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SHORT-TERM EFFECTS OF TILLAGE TREATMENTS ON SOIL MICROBIAL BIODIVERSITY UNDER SOYBEAN-CORN ROTATION

Shankar Ganapathi Shanmugam[1,4], Normie W.Buehring[2], M. Wayne Ebelhar[3], Michael S. Cox[1], James Larry Oldham[1], Daniel G. Peterson[4] and William L. Kingery[1]

[1]Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS

[2]North Mississippi Research and Extension Center, Mississippi State University, Verona, MS

[3]Delta Research & Extension Center, Mississippi State University, Stoneville, MS

[4]Institute for Genomics, Biocomputing & Biotechnology, Mississippi State University, Mississippi State, MS 39762

ABSTRACT

Soil health provides an overall picture of soil functionality that includes chemical, physical and biological features necessary for long-term, sustainable productivity. Studies have shown that tillage has profound and complex influence on soil physical, chemical and biological properties. This study was initiated to evaluate the effects of tillage, crop rotation and residue management on soil biodiversity in a soybean production system. Soil samples were collected from ongoing field experiment that studies the effects of tillage and residue on soil quality under soybean-corn rotation scheme located at North Mississippi Research and Extension Center (non-irrigated) and Delta Research and Extension Center (irrigated) in Verona and Stoneville, Mississippi, respectively. Soil samples were collected from existing experimental plots receiving four kinds of tillage: (1) reduced-tillage on old beds (RT), (2) bed-roller (BR), (3) disk + TerraTill (D+TT) and (4) TerraTill® (in-row-subsoiler-bed-roller, one-pass implement) (TT). Based on the nature of soil disturbance caused by the tillage treatments they were broadly grouped as conservation tillage systems (RT and TT) and conventional tillage systems (BR and D+TT). The bacterial community change at the Reduced-Till (RT) plots was significantly different from the other sites as explained by the axis of maximum variability from Bray-Curtis ordination (Axis 1= 76 %). There was no significant difference in the pattern of soil bacterial distribution between other tillage management practices. However, there was a moderate location effect along the minor axis of variation (Axis 2= 4 %) and bacterial community composition at Verona was significantly different from Stoneville plots. *Proteobacteria* were least abundant in the RT plots

ISSN: 2455-6939

Volume:02, Issue:05

when compared to other tillage systems at both the locations. Based on the Simpson's reciprocal index, the bacterial diversity was considerably higher in RT and Terra-Till plots. Results show that conservation tillage may create stable environments which favor diverse communities and slower nutrient turnover.

Keywords: Soil microbial Diversity, 16S rRNA gene, tillage and crop rotation, reduced tillage.

1. INTRODUCTION

Soil health provides a concept of soil functionality that includes chemical, physical and biological features necessary for long-term, sustainable productivity. Although considerable attention has been placed on the chemical and physical property impacts on soil quality, publications on sustainable land use have shown that there is a lack of research on the assessment of "soil quality" from a soil microbiological point of view (Pankhurst et al., 1997). 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 (Anderson, 2003; Doran and Zeiss, 2000). However, there is a lack of knowledge regarding the effect of tillage and residue management on soil microbial diversity under major crop rotation systems. Hence, this study was conducted to learn more about the interactions between chemical, physical and biological indicators under different tillage and residue management systems that effectively support sustainable agriculture.

Microorganisms are major factors in regulating numerous biological processes in soil (Kuramae et al., 2011). Soils are highly heterogeneous both physico-chemically and biologically, thereby encompassing a wide range of niches available to sustain microbial diversity (Reynolds et al., 2003). Numerous studies have shown the influence of both abiotic and biotic factors on microbial community structure (Brockett et al., 2012; Kuramae et al., 2011). Many factors are responsible for the composition of soil microbial community and their differences may cause variability in the distribution of microbial species. Soil type has been shown to be a primary factor determining microbial species composition (Bossio and Scow, 1998; Girvan et al., 2003). Bossio et. al (1998) found that soil type was the major determinant driving bacterial community structure in fields maintained under different management systems (organic and conventional systems). In a similar study, Girvan et al. (2003) showed that soil type was the key factor determining bacterial community composition in two cropping systems that had two major soil types. Other environmental factors, such as quantity and quality of available soil carbon (Blaalid et al., 2012), soil nitrogen (Eaton et al., 2011), soil water content (Schaaf et al., 2011), soil texture (Sun et al., 2013) and land-use history (Sun et al., 2015), either alone or in combinations, have been shown to be significant contributors in shaping the soil microbial community structure. Soil pH has been often shown as significant contributor with significant correlations

ISSN: 2455-6939

Volume:02, Issue:05

with soil microbial community structure, particularly bacteria. For example, recent studies across huge spatial scales have shown that soil pH could be a key driver that influences the bacterial community composition (Lauber et al., 2009; Rousk et al., 2010). Thus, land use management practices, such as different tillage systems and residue management practices which have direct influence on soil characteristics are important factors to be studied in order to understand soil microbiome community distribution in relation to soil functions.

Studies have shown that tillage has profound and complex influence on soil physical, chemical and biological properties. 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 (Roger-Estrade et al., 2010). These physical disturbances caused by tillage affect the organisms that inhabit these environments (Anderson, 2003; Van Bruggen and Semenov, 2000).Several authors have reported increase in soil microbial biodiversity due to conservation tillage, particularly no-tillage systems (Doran, 1980; Doran and Zeiss, 2000; Govaerts et al., 2007). This increase in microbial diversity also improves the ability of these organisms to effectively use the carbon sources. Increased microbial diversity and abundance usually accompanies increase in soil organic matter level. Thus, besides reducing cost of production, conservation tillage also increases organic matter retention –over time and help in reducing farm inputs (Mbuthia et al., 2015).

Crop rotation alters the quantity and quality of plant residues entering the field through their spatio-temperal variability of input supply (Dorr de Quadros et al., 2012). They determine the above-ground and below-ground inputs and influences soil microorganisms and soil microbial processes (Jordan and Leake, 2004). An example of this is the inclusion of legumes in crop rotation which often reduces the need for nitrogen for the subsequent crop and also helps in improving soil properties due to enhanced microbial activity. It has been estimated that biological N fixation by incorporating leguminous plants may fix more than 100 kg N ha^{-1} $year^{-1}$ (Van Bruggen and Semenov, 2000). Soil biodiversity and their ecosystem services are not commonly priced in the market; however, they have both direct (direct metabolic inputs) and indirect (long-term outcomes) cost-benefit to the farmers. So it has become indispensable to make concrete improvements in current management practices to adequately promote soil biodiversity conservation practices to achieve sustainability in crop production.

In this study, we collected soil samples from four types of tillage treatments and two types of residue management treatments. We hypothesized that the different tillage treatments would target distinct soil microbiomes and different soil properties and, therefore, would likely influence different soil microbial community assemblies. Consequently, a study was initiated in 2013 with the following objectives: (1) to study the influence of different tillage treatments under

ISSN: 2455-6939

Volume:02, Issue:05

two residue management regimes on soil microbial biodiversity and (2) to determine any correlations between the soil variables and changes in the abundance, diversity, and composition of soil bacteria under different tillage treatments.

2. MATERIALS AND METHODS

2.1 Experimental sites and tillage treatments

This study is part of a long-term trial that began in 2011 and established at two locations in Mississippi. One study was established on a Marietta loam (Fine-loamy, siliceous, active, thermic Fluvaquentic Eutrudepts) soil (non-irrigated)at the North Mississippi Research and Extension Center, Verona, MS; and another study was established on Bosket very fine sandy loam (Fine-loamy, mixed, active, thermic Mollic Hapludalfs) and Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalfs) soils (irrigated) at the Delta Research and Extension Center, Stoneville, MS. The experimental treatments combined different tillage methods on residue retention for croplands with corn-soybean rotation. The experimental design included a replicated split-plot design with residue treatments as main plots and fall tillage as sub-plots. The study compared the effects of four tillage treatments on soybean plots where sowing was carried out on the residues of previous corn crop. The tillage systems were reduced-tillage on old beds (RT), bed-roller (BR), TerraTill® (in-row-subsoiler-bed-roller, one-pass implement) (TT) and disk + TerraTill. (D+TT). The crop residue treatment included either burning (burn) or not burning (no-burn) the corn residues following harvest and before planting the soybean. Based on the nature of soil disturbance caused by the tillage treatments they were broadly grouped as conservation tillage systems (RT and TT) and conventional tillage systems (BR and D+TT). The data reported for this study were collected from October to November 2013.

2.2 Soil Sampling and physicochemical analysis

Soil samples were collected from zone of dominant root activity (A-horizon) in each site at a depth of 0 to 15 cm. Composite soil samples consisting of 12 soil cores were collected at four locations within each plot using a 32-mm diameter soil probe following the 2013 crop harvest. After sampling, soil from O- horizon was carefully removed and immediately transferred into a Ziploc® bag after mixing and homogenizing. The sample bags were frozen immediately in container filled with dry ice. Upon arrival in the laboratory, soils were thawed for 25-30 minutes. Further homogenized, extraneous roots and organic materials were removed and the samples were stored at -80˚C. A total of 48 soil samples were analyzed (four tillage treatments and two residue treatment with three replications at two locations).

Extractable phosphorus (P), potassium (K), calcium (Ca) magnesium (Mg) and sodium (Na) were analyzed based on a Mehlich 3 extraction and measured using Inductively Coupled Argon

ISSN: 2455-6939

Volume:02, Issue:05

Plasma Spectroscopy (ICAP) (Thermo Fisher, Waltham, MA). Soil pH was measured on a 1:1 soil/water suspension. Total C and N were measured using Elementar Vario EL III combustion analyzer after samples had been air dried, ground and sieved through a 30 µm sieve (Elementar Americas Inc., Mt. Laurel, NJ).

2.3 DNA sequencing details

Soil DNA was extracted from 0.5 g of soil using ZR Soil Microbe DNA Kit™ (Zymo Research). After purification the template DNA was stored in -80˚C. The 16S rRNA gene V4 variable region bacterial primers 515F/806 R with barcode on the forward primer were utilized to evaluate the microbial ecology of samples on the Illumina MiSeq platform. Briefly, a single-step 30 cycle PCR using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product was used to prepare DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed using the Illumina MiSeq sequencing platform following the manufacturer's guidelines. A total of 48 different DNA libraries were assembled from the two sites (irrigated and non-irrigated) across the treatment plots. Across all 48 samples, we obtained a total of 4,447,258 quality sequences, with a range of 21,412 to 159,907 sequences from each soil. The average read length of the sequences was ~ 430 bp). In order to verify whether the number of sequences analyzed affected the calculation of diversity indices, random sub-samples from each soil library was used to build the distance matrix.

2.4 Processing of 16S rRNA gene data

A two- step pipeline was established to analyze the 16S rRNA gene sequence data. QIIME (Quantitative Insights into Microbial Ecology) was used to quality trim the raw sequences for primers, chimeras and to sort them based on the barcodes (Caporaso et al., 2011; Caporaso et al., 2012). The denoised data was then passed through MOTHUR v1.22.0 (Schloss et al., 2009), a software for describing and comparing microbial communities. To facilitate the downstream analysis of the large sequence datasets, identical sequences or artificially duplicated sequences, which can constitute a significant fraction of the dataset, were removed (Quince et al., 2011; Schloss et al., 2011). The non-redundant sequence dataset was then aligned using SILVA reference dataset (http://www.arb-silva.de/) (Quast et al., 2013). A second pass in MOTHUR, sequence reads were assigned (clustered) to Operational Taxonomic Units (OTUs) based on pair

ISSN: 2455-6939

Volume:02, Issue:05

wise distances between all aligned sequences. A column formatted distance matrix was generated using average-neighborhood algorithm at an evolutionary distance D=0.03, which restricted the distance matrix to keep only sequence reads that had 97 % sequence similarity.

The alpha-diversity estimates such as rarefaction, richness, evenness, Shannon, Simpson's reciprocal index and Chao1 estimates were done on OTUs at D=0.03 evolutionary distances (about 97% sequence similarity). This level of DNA sequence similarity is typically used to assign sequences to the same taxa (Schloss et al., 2011). Representative sequences from dominant OTUs were assigned using SILVA.

2.5 Statistical and Multivariate analyses

Data were analyzed by Analysis of Variance (ANOVA) based on the Generalized Linear Mixed Model (GLIM Mix). Significant differences in the means of three replicates among the field treatments were considered at $P < 0.05$ using Fisher's protected LSD in SAS (SAS Institute Inc., Cary, NC, USA).In order to visualize the differences in microbial community differences among the treatment plots based on 16S rRNA gene sequences, non metric multi dimensionals caling (NMDS) ordination techniques were used. This method was used because it does not assume linear relationships between variables and is very effective method for ecological data (Clarke, 1993). Bray-Curtis ordination analysis was used to visualize bacterial community structure and to investigate the components of the structure that has the most influence on the final ordination solution. This method was used to calculate dissimilarity matrix, which is a pair-wise comparison based on Sørensen distance measure. In this method, samples are randomly placed in 1 to 3 dimensions and Euclidean distance is calculated between samples. The elements of dissimilarity (Sørensen distance) are ranked in ascending order and plotted against the ordination distance (Euclidean distance). A stress values is calculated which measures the departure of monotonicity of the above mentioned plot. Hence, lesser the stress value more is the reliability of the relation. After multiple iterations the lowest stress value can be attainted and samples containing common microbes will cluster together in ordination space(McCune and Grace, 2002). The final dimensionality of the dataset was determined based on the stress and stability measurements from the ordination analysis (McCune and Grace, 2002)using the PC-ORD software (MJM Software, Gleneden Beach, OR, USA). Mantel test was performed using the PC-ORD software to determine correlations between bacterial community composition and soil characteristics. For the Mantel test, Sørensen distance measure was used with a random starting configuration. Pearson and Kendall correlations (r^2 values)between the ordination axes and the environmental variables were calculated using the Sørensen distance measure. Hierarchical cluster analysis of the most abundant OTUs was done using the PC-ORD software (McCune and Mefford, 1999).Factors that significantly influenced community composition were used to

ISSN: 2455-6939

Volume:02, Issue:05

construct a soil variables matrix for Variance partitioning analysis in CANOCO (ver 4.5) (Ter Braak and Verdonschot, 1995).The significance of the correlations with each factor was evaluated through the Monte Carlo permutation test by applying 998 permutations.

Alpha diversity was calculated using MOTHUR to estimate richness with the Chao1 estimator and the abundance-based estimators, while we estimated diversity using the Shannon diversity and Simpson diversity indices. For beta diversity, taxonomic and phylogenetic community comparisons were performed using Bray-Curtis ordination using weighted and unweighted UniFrac distance matrices. Non-metric multi-dimensional scaling was used to illustrate the clustering of the different samples. Analysis of similarities was performed to test the significance of separation between different tillage treatments. To study the relationship between the soil characteristics and the abundance of dominant phyla, a redundancy analysis was used. Correlations between the soil bacterial community structure and soil characteristics were determined using Mantel tests with 999 permutations. The Spearman's rank correlations between the abundant phyla and soil properties were calculated using the Sigmaplot version 22.0 software packages (Systat Software Inc.). Nonmetric multi-dimensional scaling, analysis of similarities, redundancy analysis, and Mantel tests were performed using using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). A P-value of < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Tillage treatment contributes to differences in soil characteristics

Verona (non-irrigated): Crop residue (burn vs no-burn) management did not have any effect on the soil chemical characteristics. The interaction between tillage and residue management was also not significant. Therefore, the effects of tillage management on some basic soil characteristics are shown in Table 1. Tillage systems showed a significant effect on extractable Ca and P. The conservation tillage plots also had lower pH levels than conventionally tilled plots. Total C content (%) was lowest in the reduced till plots (Table 1) compared to other tillage systems.

ISSN: 2455-6939

Volume:02, Issue:05

Table 1. Analysis of variance of selected soil chemical properties for the fall Soybean, Verona, MS.

Tillage system	Ca	K	Mg	Na	P	pH	OM	CEC	Total N	Total C
			Kg ha^{-1}				(%)	meq 100g^{-1}	(%)	(%)
Reduced-tillage	4043 b	359	185	25	176 ab	6.3 b	1.1	12.3	0.05	0.25 b
Bed-roller	4019 b	321	177	24	192 a	6.2 b	1.1	12.3	0.05	0.32 a
Disk (2X) + TerraTill	4438 a	317	169	23	168 b	6.7 a	1.0	12.5	0.05	0.27 ab
TerraTill	4373 ab	343	175	23	180 ab	6.6 a	1.1	12.5	0.05	0.27 ab

Means (n=3) in a column followed by the same lower case letter are not significantly different (LSD protected, $P \leq 0.05$).

Stoneville (irrigated): There was no significant crop residue management by tillage system interactions for the soil nutrient content. There were no significant differences among tillage systems for soil extractable nutrients such as Ca, Mg, Na and P (Table 2). There was a significant tillage effect on K and soil organic matter (Table 2).

ISSN: 2455-6939

Volume:02, Issue:05

Table 2. Analysis of variance of selected soil chemical properties for the fall Soybean, Stoneville, MS.

Tillage system	Ca	K	Mg	Na	P	pH	OM	CEC	Total N	Total C
			Kg ha^{-1}				(%)	meq 100g^{-1}	(%)	(%)
Reduced-tillage	3329	419 a	597	57	114	6.6	0.91 ab	11.7	0.06	0.31
Bed-roller	3346	400 ab	585	62	112	6.5	0.89 ab	11.9	0.05	0.31
Disk (2X) + TerraTill	3335	390 ab	612	57	110	6.6	0.96 a	11.8	0.05	0.31
TerraTill	3233	369 b	561	59	110	6.5	0.86 b	11.5	0.05	0.30

Means (n=3) in a column followed by the same lower case letter are not significantly different (LSD protected, $P \leq 0.05$).

3.2 Microbial Community structure and Crop management

The distribution of 200 most abundant OTUs (Operational taxonomic Units- Bacterial species groups) across the treatment plots from Verona and Stoneville showed strong relationships between the bacterial community change and tillage management (Figure 1). The bacterial community change at the RT plots was significantly different from the other tillage plots as explained by the axis of maximum variability from Bray-Curtis ordination (Axis 1= 76 %). There was no significant difference in the pattern of bacterial OTU distribution between other tillage treatments. However, there was a moderate location effect along the minor axis of variation (Axis 2= 4 %) and bacterial community composition at Verona was significantly different from Stoneville plots (Figure 1).

ISSN: 2455-6939

Volume:02, Issue:05

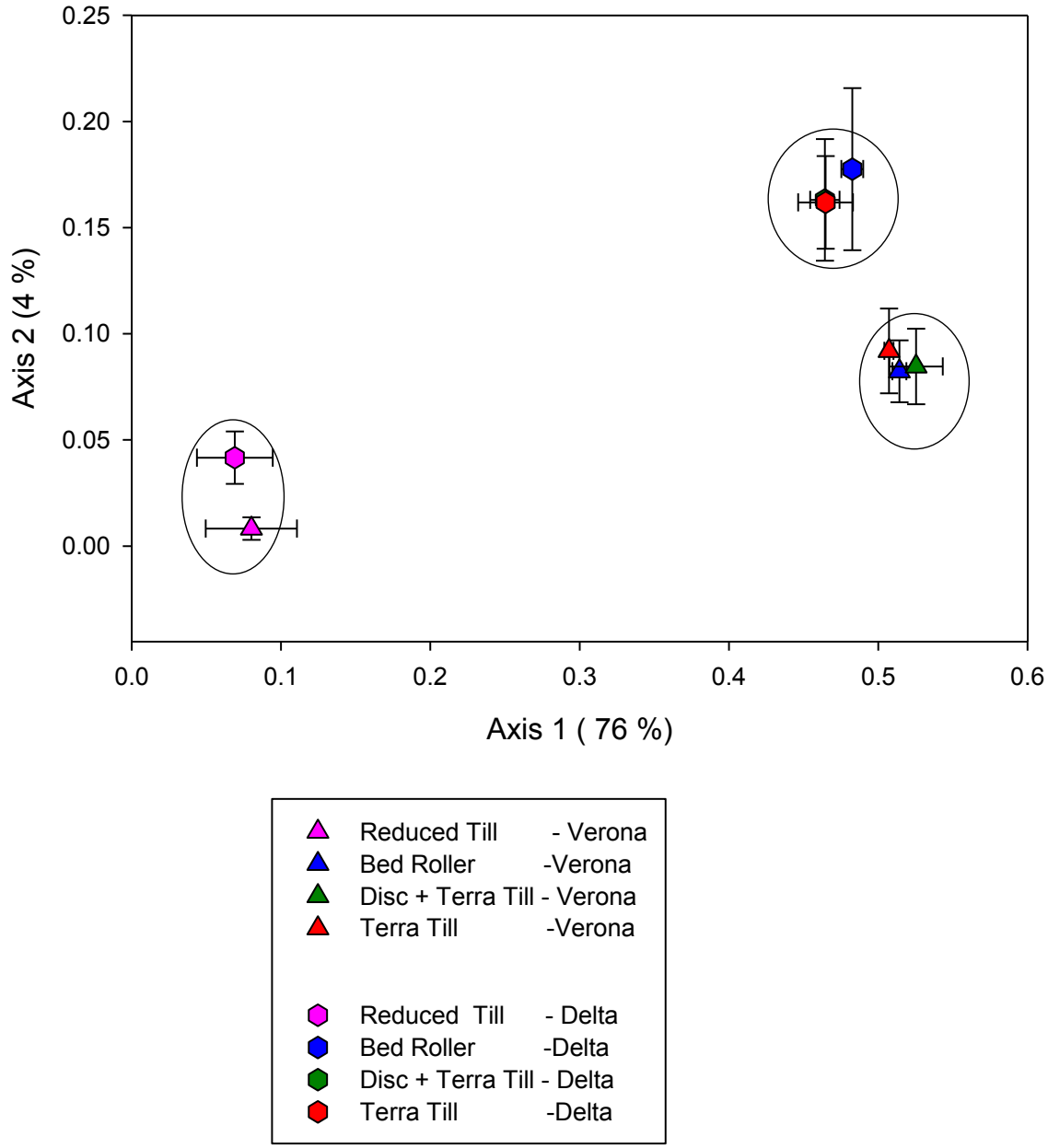

Figure1: Bray-Curtis ordination plot showing the relationship between tillage management, location and bacterial community composition. Error bars represent standard error (n=3). Percentages on each axis denote the amount of variability associated with each axis.

ISSN: 2455-6939

Volume:02, Issue:05

This pattern was confirmed by the cluster analysis (Figure 2). The number of sequences attributed to each taxon was compared between the treatments based on a Euclidean matrix of distance, estimated with the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm and represented as a phylogenetic tree (Figure 2). Two main clusters were observed, for the RT and the CT 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. However, there was no significant effect of residue management on the bacterial community distribution in either location (Table 5). A mantel test was performed to understand the correlation between the soil bacterial community distribution as affected by various factor involved in the study. It showed that there was no significant correlation between the bacterial community distribution and the residue management (Table 5). The standardized Mantel statistic (r_M) was not significant at 95 % confidence level (r_M = - 0.009, P = 0.638). Tillage treatments showed an overall significant influence on bacterial community distribution (r_M = 0.29, P <0.0001) followed by soil characteristics showing moderate effect.

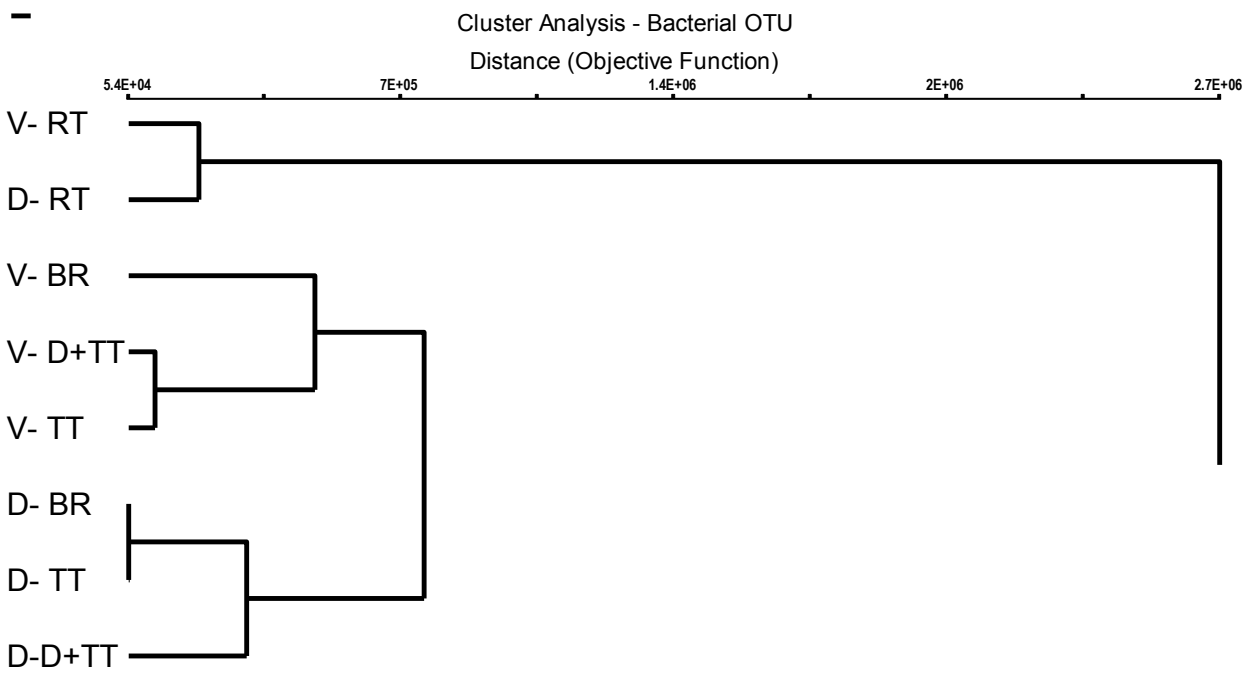

Figure 2. Hierarchical Cluster Analysis of the most abundant OUT based on Euclidean distance with UPGMA algorithm. The distance axis represents the similarity index.

ISSN: 2455-6939

Volume:02, Issue:05

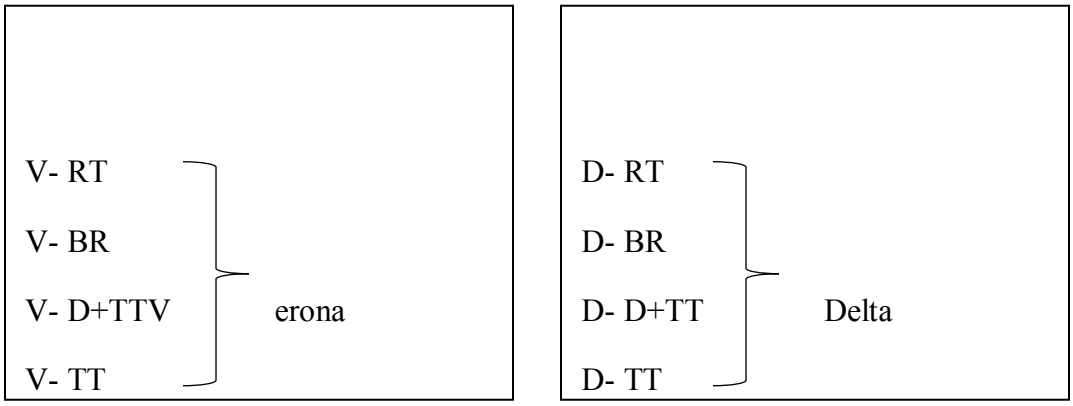

Table 5. Mantel test correlations between the bacterial community distribution and selected variables.

Variable	Mantel Test [a]	
	r_M	P-value
Tillage treatments	0.29	<0.0001
Soil chemical characteristics	0.15	0.002
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

3.3 Bacterial Phylogenetic Distribution and Crop Management

Across all soils, the broad level of phylum classification (> 97% sequence similarity) showed that *Proteobacteria* were most abundant (50.34 %), followed by *Actinobacteria* (13.13 %) and

ISSN: 2455-6939

Volume:02, Issue:05

Acidobacteria (7.29 %) covering 70 % of all the sequences (Figure 3). The other less represented phyla were *Cyanobacteria, Planctomycetes, Bacteroidetes, Chlorflexi, Verrucomicrobia* and *Firmicutes* with an overall abundance close to 5% or less. While looking at the relative abundance of individual phylum across the treatment plots at Verona (Table 3), phylogenetic groups, such as, *Actinobacteria, Proteobacteria, Firmicutes* and *Bacteroidetes*, showed a significant change across the tillage systems. At Stoneville (Table 4), *Bacteroidetes, Acidobacteria, Planctomycetes* and *Verrucomicrobia* abundance changed significantly across the treatment plots. *Proteobacteria* were least abundant in the reduced-till plots when compared to other tillage systems at both the locations (Tables 3 and 4). Although not statistically significant, *Acidobacteria,* showed an increasing abundance in reduced -till soils compared to other tillage systems at Verona (Table 3). However, reduced-till plots at Stoneville showed significantly higher *Acidobacteria* abundance compared to other tillage systems (Table 4). *Cyanobacteria* in this study appeared to be in lower abundance (not statistically significant) in the reduced-till plots than other tillage systems, at both the locations (Tables 3 and 4).

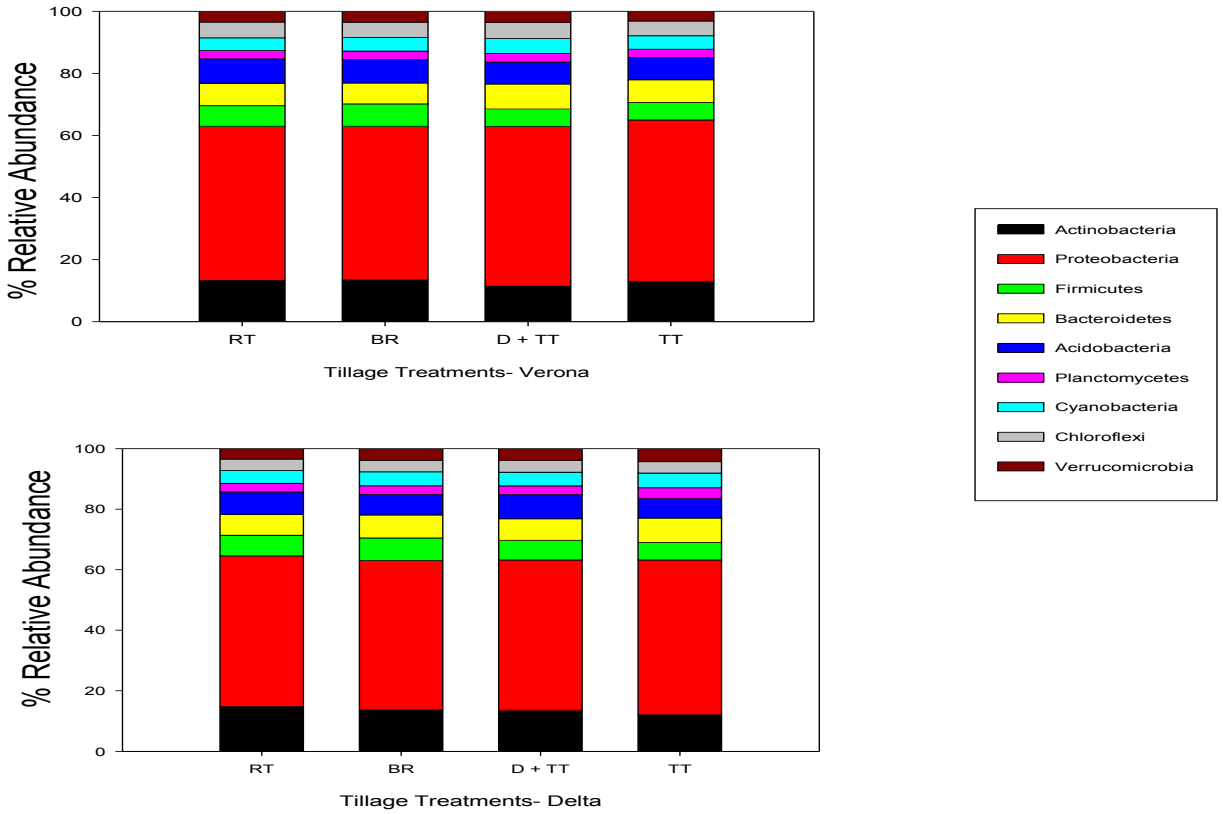

Figure 3.Relationship between the relative abundance of bacterial phyla across the tillage treatment plots.

ISSN: 2455-6939

Volume:02, Issue:05

Table 3: Relationship between tillage treatments and nine individual bacterial phyla at Verona.

Tillage	*Actinobacteria*	*Proteobacteria*	*Firmicutes*	*Bacteroidetes*	*Acidobacteria*	*Planctomycetes*	*Cyanobacteria*	*Chloroflexi*	*Verrucomicrobia*
RT	13.21 a	48.75 b	6.60 ab	7.30 b	7.81	2.66	4.08	5.08	3.50
BR	13.42 a	49.52 a	7.20 a	6.75 b	7.54	2.75	4.40	4.90	3.52
D + TT	11.39 b	51.44 a	5.62 b	8.10 a	7.07	2.77	4.80	5.21	3.59
TT	12.96 a	52.00 a	5.64 b	7.31 b	7.19	2.63	4.37	4.73	3.16

Means (n=3) in a column followed by the same lower case letter are not significantly different (LSD protected, $P \leq 0.05$).

Table 4: Relationship between tillage treatments and nine individual bacterial phyla at Stoneville.

Tillage	*Actinobacteria*	*Proteobacteria*	*Firmicutes*	*Bacteroidetes*	*Acidobacteria*	*Planctomycetes*	*Cyanobacteria*	*Chloroflexi*	*Verrucomicrobia*
RT	14.73 a	48.85 b	6.77	6.89 b	7.43 a	2.84 b	4.23	3.81	3.45 b
BR	13.68 a	49.40 b	7.40	7.63 ab	6.73 b	2.87 b	4.64	3.72	3.94 ab
D + TT	13.58 a	49.61 a	6.48	7.14 ab	6.97 b	2.87 b	4.53	3.94	3.87 ab
TT	12.06 b	51.15 a	5.79	8.04 a	6.54 b	3.48 a	4.87	3.74	4.33 a

Means (n=3) in a column followed by the same lower case letter are not significantly different (LSD protected, $P \leq 0.05$).

ISSN: 2455-6939

Volume:02, Issue:05

Proteobacteria are considered to be efficient decomposers and are functionally related to decomposition of organic matter, and carbon and nitrogen cycling (Marinari et al., 2012; Nannipieri et al., 2003).Tillage operations in conventional plots lead to breaking up and incorporation of crop residues into the surface layers of soil. Decomposition and mineralization is then accelerated as the microbial decomposers act on the readily available carbon. Thus the *Proteobacteria* were more abundant in the conventionally tilled plots than the reduced- till plots. These groups are efficient decomposers and mineralize the available C more quickly than other groups, which would support the results from our study. The relative abundance of *Acidobacteria* in reduced-till soils could be attributed to the pH difference in the soil. Many studies have shown that soil pH is a major driving force in shaping microbial diversity in soil (Fierer and Jackson, 2006; Lauber et al., 2009; Serna-Chavez et al., 2013).The reduced-till plots are subjected to reduced physical mixing which might have created localized zones with unique properties, such as, low pH (Matowo et al., 1999; Nannipieri et al., 2003). This might have selected these groups which are not only known to be ubiquitous, but are also phylogenetically diverse (Lauber et al., 2008).

The relative lower abundance of *Cyanobacteria* in reduced-till system could be attributed to soil aeration. Studies have shown that conventional tillage practices increases total soil aeration capacity up to 20 cm deep compared to reduced tillage systems (Khan, 1996).In reduced-tillage systems, the soil is not disturbed physically, and because of this, there is less aeration than in tilled soils. The phyla *Cyanobacteria* are aerobic microorganisms and that explains their lower abundance in reduced till systems. Thus the data support the notion that conventional-till systems supports greater proportion of aerobic organisms which could be explained by higher number of aerobic microsites available for these organisms to access due to increase soil aeration compared to the reduced-till systems (Mbuthia et al., 2015; Yin et al., 2010).

3.4 Effect of different tillage protocols on soil bacterial diversity indices

To calculate alpha-diversity indices (using D=0.03), the number of sequences per sample were normalized to 21412 by randomly subsampling a subset of sequences using QIIME scripts. This was done to avoid the possible influence of sample size on diversity estimates and normalized subset were used for further diversity measurements (Gihring et al., 2012). Based on the Simpson's reciprocal index, the bacterial diversity was considerably higher in reduced-till and Terra Till plots (Table 6). Species evenness showed a trend in which the conservation tillage (RT and TT) plots had more even bacterial communities compared to the conventional plots (BR and D+TT). However, the Shannon diversity showed that the bacterial diversity was not statistically different across the tillage treatments (Table 6). Although there was no significant higher Shannon's index value in conservation tilled plots, a more diverse soil bacterial community was

ISSN: 2455-6939

Volume:02, Issue:05

observed in the reduced-till and terra –till plots, which had abundant functional microorganisms and relatively large Simpson's indices. The chao1 richness predictor values showed that only 57-78% of the OTU's predicted by this estimator were actually observed indicating that the diversity was not completely sampled at evolutionary distance of 0.03.

Table 6. The alpha diversity indices of bacteria across tillage treatment plots.

Diversity Index [a]	Verona				Delta			
	RT	BR	D+TT	TT	RT	BR	D+TT	TT
N[b]	21412	21412	21412	21412	21412	21412	21412	21412
Evenness	2.01 ± 0.1	1.88± 0.2	1.74± 0.1	1.99± 0.1	2.11± 0.2	1.89± 0.1	1.79± 0.2	1.99± 0.2
Shannon	6.63± 0.4	5.22± 0.1	5.47± 0.2	5.72± 0.2	6.02± 0.2	5.79± 0.4	5.34± 0.4	6.09 ± 0.2
1/D[c]	205± 32 a	134± 12 b	125± 32 b	189± 28 ab	211± 23 a	129± 13 b	142± 28 b	192± 38 a

[a] Calculations based on the Operational Taxonomic Units (OTU) formed at an evolutionary distance of <0.03.

[b] Number of sequences in the library.

[c] Simpson's reciprocal index.

Means (n=3) with standard error in a row followed by the same lower case letter are not significantly different (LSD protected, $P \leq 0.05$).

The diversity indices are dependent both on richness and evenness of a community. These indices provide a link between diversity, richness, and evenness of the microbial communities. Our results show that bacterial community in conservation tillage (RT and TT) plots were more diverse in terms of evenness and richness. Tillage reduced microbial diversity not only through

ISSN: 2455-6939

Volume:02, Issue:05

reduced richness (Simpson index), but also through increase in dominance of few groups (i.e. reduces evenness).These results are consistent with studies that reported reduced microbial diversity under conventional tillage systems (Feng et al., 2003; Hassink et al., 1991; Lienhard et al., 2014; Wang et al., 2016).In a review investigating various factors that influence soil microbial diversity, (Giller et al., 1997) reported that soil disturbance through tillage was the major factor affecting soil microbial diversity. Various factors, such as, desiccation, mechanical disruption, soil compaction, reduced pore volume and reduction of access to food sources have been attributed to the reduction in diversity (Ladd et al., 1994; Spedding et al., 2004; Wang et al., 2016). The conservation tillage, which caused less soil disturbance than the conventional tillage, could have provided a suitable environment for multiplying soil bacteria. Consequently, our study supports the view that conservation tillage treatments affects soil characteristics, causing changes in alpha diversity and the abundance of soil bacteria.

3.5 Effects of soil properties on soil bacterial taxonomic distribution

Considering the entire bacterial community composition, the study revealed a significant correlation between soil properties and the dominant phyla identified ($r = 0.602$, $P= 0.001$; Figure 4). Soil properties explained the variation (62 %, Figure 4) in bacterial composition between the tillage treatments. The soil pH had stronger effects on the composition of bacterial communities (Longer line- Figure 4). Indeed, soil pH was significantly correlated with the relative abundances of the dominant bacterial phyla including *Proteobacteria, Acidobacteria, Firmicutes, Planctomycetes* and *Cyanobacteria*. Especially *Acidobacteria* had a strong significant negative correlation with soil pH (Table 7). These results partially support the notion that soil pH is one of the factors in structuring bacterial communities in soil under different management systems (Lauber et al., 2008; Lauber et al., 2009). Soil organic matter content and total Carbon had a significant influence on the *Proteobacteria* abundance. Both % OM and % C had a strong significant correlation on *Proteobacteria* abundance across the tillage treatments at both the locations (Figure 4; Table 7).

ISSN: 2455-6939

Volume:02, Issue:05

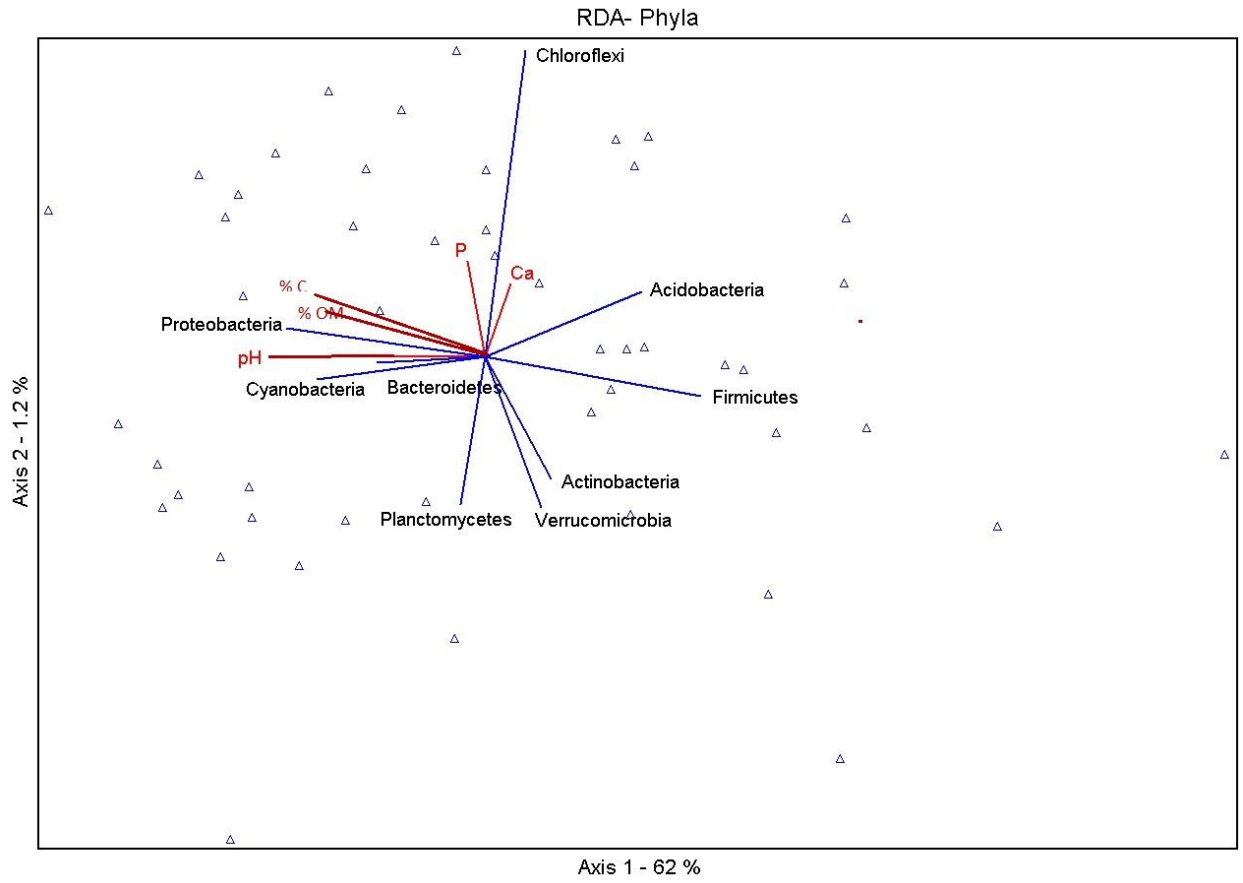

Figure 4: Redundancy analysis of abundant phyla (Blue lines)and a vector overlay of the significantly correlated soil characteristics (Red lines). Length of the vectors indicates influence of each on the ordination.

ISSN: 2455-6939

Volume:02, Issue:05

Table 7. Spearman rank correlations between soil properties and the relative abundance of dominant bacterial phyla across tillage treatments in both locations.

Bacterial Phyla	pH	P	K	Ca	Mg	CEC	% OM	% C
Actinobacteria	-0.10	-0.32*	-0.15	-0.28	0.24	-0.18	-0.14	0.02
Proteobacteria	0.32*	0.08	-0.06	-0.25	-0.32*	-0.33*	0.31**	0.37**
Firmicutes	-0.44*	-0.12	-0.13	0.02	0.18	0.12	0.20	0.21
Bacteriodetes	0.39*	-0.05	-0.03	-0.06	-0.02	-0.13	-0.17	-0.12
Acidobacteria	-0.35**	0.14	0.10	0.39*	0.08	0.42*	0.37*	0.17
Plancomycetes	0.18*	-0.33*	0.36*	-0.05	0.40*	0.08	-0.23	0.24
Cyanobacteria	0.34*	0.10	0.26	0.03	0.08	0.01	0.02	-0.02
Chloroflexi	-0.07	0.60	-0.17	0.52*	-0.52*	0.20	0.47*	0.15
Verrucomicrobia	-0.10	-0.12	0.44*	0.10	0.45*	0.28	0.07	0.21

*P <0.05; ** P <0.01.

ISSN: 2455-6939

Volume:02, Issue:05

Proteobacteria are described as fast-growing copiotrophs that are stimulated in carbon-rich environments (Venter et al., 2016). This supports why *Proteobacteria* was found to be positively correlated with soil organic matter content and total soil carbon content. Ploughing in the conventional till helped to incorporate crop residues into the surface layers of the soil and expose the labile carbon pool for easy access to microbial decomposers (Sengupta and Dick, 2015).Studies have shown that addition of labile plant residues with immediately available organic carbon source was correlated with increase in abundance of *Proteobacteria* under different land management systems (Bissett et al., 2011; Drenovsky et al., 2010). *Acidobacteria* are acidophilic organisms and tend to have strong negative correlations with soil pH (Lauber et al., 2009). Soil pH affects both the abiotic (nutrient availability, solubility of metals) and biotic conditions of soil (Kemmitt et al., 2006).The pH affected biotic factors include biomass, diversity and enzymatic activity of soil microbes (Drenovsky et al., 2010). As a result, soil microbes could be strongly affected by these changes in soil pH. *Acidobacteria* are considered to be oligotrophic and manage to survive these harsh environment (Lauber et al., 2008).

3.6 Relative contributions of tillage and soil parameters on soil bacterial community

Variance partitioning analysis (VPA) was performed to quantify the relative contributions of tillage systems and soil parameters to the taxonomic structure of the bacterial communities. A subset of soil parameters (pH, Ca, pH, Total Carbon (%) and organic matter (%) that had the highest Pearson correlation with the bacterial communities were selected by the RDA (Redundancy Analysis) procedure. The combination of selected soil characteristics and tillage regimes showed a significant ($p = 0.004$) correlation with the bacterial community structure. These factors explained 52.9 % of the observed variation, leaving 47.1 % of the variation in data unexplained. The soil factors explained 18.6% ($p = 0.024$), and tillage treatments alone explained 34.3 % ($p = 0.009$) variations, and no interaction effect was detected (Figure 5). These factors act together and determine the availability of nutrients and which in turn decides the relative abundance of oligotophs or copiotrophs in a soil system. Although soil factors and tillage effects tend to interact with each other, VPA was able to differentiate the factors that significantly influenced the microbial composition data. In this work, the tillage system had a greater impact than soil chemistry on microbial abundance. Dorr de Quadros et al. (2012) found similar results in which soil management influenced soil biodiversity more than the soil chemistry and rotation. Many other studied have also shown the dominant influence of tillage practices on altering soil microbial communities when studied under various land management practices (Sengupta and Dick, 2015; Wang et al., 2016; Zhang et al., 2014). However, other studies have also found high correlations between soil bacterial groups and soil chemistry as opposed to correlations with vegetation type (Kuramae et al., 2011) and land-use types (Bissett et al., 2011).

ISSN: 2455-6939

Volume:02, Issue:05

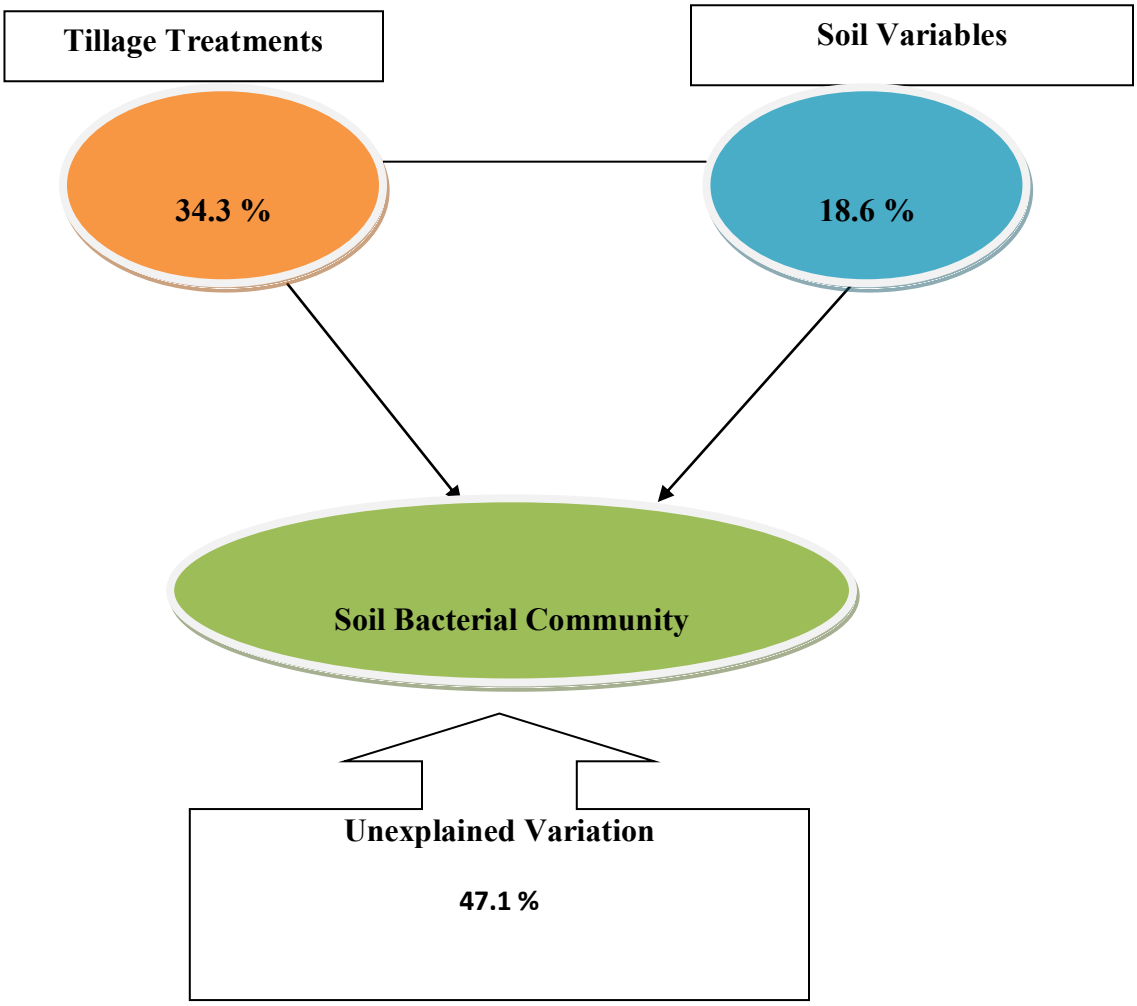

Figure 5. Variance partition analysis of the effects of tillage treatments and soil variables on the bacterial community structure

4. CONCLUSIONS

Soil biodiversity and their ecosystem services are not commonly priced in the market. However, they play an important role in sustaining life on earth and play crucial role in nutrient cycling in biosphere. However, the mechanism of how soil microbes are affected by agricultural management appear to be complex and remain poorly understood. This study showed diversity components favoring both the conservation and conventional tillage. In general, the conventional

ISSN: 2455-6939

Volume:02, Issue:05

tillage showed higher abundance of a few species, while conservation tillage favored lower abundance of many species. For example, *Proteobacteria* were abundant in conventional till plots which would have been correlated to the availability of labile carbon pool. However, with time, carbon stock would deplete in conventionally tilled plots and microorganism that can utilize a variety of carbon sources will be selected. It can be conclude that physical disturbance caused by secondary tillage may be a crucial factor in homogenizing the microenvironments that harbor unique microorganisms. This might lead to decreasing of soil bacteria species diversity. Results show that conservation tillage may create stable environments which favor diverse communities and slower nutrient turnover.

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 to adequately promote soil biodiversity conservation practices to achieve sustainability in crop 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. The findings in this study can enhance the understanding of the role of conservation tillage in altering soil bacterial community and contribute to building a stable and functional soil environment.

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