepts (Marietta series), which is in the upper Southern Coastal Plain (SCP) major land resource area (MLRA);

and another at Delta Research and Extension Center, Stoneville, MS on an irrigated Typic Hapludalfs (Dubbs series) in the Southern Mississippi River Alluvium (SMRA) MLRA. The experimental method combined different tillage intensity treatments on residue retention in a corn-soybean rotation. The experimental design was a replicated split-plot design with residue treatments as main plots and fall tillage as sub-plots with three replicates for each tillage treatment. The study compared the effects of four tillage treatments on soybean plots, where soybean was sown on the residues of previous corn crop. In 2013, soybean was planted to soil that was under corn the previous year. These plots were rotated to corn in 2014 and to soybean again in 2015. Therefore, year 2013 marked cycle 1 of rotation (Corn-Soybean) and 2015 marked cycle 2 (Soybean-Corn-Soybean). The two residue management treatments were (1) fall burning of corn residues and (2) no burning of corn residues. The four tillage treatments were (1) Reduced till treatment where the beds were reshaped every year only when spring bed heights were less than 7.6–10.2 cm; referred as minimum intensity tillage (Min); (2) One pass of in-row subsoil bed-roller TerraTill® in fall; referred as reduced intensity tillage (Red); (3) One pass of bed-roller and disk in fall; referred as moderate intensity tillage (Mod) and (4) A combination of disking (twice) and in-row subsoil bed-roller TerraTill®; referred as high intensity tillage (High). Based on the tillage intensity and the amount of soil disturbance incurred, Min and Red are considered conservation tillage treatments while Mod and High are designated as conventional tillage treatments.

2.2. Soil Sampling and Characterization

Soil samples were collected from zone of dominant root activity (A-horizon) in each site at a depth of 0 to15 cm from the middle of each planted row in a plot and homogenized. Composite soil samples consisting of soil samples collected at four locations within each plot following the 2013 and 2015 soybean crop harvest. After sampling, visible roots and plant residue were removed and immediately transferred into soil bags after homogenizing. The sample bags were frozen immediately in cooler pack filled with dry ice. Upon arrival in the laboratory, soils were thawed for 25–30 min, further homogenized, and divided into two subsamples. One was stored at 4 °C to determine soil physicochemical properties, and the other was stored in −80 °C ultra-low freezer for extracting DNA and microbial analysis. We analyzed 96 soil samples in total (four tillage treatments and two residue treatment with three replications at two locations for two years).

Extractable phosphorous (P), potassium (K), and zinc (Zn) were analyzed based on a Mehlich 3 extraction and measured using an Inductively Coupled Argon Plasma (ICAP) unit. Soil pH was measured in a soil suspension with deionized water (1:10, w/v). Total C and N were measured using combustion analyzer (Elementar Vario EL III) after samples had been air dried, ground and sieved through a 30 µm sieve.

Soil penetration resistance was determined for each tillage plots by measuring the soil penetration resistance using a soil cone penetrometer (Eijkelkamp, with a cone diameter amounting to 2.00 cm^{-2} and 60° cone angle). The penetration resistance measurement was taken by recording resistance (in psi) up to 10 cm soil depth at 10 places within each plot and the readings were averaged. Percent water stable aggregate (aggregate stability) was measured by using the wet-sieving method [23]. Four grams air-dried soil sample (1.0 mm–2.0 mm) was placed in a sieve of a wet sieving apparatus and wet-sieved in a can of deionized water for 3.0 min. This can was replaced by another can with a dispersing solution (2.0 g of NaOH/L) and sieved until only sand particles were left in a sieve. Soil collected in the can was oven-dried overnight at 110 °C. The weight of soil in the can was measured. Water-stable aggregate was calculated based on different fractions obtained during wet sieving procedure [23].

2.3. Soil Basal Respiration and Substrate Induced Respiration Rate

Soil basal respiration (BR) was measured as CO_2 evolved from soil incubated in mason jars using NaOH traps. Soil samples (20 g) were measured into a major jar and adjusted

to 60% water holding capacity. The jars were incubated for seven days at 28 °C in an incubator. The CO_2 evolved during incubation was trapped in a beaker containing 0.5 N NaOH (10 mL) placed inside the mason jars. The carbonate in the alkaline traps were precipitated with 50% $BaCl_2$ and back titrated with 0.5 N HCl using phenolphthalein as indicator. Based on the quantification of CO_2 released, the basal soil respiration was calculated as μg of CO_2 released per gram of dry soil per day [2].

Glucose was used as energy source for the substrate induced respiration (SIR) rate measurement [2,24]. Soil samples (20 g) were measured into a major jar and adjusted to 60% water holding capacity with water. Then, the soils were amended with a substrate mixture containing 75 mg glucose and additives [2]. After the substrate addition, a beaker containing 10 mL of 0.5 N NaOH was placed inside each jar, sealed tight and incubated at 28 °C. Mason jars without substrate addition was maintained as control. The amount of CO_2 evolved was measured as described before and SIR was calculated based on CO_2 quantification and expressed as μg of CO_2 released per gram of dry soil per day.

2.4. DNA Extraction and 16S rRNA Gene Sequencing

Total soil genomic DNA was extracted from 0.5 g of fresh soil using a FastDNA™ SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. NanoDrop™ (Thermo Scientific, Wilmington, DE, USA) was used to measure the purity and concentration of the DNA spectrophotometrically. Universal primer pairs 515FB (GTGYCAGCMGCCGCGGTAA) and 926R (CCGYCAATTYMTTTRAGTTT) were used to amplify the V4-V5 hypervariable regions of 16S rDNA gene. Illumina adaptor and barcode sequences were added using PCR steps [25,26]. The PCR products were further purified using Agencourt AMPure XP PCR purification system (Beck-man-Coulter, CA, USA). Equimolar amounts of purified products were pooled and submitted to the Dalhousie university Centre for Comparative Genomics and Evolutionary Bioinformatics (CGEB, Canada) for sequencing on a Illumina MiSeq platform (250-bp paired-end sequencing).

2.5. Data Processing and Bioinformatics Analysis

We used QIIME (Quantitative Insights into Microbial Ecology) (v 1.9.1) to denoise the sequence data of chimeras and to separate the sequences bioinformatically based on barcodes [27,28]. Downstream sequence analysis was done using MOTHUR v1.43.0 [29], a software for describing and comparing microbial communities. The sequences were further processed to remove identical sequences or artificially duplicated sequences, which can constitute a significant fraction of the dataset [30,31]. Unique sequences were then was then aligned using SILVA (v 123) reference dataset (http://www.arb-silva.de/, accessed on 17 February 2017) [32]. MOTHUR was used to assign (cluster) sequence reads to OTUs (Operational Taxonomic Units) with a threshold of 97% pair-wise identity. Number of sequences per sample were rarefied by randomly picking sequences, based on lowest sequence read sample, to normalize sequence depth [33]. Simpson's reciprocal index was used to estimate the alpha-diversity using OTUs at D = 0.03 evolutionary distances (about 97% sequence similarity) [29]. MOTHUR was used to assign phylogeny to sequences using SILVA database. The 16S rRNA gene sequences generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) database under BioProject PRJNA685638.

2.6. Statistical and Multivariate Analyses

Non-metric multidimensional scaling (NMDS) analysis was performed based on a Bray-Curtis dissimilarity matrix for beta-diversity analysis [34,35]. This method was used to calculate dissimilarity matrix, which is a pair-wise comparison based on Sørensen distance measure [36]. The final dimensionality of the dataset was determined based on the stress and stability measurements from the ordination analysis [36] using the PC-ORD software (MJM Software, Gleneden Beach, OR, USA). Mantel test was performed using the PC-ORD software (MJM Software, Gleneden Beach, OR, USA) to determine correlations between bacterial community composition and soil characteristics. For the Mantel test,

Sørensen distance measure was used with a random starting configuration for the bacterial community matrix. Canonical correspondence analysis (CCA) was performed to explore the change in bacterial community composition in relation to environmental variables to establish the directional changes of bacterial community composition constrained by the selected factors. Statistical comparison for difference in the effect of tillage treatments on soil properties were conducted using GLIMMIX procedure and the statistical significance was measured using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). PROC GLIMMIX procedure in SAS software was used to fit linear general linear mixed model (GLMM) analyses on each of the response variables. The procedure was used to evaluate the effect of tillage, residue management and their interaction effects on soil parameters. Blocks and years were considered random effects and tillage, residue management and their interaction, as fixed effects. Hierarchical cluster analysis of the most abundant OTUs was done using the PC-ORD software [37]. Data visualization of the multivariate analysis was done using the Sigmaplot version 11.0 software packages (Systat Software Inc.). Factors that significantly influenced community composition were used to construct a soil variables matrix for Variance partitioning analysis in CANOCO (ver 4.5) to resolve the explanatory power of different factors (tillage and soil parameters) in relation to bacterial community [38].

3. Results

3.1. Soil Physicochemical Properties

Crop residue (burn vs. no-burn) management and tillage treatments did not have any effect on the soil chemical characteristics at both the locations. The interaction between tillage and residue management was also not significant. Therefore, we are showing the results of some basic soil characteristics averaged over years (Table 1). Changes in soil aggregate stability (% water stable aggregates) and penetrometer resistance in response to different tillage treatments are shown in Table 2. At both the locations (SCP and SMRA), conservation tillage plots (Min and Red) showed a significant higher water stable aggregate content than the conventionally tilled plots (Mod and High), especially in the second crop rotation cycle (2015) and registered significant higher soil resistance at Min-till plots compared to all the other tillage treatments (Table 2).

Table 1. Selected soil chemical properties following residue management-tillage treatments (averages across years) for Southern Coastal Plain (SCP) and Southern Mississippi River Alluvium (SMRA) study sites.

	pH	C	N	P	K	Zn
		%	%	(kg ha^{-1})	(kg ha^{-1})	(kg ha^{-1})
Verona (SCP)						
2013 *	6.5 (0.3)	0.05 (0.01)	0.27 (0.05)	182 (31)	344 (68)	3.1 (0.4)
2015 *	6.3 (0.2)	0.09 (0.06)	0.32 (0.06)	175 (25)	312 (62)	2.4 (0.5)
Stoneville (SMRA)						
2013	6.5 (0.3)	0.31 (0.01)	0.05 (0.1)	116 (14)	413 (72)	3.4 (0.4)
2015	5.8 (0.6)	0.28 (0.05)	0.11 (0.1)	119 (13)	435 (12)	2.9 (0.9)

* 2013-cycle 1; 2015-cycle 2; mean value (n = 24) followed by standard deviation in parenthesis.

3.2. Residue Management

The two residue management treatments were as (1) fall burning of corn residues and (2) no burning of corn residues in the fall. These crop residue (burn vs. no-burn) management treatments did not have any statistically significant effect on the soil biological, microbial diversity or functional characteristics at both the locations. The interactions between tillage and residue management for all the measured variables were also not statistically significant for both the locations and rotation cycles. Hence, all the results presented here are arranged as mean comparisons of the tillage treatments.

Table 2. Analysis of variance of selected soil physical properties as affected by tillage intensity treatments for Southern Coastal Plain (SCP) and Southern Mississippi River Alluvium (SMRA) study sites.

	Tillage Intensity	Water Stable Aggregates (%)	Penetrometer Resistance (psi)
Verona (SCP)			
2013	Min	54 a	129 a
	Red	44 a	107 ab
	Mod	51 a	103 ab
	High	45 a	93 b
2015	Min	57 a	125 a
	Red	47 ab	103 b
	Mod	44 b	104 b
	High	43 b	116 ab
Stoneville (SMRA)			
2013	Min	46 ab	153 a
	Red	49 a	152 a
	Mod	44 ab	148 a
	High	42 b	178 a
2015	Min	56 a	150 a
	Red	51 a	113 b
	Mod	40 b	120 b
	High	39 b	111 b

Means (n = 3) followed by the same lower-case letter within year are not significantly different (LSD protected, $p < 0.05$).

3.3. Soil Biological Properties

Tillage systems indicated significant effect on both soil basal respiration and substrate induced respiration (Table 3) at both the locations. Min -till plots showed a significant higher overall respiration rates than the other tillage treatments. At SMRA, Min -till plots showed a significant higher respiration rates in both the years (Table 3). At SCP, this effect of conservation till systems were pronounced during third year of crop cycle (2015). Red-Till plots showed equally higher soil basal respiration and substrate induced respiration rates at both the locations. Year and tillage interactions also had a significant effect on these soil biology measurements at both the locations.

3.4. Sequencing Depth and Alpha Diversity

Using Illumina MiSeq sequencing of 16S rRNA gene amplicons, a total of 3,643,332 quality sequences (ranging from 30,221 to 38,694 reads per sample) were generated from 96 soil samples for further bioinformatic analysis. All these sequences were subsequently clustered into operational taxonomic units (OTUs) based on 97% sequence similarity. The number of observed OTUs detected in each individual sample ranged from 2964 to 3874. There was significant influence of tillage treatments on the alpha diversity of bacterial populations based on the Simpson's reciprocal index (Table 4). Diversity index analysis showed a trend in which the conservation tillage (Min and Red) plots had more bacterial community diversity compared to the intensive tilled plots (Mod and High). This was consistent across years and at both the locations (Table 4).

Table 3. Soil basal respiration (BR) and substrate induced respiration (SIR) as affected by tillage intensity treatments for Southern Coastal Plain (SCP) and Southern Mississippi River Alluvium (SMRA) study sites.

		BR	SIR
	Tillage Intensity	**(μg of CO_2 g^{-1} of Soil Day^{-1})**	**(μg of CO_2 g^{-1} of Soil Day^{-1})**
Verona (SCP)			
2013	Min	32.31 a C	33.94 ab AB
	Red	40.66 a BC	20.69 b C
	Mod	41.38 a BC	35.03 a AB
	High	39.02 a BC	32.31 ab B
2015	Min	60.80 a A	43.02 a A
	Red	54.09 b AB	36.84 b AB
	Mod	50.28 bc AB	29.58 d BC
	High	46.65 c ABC	32.13 c B
Stoneville (SMRA)			
2013	Min	53.36 a BC	42.65 a A
	Red	42.29 a C	34.49 a AB
	Mod	43.02 a C	40.66 a A
	High	45.38 a C	33.76 a AB
2015	Min	73.33 a A	41.38 a A
	Red	64.61 a AB	31.94 b AB
	Mod	46.28 b C	25.05 b B
	High	53.18 b BC	30.13 b AB

Means (n = 3) followed by the same lower-case letter within year are not significantly different (LSD protected, $p < 0.05$). Means followed by the same upper-case letter are not significantly different between years within each location (LSD protected, $p < 0.05$).

Table 4. The Simpson diversity index of bacteria across tillage treatment plots for Southern Coastal Plain (SCP) and Southern Mississippi River Alluvium (SMRA) study sites.

	Tillage Intensity	**Simpson (1/D)**
Verona (SCP)		
2013	Min	205 a
	Red	189 a,b
	Mod	134 b
	High	125 b
2015	Min	216 a
	Red	217 a
	Mod	141 b
	High	136 b
Stoneville (SMRA)		
2013	Min	211 a
	Red	192 a
	Mod	129 b
	High	142 b
2015	Min	234 a
	Red	202 a
	Mod	139 b
	High	151 b

Means (n = 3) followed by the same lower-case letter within year are not significantly different (LSD protected, $p < 0.05$).

3.5. Relative Abundance of Bacterial Phyla as Affected by the Tillage Management

Across all soils, the broad level of phylum classification (>97% sequence similarity) showed that *Proteobacteria* were most abundant phyla (50.29%), followed by Actinobacteria (13.33%), *Acidobacteria* (7.36%), Bacteroidetes (7.34%) and Firmicutes (6.5%), covering 85% of all the sequences (Table 5). The other less represented phyla were *Cyanobacteria*,

Planctomycetes, *Chlorflexi*, and *Verrucomicrobia* with an overall abundance close to 5% or less. Proteobacteria abundance was significantly higher in moderate and high intensity tillage plots at both the locations compared to conservation tillage plots (Min and Red) (Table 5). This trend was more pronounced in year 2015. Actinobacteria and *Acidobacteria*, were significantly abundant in samples from conservation tillage plots at both SCP and SMRA locations (Table 5). While looking at the relative abundance of individual phylum across the treatment plots at SCP (Table 5), phylogenetic groups, such as, *Firmicutes* and *Bacteroidetes*, showed a significant change across the tillage systems both the years. At SMRA, *Bacteroidetes* abundance changed significantly across the treatment plots. Although these phyla changed across tillage treatment plots, there was no significant trends that could be discerned.

Table 5. Relative abundance (percentage) of five most abundant bacterial phyla) as affected by tillage intensity treatments for Southern Coastal Plain (SCP) and Southern Mississippi River Alluvium (SMRA) study sites.

	Tillage Intensity	***Actinobacteria* (%)**	***Proteobacteria* (%)**	***Firmicutes* (%)**	***Bacteroidetes* (%)**	***Acidobacteria* (%)**
Verona (SCP)						
2013	Min	13.21 a	48.75 a	6.60 a b	7.30 b	7.81 a
	Red	12.96 a	52.00 a	5.64 b	7.31 b	7.19 a
	Mod	13.42 a	49.52 a	7.20 a	6.75 b	7.54 a
	High	11.39 b	51.44 a	5.62 b	8.10 a	7.07 a
2015	Min	15.62 a	49.01 b	7.52 a	7.14 a	8.56 a
	Red	13.67 b	49.08 b	4.78 b	7.14 a	8.96 a
	Mod	11.66 b	52.24 a	6.41 b	6.12 a	7.46 b
	High	13.44 b	51.57 a	5.23 b	7.59 a	7.85 b
Stoneville (SMRA)						
2013	Min	14.73 a	48.85 a	6.77 a	6.89 b	7.43 a b
	Red	12.06 b	51.15 a	5.79 a	8.04 a	6.54 b
	Mod	13.68 a	49.40 a	7.40 a	7.63 a b	6.73 b
	High	13.58 a	49.61 a	6.48 a	7.14 a b	7.97 a
2015	Min	14.77 a	48.95 b	7.89 a	6.95 b	7.77 a
	Red	13.27 a	46.96 b	7.56 a	7.13 b	6.59 a
	Mod	12.89 b	52.56 a	6.56 a	7.56 a b	5.96 b
	High	12.84 b	53.63 a	6.49 a	8.59 a,b	6.23 b

Means (n = 3) followed by the same lower-case letter within year are not significantly different (LSD protected, $p < 0.05$).

3.6. Beta Diversity and Microbial Community Composition

The distribution of the most abundant OTUs across the treatment plots from SCP and SMRA showed strong dissimilarity in bacterial community structure across the different tillage treatments. (Figure 1). Tillage treatments explained 67% of the variation in the observed bacterial communities in 2013 and 80% in 2015 (Figure 1). In 2013, the bacterial community change in the minimum tillage intensity plots (Min) was significantly different from the other tillage plots as explained by the first axis (56.9%). There was no significant difference in the pattern of bacterial OTU distribution between other tillage treatments. However, location showed a moderate effect along the minor axis of variation (Axis 2 = 10.4%) and bacterial community composition at SCP was significantly different from SMRA plots (Figure 1a). In 2015, the clustering pattern showed that the soil bacterial communities in reduced till plots (Red) were most similar to the minimum-till plots (Min) than any other tillage treatments along the major NMDS axis (65.5%) of variability (Figure 1b).This indicated that the conservation tillage practices (Min and Red) had a significant effect on the bacterial community composition through the annual crop rotation cycles in these corn-soybean production systems.

This pattern was confirmed by the cluster analysis of the soil bacterial communities (Figure 2). UPGMA algorithm based on the Euclidean matrix of distance was used to construct cluster dendrograms to compare the number of sequences attributed to each taxon between tillage treatments (Figure 2). In the year 2013 (Figure 2a), two main clusters were observed, one for the minimum tillage (Min) and the other cluster for all the other tillage (Red, Mod and High) systems, clearly indicating differences in microbial-community

structure resulting from the absence or presence of plowing and disking (Figure 2). Within each soil- management system, two sub-clusters were formed, related to location. In 2015, the bacterial communities clustered into two major groups as conservation tillage (Min and Red) and conventional tillage (Mod and High) practices (Figure 2b).

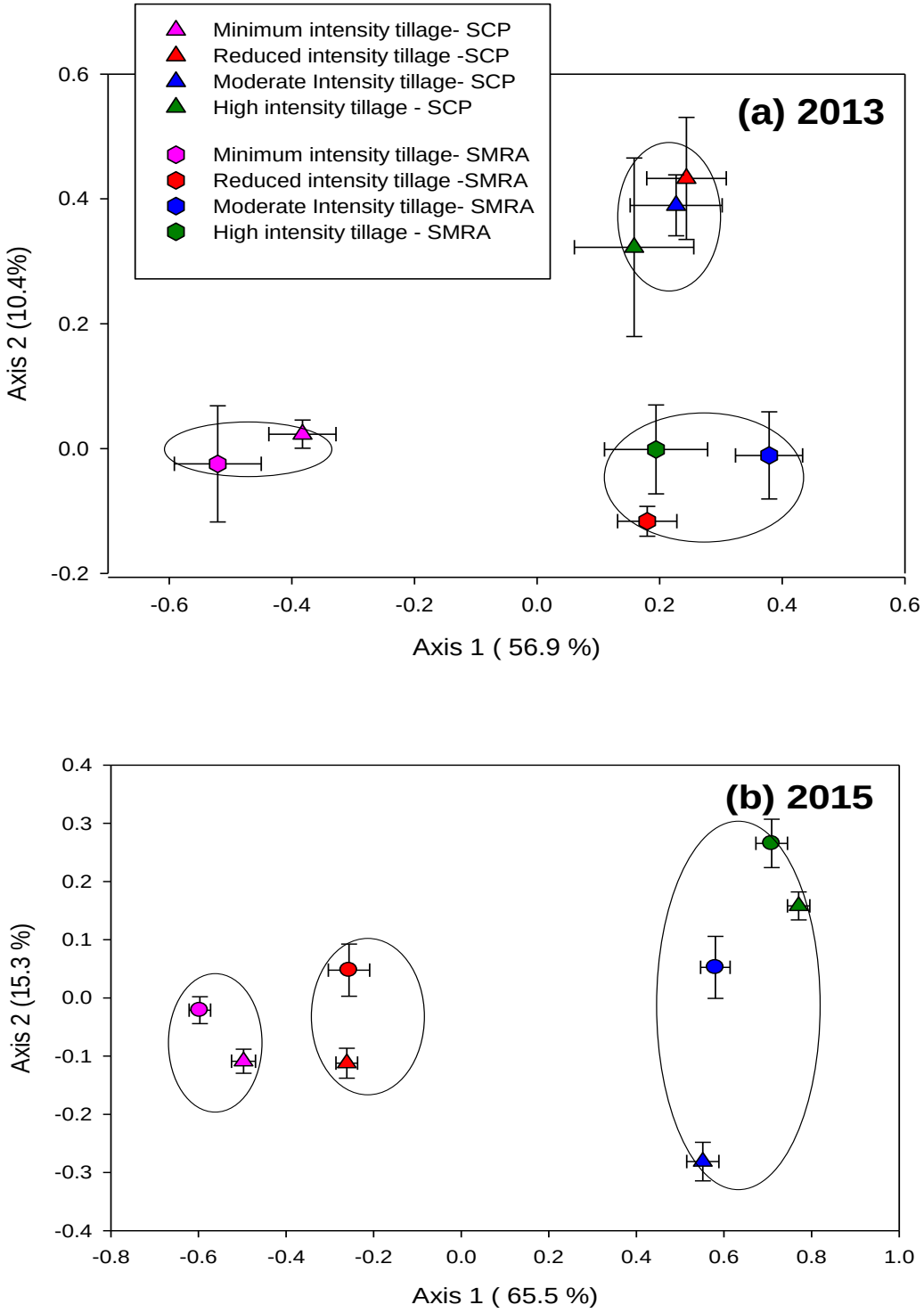

Figure 1. Non-metric multidimensional scaling plot (NMDS) based on the calculated Bray-Curtis distance showing the relationship between tillage management, location (Southern Coastal Plain—SCP and Southern Mississippi River Alluvium -SMRA) and bacterial community composition. ((**a**) = rotation cycle 1-2013 and (**b**) = rotation cycle 2-2015). The distance between symbols is inversely proportional to the degree of similarity between the communities.

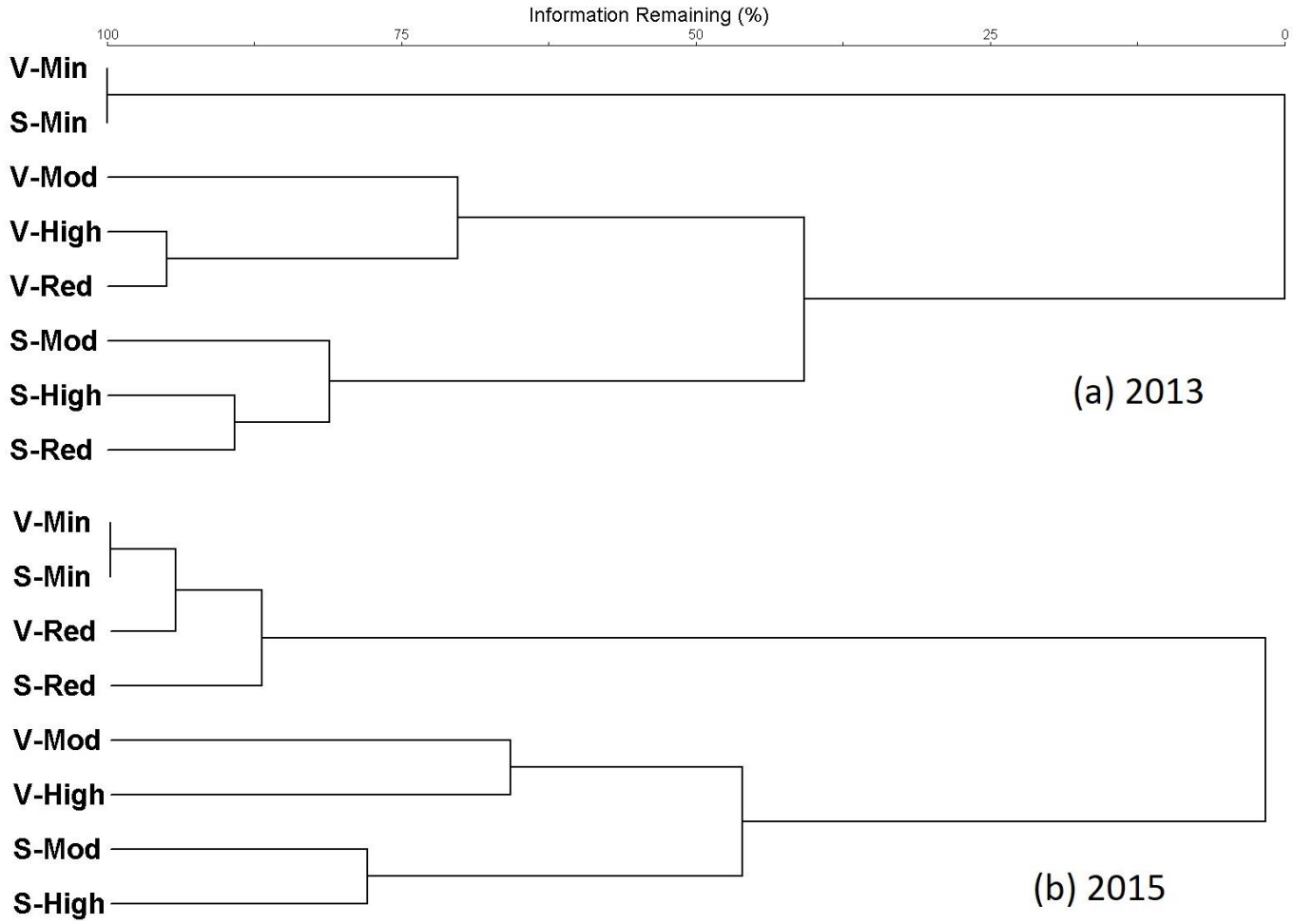

Figure 2. Cluster analysis comparing the influence of different tillage practices (Min- Minimum intensity tillage, Red-reduced intensity tillage, Mod-moderate intensity tillage and High- high intensity tillage) on the bacterial community composition during (**a**) 2013—cycle 1 of rotation (Corn-Soybean) and (**b**) 2015—cycle 2 of rotation (Soybean-Corn-Soybean) at both the locations (V-Verona and S-Stoneville). Hierarchical cluster analysis of the most based on Euclidean distance with UPGMA algorithm.

3.7. Relationships between Soil Parameters and Microbial Community Structure

Mantel test at 999 permutations was used to understand the correlation between the soil bacterial community distribution as affected by various factor involved in the study (Table 6). Overall, tillage treatments showed a strong influence on bacterial community distribution (rM = 0.39, $p < 0.0001$). Crop rotation cycle also had a significant influence on soil bacterial community composition (rM = 0.15, $p < 0.0001$). Soil physical properties (aggregate stability and penetrometer resistance) also showed a moderate effect on the soil bacterial composition (rM = 0.09, p =0.024). However, there was no significant correlation between the bacterial community distribution and the residue management (Table 6). The standardized Mantel statistic (rM) was not significant at 95% confidence level (rM = −0.009, $p = 0.638$).

The correlations between the bacterial community structures and the soil properties were analyzed using the distance-based canonical correspondence analysis (CCA) (Figure 3). The CCA biplot was constructed to illustrate the correlation between selected soil factor and soil microbial diversity over the two-year cycle (2013–2015). Based on this model, the first two constrained axes of the CCA showed a strong association between soil variables and the change in soil bacterial community. The first and second axes explained 21.2% and 11.34% of the total variation of bacterial community structure, respectively. Soil substrate-induced respiration (SIR) had a strong influence on bacterial community structure

followed by soil basal respiration (BR) and aggregate stability (AGG). The projected length and direction of these vectors showed a positive correlation with the conservation tillage plots (Min and Red) at both the locations (Figure 3).

Table 6. Mantel test results for the correlation between the bacterial community composition and selected variables.

Factors	r_M	*p*-Value
Tillage treatments	0.39	<0.0001
Rotation Cycle (Year)	0.15	<0.0001
Soil Physical Properties	0.09	0.024
Residue Management	−0.009	0.638

Mantel test of relationship between bacterial community similarity matrix, tillage treatments, residue management and environmental characteristics. r_M Standardized Mantel statistic.

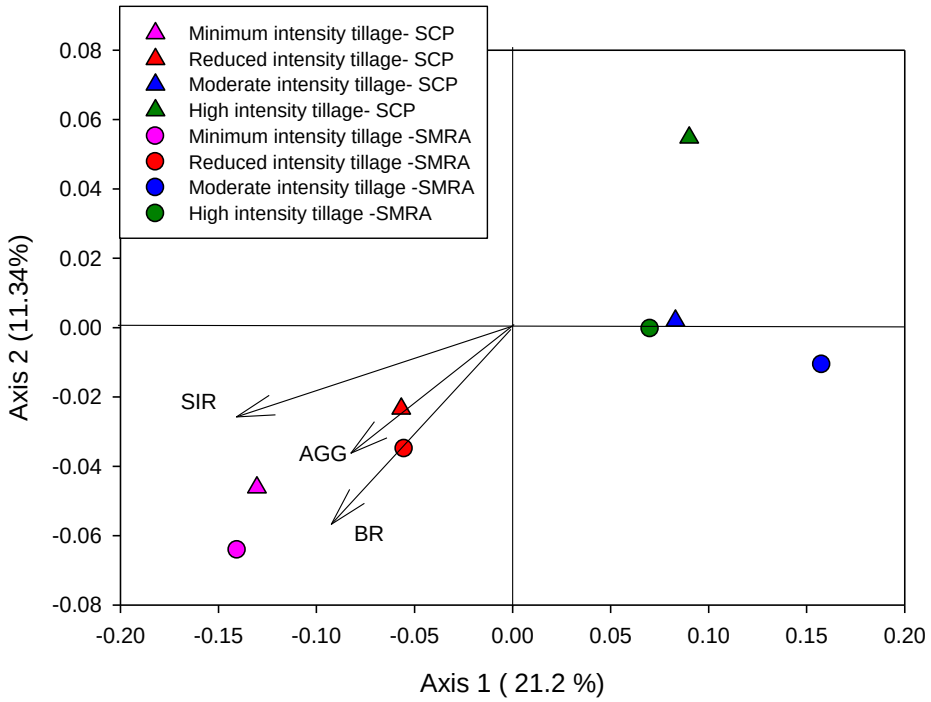

Figure 3. Canonical correspondence analysis (CCA) ordination biplots showing the association between the bacterial composition and soil biological indicators SIR (Substrate induced respiration), AGG (water stable aggregates) and BR (soil basal respiration) in 2015.The projected length and direction of the vectors centered in the panel on the two axis represents the strength and magnitude of the soil factor influence on microbial community composition. Notes: All environmental variables were significant ($p < 0.05$, Monte Carlo test of significance).

3.8. Relative Contribution of Management Factors on Bacterial Community Structure

To partition and quantify the relative explanatory power of tillage and soil physical properties on bacterial community structure, we used variance partitioning analysis (VPA) on those variables. For this analysis, soil parameters that had significant correlation with the bacterial community structure (aggregate stability, BR, SIR, and penetrometer resistance) were chosen based on the CCA plots. The combination of selected soil characteristics and tillage regimes showed a significant ($p = 0.003$) correlation with the bacterial community structure. The soil physical properties explained 22.7% ($p = 0.017$), and tillage treatments alone explained 52.8% ($p = 0.001$) variations in the soil bacterial community data (Figure 4).

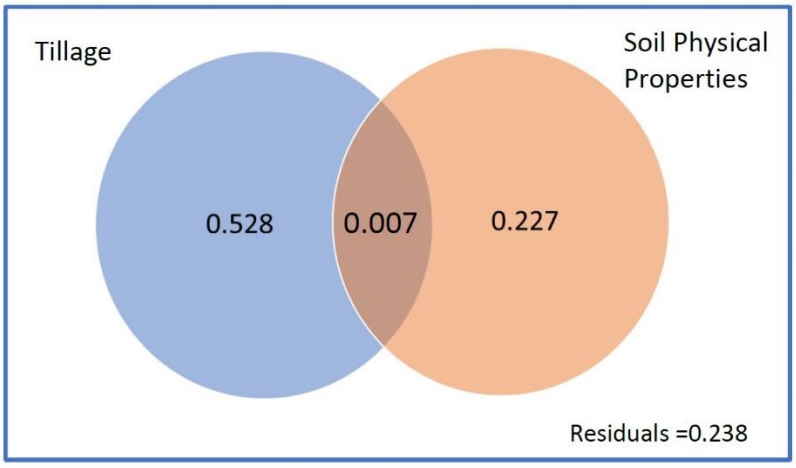

Figure 4. Variance partition analysis of the effects of tillage treatments and soil variables on the bacterial community structure.

4. Discussion

4.1. Soil Characteristics as Affected by the Tillage Systems

Unlike the strong relationship between the soil microbiome and tillage systems that we detected; soil chemical properties were not influenced by tillage practices (Table 1). However, the tillage treatments had a significant influence on soil physical properties (Table 2). Our study showed higher percentages of stable aggregates for the least intensive tillage practices (Table 2), which was consistent with increase in substrate quality (Table 3) and microbial diversity (Table 4). The stability of aggregates is mainly determined by quantity and nature of organic compounds involved in binding soil particles together [3]. The sources of these binding agents are by-products of decomposition of soil organic matter and crop residues, and secretions by microorganisms [39]. It has been shown previously that the aggregates and the soil structure are maintained due to the lack of plowing, which, over time, results in an increase of macro-aggregates and helps in reducing water loss [16,17,40]. Soil tillage system influenced the penetration resistance more significantly (Table 2). This could be because the soil penetrometer resistance is a complex measurement that depends on the condition of soil compaction, soil structure and soil moisture conditions which are strongly determined by soil disturbance [1,41,42].

4.2. Soil Bacterial Communities as Affected by the Tillage Systems and Carbon Inputs

Proteobacteria are shown to be efficient decomposers and are functionally related to decomposition of organic matter, and soil carbon [43,44]. Although many studies have shown conservation tillage favoring *Proteobacteria* [15,45], our study showed higher abundance of *Proteobacteria* in conventionally tilled plots (Mod and High) (Table 5). In soil, studies have shown that Proteobacteria (β) are one of the initial metabolizers of labile carbon inputs and they respond well to labile carbon sources [46]. They are generally considered fast-growing r-selected microbes that fluctuate opportunistically based on the carbon availability in the system. Tillage operations in conventionally tilled plots leads to breaking up and incorporation of crop residues into the surface layers of soil. Decomposition and mineralization processes are then accelerated as the microbial decomposers act on the readily available carbon [12,40]. Thus, Proteobacteria were more abundant in the conventionally tilled plots (Mod and High) than the conservation- till plots (Min and Red). Our study also showed higher abundance of *Actinobacteria* and *Acidobacteria* under conservation tillage practices. *Actinobacteria* are considered important in the cycling of organic materials such as cellulose [18,47] and their relative abundances may have been increased due to crop residue availability in conservation tillage plots; similar results have been reported by others [14,44,45]. Tillage also have shown to help mobilize nutrients and thereby appears to enrich for competitors under a C-S-R strategy model [18,48]. Indirectly, tillage would have

reduced habitat heterogeneity which would have favored these competitors and helped them outgrow other competitors [49]. This would have eventually led to reduced overall diversity in the system.

In contrast to these results, the metabolic quotients were increased under conservation tillage (Min and Red) compared to conventional tillage plots (Table 3). This could be due to the quantity and quality of the carbohydrates entering these systems and how the microbes are partitioning the carbon resources. Although microbial metabolic quotient measured through soil respiration is a good indicator of microbial efficiency, it has been shown to have limitations in capturing subtle changes in microbial metabolism caused by soil disturbance [50]. Based on these results, we incur that conservation tillage (Min and Red) might favor microorganisms of higher metabolic efficiency that can metabolize complex carbon sources while the conventional tillage (Mod and High) plots support organisms that can proliferate utilizing a broad pool of labile carbon sources.

4.3. Influence of Tillage Treatments on Bacterial Alpha and Beta Diversity

Bacterial diversity, measured by Simpson's index, was significantly different across tillage treatments (Table 4). Our results showed that bacterial community in conservation tillage (Min and Red) plots were more diverse in terms of evenness and richness. Tillage reduced microbial diversity through reduced richness (Simpson's index) consistent with studies that reported reduced microbial diversity under conventional tillage systems [51–53]. In a review investigating various factors that influence soil microbial diversity, [5] reported that soil disturbance through tillage was the major factor affecting soil microbial diversity. Tillage practices could change the soil physicochemical properties and thereby alter the microhabitat for microorganisms, which might change carbon utilization pattern and activity of soil microbes [54–56]. Various factors, such as, desiccation, mechanical disruption, soil compaction, reduced pore volume and reduction of access to food sources have been attributed to tillage-related diversity changes in soil [4,57]. Although not significantly, bacterial diversity increased from 2013 to 2015 season along the crop rotation cycles (Table 4). The conservation tillage practices across crop rotation cycle years, would have caused the strongest and more adaptable communities to establish and flourish by providing a suitable environment for the multiplying soil bacteria. Our results imply that conservation tillage treatments strongly affect soil characteristics and thereby significantly influencing changes in alpha diversity.

Our results also showed that bacterial community structure (estimated with the Bray-Curtis Dissimilarity Index) differed significantly across tillage treatments (Figure 1). In agreement with our results, previous studies reported on the potential of conservation tillage (and no-till) management to significantly affect microbial community composition [1,14,58]. We also found that the β-diversity results were tracking the changes in bacterial phyla responding to tillage treatments (Table 5). Our findings suggest that the soil bacterial β-diversity was significantly influenced by conservation tillage practices through considerable changes it imparts in the substrate availability to soil bacterial communities.

4.4. Strategic Tillage Recommendation

Based to our results, the soil bacterial community composition in reduced till plots (Red) were becoming more similar to the minimum-till (Min) plots (Figures 1 and 2) during the crop rotation cycles (year 2013–2015) as a result of the accumulated tillage effect. We also noted that all other soil physical and biological properties were consistent with this trend. Based on the amount of soil disturbance it imparts, reduced intensity tillage (Red) can be considered as a strategic tillage treatment in these corn-soybean production systems [1,59]. This tillage treatment (Red) could be considered as an alternative approach to manage some of the shortcomings of the no-till farming systems such as managements of weed populations and alleviating the pressure of stubble-borne diseases [13]. According to our results, this could be achieved without altering the structural and functional diversity of soil microbes and the associated soil quality benefits of a no-till farming system.

5. Conclusions

It has become imperative to examine the effects of specific soil attributes on soil microbial diversity that would help understand what drives their population changes. This would help to make concrete improvements in current management practices in soybean production systems to adequately promote soil biodiversity conservation practices to achieve sustainability in soybean production. This study was a preliminary attempt to understand bacterial biodiversity as affected by contrasting tillage regimes under a long-term corn-soybean rotation system. We conclude that physical disturbance caused by secondary tillage may be a crucial factor in homogenizes the microenvironments that harbor unique microorganisms. This might lead to decreasing of soil bacteria species diversity. Results also showed that conservation tillage may create stable environments which favor diverse communities and slower nutrient turnover. The results from this study could add to other research works in better understanding the role of conservation tillage in altering the soil microbial habitat and eventually help enhancing soil ecological functions in building a stable soil environment. These findings might help providing insights to design strategic tillage practices in these crop production systems for sustainable productivity.

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