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Flowering Stage Soil Bacterial Diversity as Affected by Long-term Tillage and Crop Residue Retention

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Abstract Tillage practises can affect the soil microbes and edaphic properties. The research was aimed to assess the influence of tillage and stubble retention on the soil bacterial diversity and soil properties at the flowering stage of the field pea (Pisum arvense L.) in a rotation system with spring wheat (Triticum aestivum L.). The experiment had four treatments; no-tillage with stubble removed (NT), no-tillage with stubble retained (NTS), conventional tillage with stubble removed (T), and conventional tillage with stubble incorporated (TS). Microbial genes in top bulk soil and rhizosphere soils were sequenced using bacterial 16S rRNA (V3V4) genes. Soil from NT and NTS recorded high number of bacterial 16S rRNA operational taxonomic units (OTUs) and the bacterial community in the 0-10 cm top soil varied significantly. Bacterial diversity indices in the bulk soil were greater compared to the rhizosphere. The predominant bacterial groups were Actinobacteria, Proteobacteria, and Acidobacteria. Bacterial classes correlated with soil temperature, nitrogen, and organic carbon, Olsen phosphorus and microbial biomass carbon in bulk and rhizosphere soil. The results showed the benefits of long-term tillage and crop residue and their influence on soil properties and microbial diversity in semi-arid environments.

Keywords: *bacterial diversity, 16S rRNA, tillage, crop residue, soil properties*

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1. Introduction

Tillage is used to optimize soil conditions such as water, temperature, and nutrient availability for crop production [1]. However, tillage can have a harmful impact on soil physicochemical properties by aggravating soil erosion and degradation and cause changes in the composition and structure of soil microbiome [2]. Crop residue retention, crop rotation, and no-tillage on the other hand, are practises that protect against soil erosion, conserve soil water and nutrients, and enhance microbial biomass and processes [3]. Authors [4,5] have found increased bacterial alpha diversity in no-tillage relative to conventional tillage. Furthermore, Author [6] reported that no-tillage with stubble retained treatment had significantly improved bacterial diversity in top soil compared to conventional tillage with all stubble removed, but the composition of the microbial community was not significantly different. The effects of conservation tillage practices such as crop residue retention and no-tillage on soil microbes are complex to explain [7]. However, residue retention in no-tillage system has proven to be an effective method for reusing crop residues, improve soil fertility and boost crop productivity [8]. Many studies have showed that residue retention not only increases the soil carbon [9] but improves the soil function. Conservation tillage methods improve soil carbon through minimal soil disturbance and slow organic carbon decomposition rate [10]. Thus, agriculture practices that create changes in soil nutrient levels may influence microbial communities and the nutrients [11]. The microbial community is critical in determining soil function and the sustainability of agroecosystems because it plays a key role in organic matter breakdown and mineralization [12]. Change in the soil microbiome could affect soil chemical properties such as pH and other intermediate nutrient cycle processes [13,14].

The Loess Plateau of China is one of the major regions selected for national conservation because region is affected by soil erosion [15], low precipitation and high evaporation which causes crop yield [16]. Therefore, the application of conservation tillage methods has become an effective means to improve soil quality for crop production in the region. Past research in the Loess Plateau has employed conservation tillage methods to improve soil physical properties and nutrient levels for sustainable crop production [17]. Authors [18] found that no-tillage with crop residue retained significantly increased soil microbial biomass carbon [19]. However, there is limited research on the effects of tillage practices on microbial diversity indices and their relation with soil physical (moisture and temperature) and chemical (major

soil nutrients) changes during the crop growing season. There is also, the need to understand the relationship between tillage practice and microbial diversity in the region in order ensure sustainable management of the soil physical and chemical environment of the semi-arid Loess Plateau. Therefore, the study aimed to (i) determine the diversity of bacteria in the flowering stage bulk soil and rhizosphere as affected by crop residue and tillage, and (ii) explore the relationships among soil bacterial composition and physicochemical properties. We hypothesized that no-tillage and crop residue retention increase microbial diversity and also improve soil physicochemical properties. This research will help explain the relationships between soil indices and changes in bacterial community composition as affected by different tillage practices studied.

2. Materials and Methods

2.1. Site Description

This study was conducted in the year 2018 at the Rainfed Agricultural Experimental Station (35°28′N, 104°44′E, elevation 1971 m above sea level) of Gansu Agricultural University, Dingxi, P.R. China, within the long-term experiment that was initiated in 2001. Before the initiation of the experiment, the site had a long history of continuous of flax (*Linum usitatissimum* L.) cropping under traditional tillage method. The soil in the area has a sandy loam texture and is a Calcaric Cambisol [20]. The soil at depth of 0-30 cm had <14.79 g kg^{-1} organic carbon and a pH of 8.45 in 2015. The study area has a mean long-term (2001 to 2018) annual rainfall of 390.9 mm, a minimum monthly air temperature of -22°C in January, and a maximum monthly air temperature of 38°C in July.

2.2. Design of Experimental

The experiment utilised a factorial arrangement of two levels of tillage (T), two levels of stubble management (S), and two phases of field pea (*Pisum arvense* L.) and spring wheat (*Triticum aestivum* L.) crop rotation within a complete randomised block with three replications. The four combinations of tillage and stubble treatments were, no-tillage with stubble removed (NT), no-tillage with stubble retained (NTS), conventional tillage with stubble removed (T) and conventional tillage with stubble incorporated (TS). For the T treatment, all crop residues (i.e., stubble) were removed immediately after grain harvest, and tillage involved moldboard plowing in the fall to a depth of 20 cm and harrowing in the spring before sowing. In the plots with the TS treatment, all crop stubble was returned to the plots and was incorporated into the soil by moldboard plowing in the fall to a depth of 20 cm and harrowing in the spring before sowing. For the NT treatment, all crop stubble was removed immediately after harvest and there were no tillage operations in the fall or spring. In plots with the NTS treatment, all crop stubble from previous season's crops were returned to the plots and retained on the soil surface and there was no tillage operation in the fall or spring. Each year, pea (cv. Yannong) and spring wheat (cv. Dingxi No. 35) were seeded in a two-year rotation, with the two phases of rotation present.

Each year, field peas were planted in April at a rate of 180 kg ha^{-1} with a row spacing of 24 cm and harvested in July. Spring wheat was sown in mid-March at a rate of 187.5 kg ha^{-1} with a row spacing of 20 cm and harvested in late July to early August. Chemical nitrogen and phosphorus fertilisers were applied as urea (46% N) and calcium superphosphate (6.1% P_2O_5) were applied in sowing in all plots with no-tillage seeder at a rate of 105 kg N ha^{-1} and 45.9 kg P_2O_5 ha^{-1} for spring wheat and 20 kg N ha^{-1} and 45.9 kg P_2O_5 ha^{-1} for pea.

2.3. Soil Sample

Soil was collected from plots at the 60-70% flowering stage of the pea phase planted to pea in 2018. A total of three soil cores were randomly collected from each plot from the depths of rhizosphere and the 0-10 and 10-30 cm of bulk soil. A composite sample was obtained for each plot. The composite sample was stored on dry ice and taken to the laboratory, where they were frozen at -80°C until molecular analysis. Other sub-samples were stored at 4°C for biological property analysis while other sub samples apportioned for chemical analyses were air-dried.

2.4. Soil Moisture and Temperature

Moisture content and temperature were measured throughout the planting season of the experimental year. Soil moisture in the 0–5 and 5–10 cm soil was estimated using the oven-drying method at 105°C for 24 hours. A Trime–Pico IPH (Precise Soil Moisture Measurement, IMKO Micromodultechnik GmbH, Ettlingen, Germany) was used to determine volumetric soil water in 10-30 cm soil. Soil temperature at the 5, 10, and 15 cm depths was determined employing geothermometers fixed at random locations in each plot.

2.5. Soil Chemical Analyses

The soil pH was determined in a soil:water ratio of 1:2.5 (water:volume) [21]. The pH metre (Sartorius PB–10, Sartorius, Göttingen, Germany) was used to obtain the values. A modified wet oxidation method described by Walkley-Black was used to determine total organic carbon (TOC) [22]. Total nitrogen (TN) was determined by Kjeldahl Method [23]. The chloroform fumigation and extraction method was used to determine soil microbial biomass nitrogen and carbon (SMBN /SMBC) [24]. The Olsen phosphorus (P) method was used to determine the available P in the soil [25]. The protocol in [26] was used to determine soil nitrate-nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) based on 2M KCl extraction.

2.6. DNA Sequencing

Illumina HiSeq sequencing was used to sequence the V3V4 16S rRNA of bacteria in each replicate sample. Assemblage of paired-end reads was done with PANDAseq software [27]. Chimera was removed with

USEARCH v7 [28] using the Denovo method. Unique sequences were clustered at a cutoff of 97% similarity into representative operational taxonomic units (OTUs) using UPARSE software [29]. UCLUST was used to compare the representative sequences with bacterial 16S rRNA databases to classify OTUs. The SILVA rRNA databases were used to match 16S rRNA sequences. Diversity indices such as Chao1 richness, observed species, Shannon, and Simpson were estimated using QIIME software. Paleontological Statistics Software Package (PAST) version 3 [30] was used to perform permutation analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM) to measure the effects of treatments on the microbial community. Mantel tests [31] were performed in PAST to identify soil physical and chemical properties attributes that correlated with microbiome composition (abundance of OTUs).

2.7. Data Analysis

The software SPSS version 19.0 (IBM Corp., Chicago, IL, USA) was used to perform a variance analysis of variance at $P < 0.05$ and compare treatment means for soil chemical properties and microbial diversity indices. Treatment means were compared by post hoc analysis using Tukey's HSD Test ($P < 0.05$). Correlation analysis was performed between tillage and stubble effects, soil physicochemical properties, soil respiration, total carbon emission, and class level bacterial abundance.

3. Results

3.1. Soil Physicochemical Properties as Affected by Treatments

The management of tillage and stubble influenced soil moisture during the planting season (Figure 1a). Soil moisture content in the 0-5, 5-10, and 10-30 cm soil was significantly different among treatments. No-tillage with stubble retained increased soil moisture content by 7.5% in the 0-5 cm and by 10.0% in the 5-10 cm soil and decreased it in the 10-30 cm by 11.0% compared with T. The mean soil moisture at depths did not differ significantly between treatments. Tillage and stubble influenced soil temperature and the temperature increased progressively during the growing season (Figure 1b). The soil temperature in the 0-5, 5-10 and 10-15 cm soil did not differ significantly with the treatments. No-tillage with stubble retained decreased soil temperature in the 0-5, 5-10, and 10-15 cm soil by 4.9, 1.4, and 3.6°C, respectively, compared with T. The mean soil temperature at the depths were not significantly different among treatments.

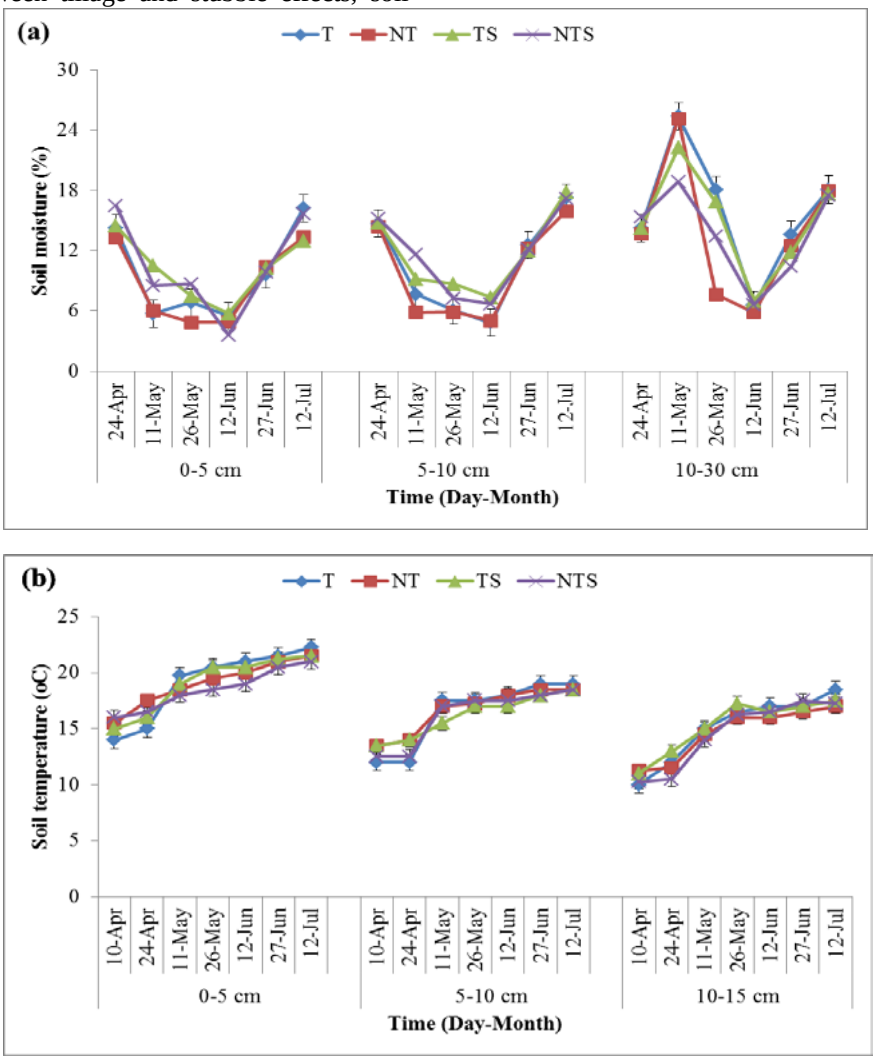


Figure 1. Soil moisture content (a) and temperature (b) as affected by tillage and stubble treatment during the growing season.

Most chemical soil indices determined in the bulk soil varied significantly with treatments (Table 1). However, NO_3^--N, soil pH and NH_4^+-N in the 0-10 cm depth and soil pH in the 10-30 cm soil were not significantly different among treatments. Soil Olsen P, TN, TOC, SMBN and SMBC in the soil 0-10 cm were significantly higher with conservation tillage treatments (NTS and NT), while TN, TOC, Olsen P, SMBN and NO_3^--N in the 10-30 cm soil were greater with TS. In the soil of 0-10 cm, NTS increased TOC by 33% compared to T, NT increased TN by 24% and SMBN by 21.5% compared to TS, NT increased Olsen P by 11.9% compared to T, and NTS increased SMBC by 17.1% compared to TS. However, in the 10-30 cm soil, TS increased TOC by 12.1%, TN by 11.6%, and SMBN by 54.9% compared to T, TS increased Olsen P by 27.7% and NO_3^--N by 20% compared to NT, T increased NH_4^+-N by 73.9% compared to TS, and NT increased SMBC by 35.6% compared to TS. T.

3.2. Soil Microbial Community Diversity as Affected by Treatments

The number of high-quality gene sequences obtained was 35,307-46,949 for bacterial 16S rRNA in bulk soil and 31,352-55,960 for bacterial 16S rRNA in rhizosphere soil. At 97% similarity cutoff, a number of subsampled sequences equivalent to the minimum number of reads per sample gave the representative OTUs used for the statistical analysis. Bacterial OTUs in the 0-10 cm top soil was 5.5-10.4% higher in NTS compared to T (Table 2). In the 10-30 cm soil, NTS increased bacterial 16S OTUs by 1.9-9.7% compared with TS. However, soil sampled from the 10-30 cm soil in all treatments had 7.6-8.3% greater 16S OTUs which mostly translated into corresponding higher diversity indices. For soil in the rhizosphere, 16S OTUs increased 4.6-5.1% with NT when compared to T (Table 3). Bacterial 16S OTUs in the 0-10 cm top soil differed significantly between treatments. Bacterial 16S observed species and Shannon index in the 0-10 cm top soil and Chao1 richness of rhizosphere soils differed significantly among treatments. The 16S OTUs in the 0-10 cm soil was 14% (T), 14.1% (NT), 15% (TS), and 21.1% (NTS) higher compared with rhizosphere soil. Tillage significantly affected bacterial 16S number of OTU, observed species, chao1 richness, and Shannon index in the 0-10 cm top soil (Table 3). In the 10-30 cm soil, tillage significantly influenced bacteria observed species and the Shannon and Simpson indices.

Table 1. Effect of tillage (T) and stubble (S) treatment on soil chemical properties in the bulk soil

Depth	Treatments	pH	TOC (g kg^{-1})	TN (g kg^{-1})	Olsen P (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	SMBC (mg kg^{-1})	SMBN (mg kg^{-1})
0-10 cm	T	8.48a	7.14b	0.60c	14.55b	8.83a	12.11a	163.5b	24.79ab
	NT	8.42a	7.03b	0.77a	16.52a	5.80a	14.8a	174.6b	28.18a
	TS	8.50a	8.33b	0.58c	14.77b	3.57a	13.77a	161.1b	22.12b
	NTS	8.45a	10.66a	0.66b	15.59ab	8.57a	15.05a	194.3a	22.50b
	T	*	ns	***	**	ns	*	**	ns
	S	ns	**	**	ns	ns	ns	ns	**
	T × S	ns	ns	**	ns	ns	ns	ns	ns
10-30 cm	T	8.42a	6.74c	0.61b	16.81ab	9.3a	13.33b	165.6b	15.6c
	NT	8.45a	6.79bc	0.61b	16.19b	4.5b	12.92b	204.4a	32.1a
	TS	8.43a	7.67a	0.69a	22.39a	2.43b	16.15a	131.7c	34.6a
	NTS	8.45a	7.02b	0.64b	16.29b	4.57b	14.55ab	199.3a	24.3b
	T	ns	***	ns	*	ns	ns	***	ns
	S	ns	***	**	ns	*	**	*	**
	T × S	ns	***	*	ns	*	ns	*	***

Means followed by different letters are significant at $P<0.05$. *, **, *** indicate significant at 0.05, 0.01, and 0.001 probability. ns: not significant at $P>0.05$ level.

Table 2. Effect of tillage (T) and stubble (S) treatment on bacterial 16S rRNA diversity indices in bulk soil

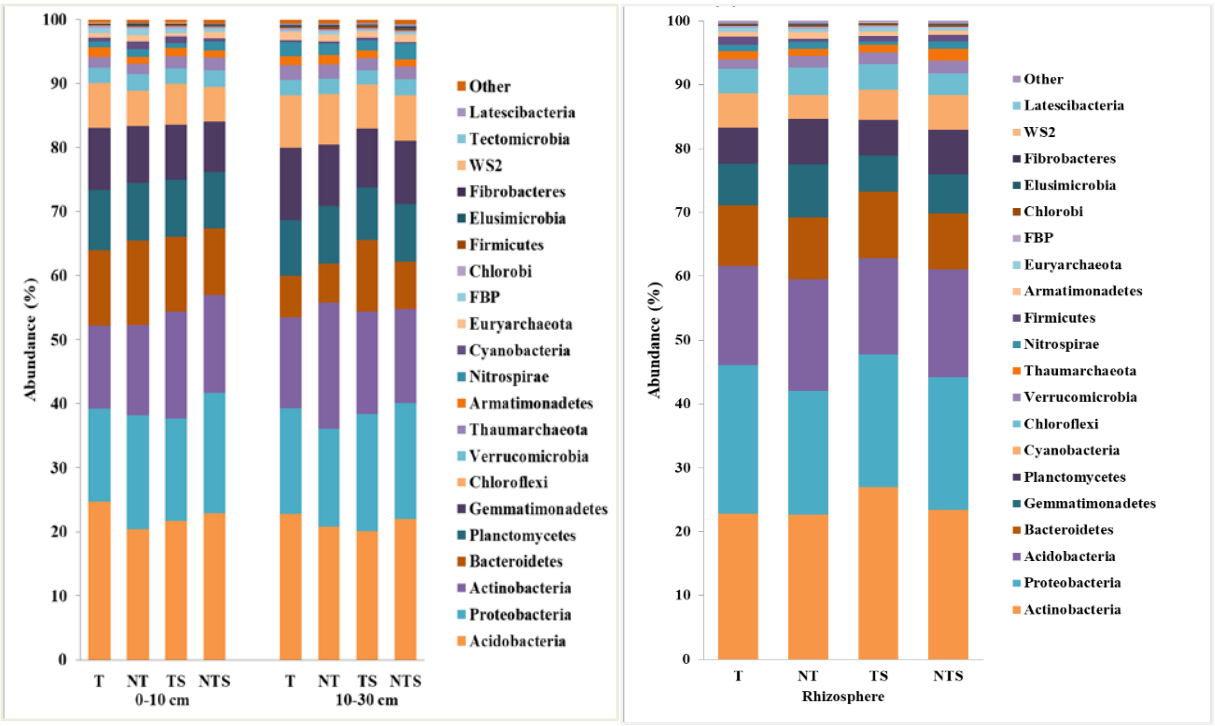
Depth	Treatments	OTUs	Coverage	Observed species	Chao richness	Shannon index	Simpson index
0-10 cm	T	1661.3b	0.987a	1661.0b	2003.1a	8.6b	0.993a
	NT	1753.3ab	0.987a	1753.0ab	2075.3a	8.68ab	0.994a
	TS	1691.0b	0.986a	1690.7b	2045.2a	8.5b	0.991a
	NTS	1854.3a	0.987a	1854.3a	2164.0a	8.9a	0.995a
	T	*	-	*	*	**	ns
	S	ns	-	ns	ns	ns	ns
	T × S	ns	-	ns	ns	ns	ns
10-30 cm	T	1865.0 a	0.986a	1858.0a	2240.0a	8.8a	0.9941a
	NT	1969.7a	0.986a	1965.7a	2309.4a	9.0a	0.995a
	TS	1812.3a	0.987a	1804.3a	2193.7a	8.8a	0.9944a
	NTS	2007.7a	0.986a	2003.3a	2357.1a	9.0a	0.995a
	T	*	-	*	ns	*	*
	S	ns	-	ns	ns	ns	ns
	T × S	ns	-	ns	ns	ns	ns

Means followed by different letters are significant at $P<0.05$. *, ** indicate significant at 0.05 and 0.01 probability. ns: not significant at $P>0.05$ level.

Table 3. Effect of tillage (T) and stubble (S) treatment on bacterial 16S rRNA diversity indices in rhizosphere soil

	Treatments	OTUs	Coverage	Observed species	Chao richness	Shannon index	Simpson index
16S rRNA	T	1428.3a	0.985a	1413.0a	1742.5b	8.0a	0.986a
	NT	1505.3a	0.985a	1492.3a	1827.1a	8.3a	0.989a
	TS	1436.7a	0.985a	1420.7a	1755.5ab	8.0a	0.985a
	NTS	1463.0a	0.986a	1448.0a	1724.5b	7.9a	0.976a
	T	ns	-	ns	ns	ns	ns
	S	ns	-	ns	ns	ns	ns
	T × S	ns	-	ns	*	ns	ns

Means followed by different letters are significant at $P<0.05$. *, ** indicate significant at 0.05 and 0.01 probability. ns: not significant at $P>0.05$ level.



(a) (b)

Figure 2. Operational taxonomic units abundance of Phylum level of bacterial 16S rRNA in the rhizosphere soil (a) and top bulk soil (b) as affected by tillage and stubble treatment

3.3. Soil Microbial Community Composition

The main representative bacterial phyla identified across treatments in bulk soil and rhizosphere soil were Acidobacteria (15.1-24.7%), Proteobacteria (14.5-23.4%), Actinobacteria (13.0-27.0%), Bacteroidetes (6.1-13.2%), Planctomycetes (9.4-5.6%) and Gemmatimonadetes (5.7-11.3%) (Figure 2a, b). Minor representative bacterial phyla such as Chloroflexi, Verrucomicrobia, Armatimonadetes, Nitrospirae, Cyanobacteria, Chlorobi, Firmicutes, unidentified bacteria were also identified in low abundance.

The five predominant bacterial classes in the 0-30 cm top bulk soil and rhizosphere soil according to percentage abundance were class Subgroup 6, Alphaproteobacteria, Gemmatimonadetes, Sphingobacteriia and Actinobacteria. The dominant bacterial genera included *RB41*, *Sphingomonas*, *Streptomyces*, *Adhaeribacter*, and *Nocardioides*. The abundance of bacterial classes was usually <21.5 whereas the abundance of bacterial genera was <12% in the rhizosphere and bulk soils but was not significantly different between treatments.

3.4. Association Between Soil Microbial Diversity and Soil Properties

PERMANOVA pairwise tests did not show a significant influence of treatments on soil bacterial communities (Table 4). The ANOSIM showed that bacterial communities were highly separated among treatments. Mantel tests showed few significant correlations among microbial OTUs abundance and the measured parameters. The bacterial community had a significant correlation (Mantel tests) with TOC in the 0-10 cm soil and SMBC in both soil depth (Table 5). Pearson's correlation analyses were also conducted to further assess the association between soil properties and bacterial classes (Table 6, and 7). Several bacterial classes had significant positive and negative correlation ($r \geq 0.578$ or $r \leq -0.588$ and $P < 0.01$ or $P < 0.05$) with the soil properties studied.

Table 4. Matrices of PERMANOVA and ANOSIM for tillage and crop residue effects on bacterial and fungal communities in the top bulk soil and rhizosphere soil

		PERMANOVA			ANOSIM		
	Treatment	TS	T	NTS	TS	T	NTS
	Bacteria 16S rRNA						
0-10 cm	T	0.7982	-	-	1	-	-
	NTS	0.7085	0.0991	-	0.3015	0.1023	-
	NT	0.8957	0.6035	0.9034	0.7036	0.0991	0.8046
10-30 cm	T	0.5056	-	-	0.6965	-	-
	NTS	0.6995	0.5016	-	0.7081	0.6978	-
	NT	0.5972	0.5035	0.6041	0.7072	0.4952	0.7035
Rhizosphere							
	Bacteria 16S rRNA						
	T	0.2929	-	-	0.3995	-	-
	NTS	0.3868	0.6993	-	0.1967	0.392	-
	NT	0.1027	0.3984	0.6026	0.1057	0.4025	0.803

P-value significant at $P<0.05$.

Table 5. Correlations (*R*) and significance (*P*) determined by Mantel tests between soil microbial community composition (abundance of operational taxonomic units) and soil properties in the top bulk soil and in rhizosphere soil

			pH	TOC	TN	Olsen P	NH_4^+-N	NO_3^--N	SMBC	SMBN	Moisture	Temperature
Bacterial 16S rRNA	**0-10 cm**	*R*	0.092	0.341	0.175	0.097	0.033	-0.008	0.278	-0.133	-0.196	-0.059
		P	0.194	**0.021**	0.102	0.218	0.311	0.459	**0.038**	0.855	0.196	0.592
	10-30 cm	*R*	-0.053	0.056	0.02	-0.096	-0.083	0.165	0.481	-0.045	0.073	-0.142
		P	0.549	0.325	0.357	0.551	0.555	0.14	**0.003**	0.546	0.274	0.766
	Rhizosphere	*R*	-0.051	0.32	-0.172	0.167	-0.108	-0.042	0.052	0.052	0.148	-0.048
		P	0.574	0.082	0.827	0.166	0.666	0.517	0.339	0.298	0.179	0.549

P-value significant at $P<0.05$.

Table 6. Pearson's correlation between bacterial classes, tillage (T) and stubble (S) effect, temperature, moisture and chemical properties in bulk soil

	Bacterial Classes	T	S	Temperature	Moisture	pH	TOC	TN	Olsen P	NH_4^+-N	NO_3^--N	SMBC	SMBN
0-10 cm	Subgroup 6	-0.498	0.078	0.382	0.308	0.415	-0.265	-.656*	-.645*	0.045	-0.436	-0.187	-0.231
	Phycisphaerae	-0.354	0.24	0.556	0.27	0.197	-.621*	-0.315	-0.288	0.106	-0.235	-0.204	0.145
	Acidimicrobiia	-0.005	0.136	-0.114	-0.281	-0.054	-0.2	-0.197	-0.125	.585*	-0.038	0.264	-0.135
	Deltaproteobacteria	0.395	-0.3	-0.49	-0.057	-0.149	0.293	-0.006	-0.043	0.08	0.399	0.474	-0.385
	Holophagae	0.528	-0.349	-0.264	-0.227	-0.098	0.401	0.017	0.114	0.344	0.415	.750**	-0.275
	Gammaproteobacteria	0.497	-0.209	-0.381	-0.338	-0.349	.589*	0.474	0.452	-0.381	0.486	0.318	0.182
	Anaerolineae	-0.511	-0.066	0.166	0.573	0.343	-0.232	-.588*	-.652*	-0.221	-0.358	-0.41	-0.341
	Thermomicrobia	-.675*	0.421	0.541	-0.01	0.25	-.596*	-0.369	-0.22	0.143	-.603*	-0.552	0.363
10-30 cm	Alphaproteobacteria	0.048	-0.248	.610*	0.139	-0.089	0.073	-0.064	0.326	-0.294	0.087	-0.073	0.248
	Blastocatellia	-0.138	0.346	.662*	0.262	-0.131	-0.223	-0.204	-0.096	0.515	-0.042	-0.04	-0.355
	Phycisphaerae	-0.201	0.209	.674*	0.223	-0.167	-0.034	-0.141	0.229	0.199	0.097	-0.168	0.036
	Cytophagia	-0.356	-0.345	.578*	0.457	0.021	0.352	0.291	0.457	-0.185	0.411	-0.542	0.218
	Thermoleophilia	0.294	0.231	-.791**	-0.352	0.032	-0.143	-0.042	-0.093	-0.211	-0.39	0.334	0.19
	Planctomycetacia	0.573	0.088	-0.511	-0.497	-0.213	-0.353	-0.326	-0.434	-0.123	-0.404	.728**	-0.066
	Holophagae	0.5	-0.004	-0.241	-0.462	0.071	-0.267	-0.29	-0.382	0.121	-0.242	.604*	-0.24
	Anaerolineae	-0.286	0.185	0.558	-0.014	-.661*	-0.207	-0.287	-0.019	0.421	-0.076	0.075	-0.546
	MBA2108	0.084	0.028	-.802**	-0.441	0.204	-0.012	0.03	-0.1	-0.139	-0.193	0.132	0.029

* $P<0.05$; ** $P<0.01$.

Table 7. Pearson's correlation between bacterial classes, tillage (T) and stubble (S) effect, temperature, moisture and chemical properties in rhizosphere soil

Bacterial Classes	T	S	Temperature	Moisture	pH	TOC	TN	Olsen P	NH_4^+-N	NO_3^--N	SMBC	SMBN
Subgroup6	.718**	0.127	-.602*	-0.563	0.008	0.028	0.142	.673*	-0.236	0.317	0.405	0.128
Alphaproteobacteria	-0.543	0.377	0.473	0.016	-0.179	-0.145	0.352	-0.168	0.37	-0.274	-0.506	-0.281
Phycisphaerae	0.559	0.119	-0.267	-0.238	-0.306	-0.206	0.22	.754**	0.118	0.275	0.158	-0.052
Thermoleophilia	0.37	0.323	-0.342	-0.141	0.401	0.283	0.044	0.4	-0.151	-0.069	0.214	0.238
Planctomycetacia	0.524	0.367	-0.332	-0.355	0.169	-0.085	0.145	.598*	-0.287	0.154	0.288	0.033
Thermomicrobia	-0.186	.630*	-0.093	-0.35	0.138	0.035	0.233	0.402	0.408	-0.463	-0.358	-0.179

* $P<0.05$; ** $P<0.01$.

4. Discussion

4.1. Effect of Tillage and Crop Residue Soil Microbial Diversity

In this study, soil microbial OTUs and diversity indices such as Chao1 richness, observed species, Simpson and Shannon indices were generally greatest with NTS and NT for bacterial 16S genes in the top soil and rhizosphere. Similar findings from previous studies were reported by [32]. The alpha diversity was increased in the 10-30 cm soil for all treatments when compared to 0-10 cm soil and this could be attributed to the relatively undisturbed subsoil layer; thus, soil depth and tillage may affect microbial diversity [33]. Tillage significantly influenced the soil bacterial community in the top soil. The main effect of stubble management and the interaction between stubble management and tillage significantly influenced bacterial community composition during the growing season. Bacteria associate with highly disturbed ecosystems with rapid nutrient recycling, whereas fungi tend to be associated with soil that has been undisturbed [34]; this may help explain the tillage and stubble management effects in this study. Author [35] found that soil bacteria exhibited temporal dependency on tillage and crop residue management has less influence on the soil bacterial community composition. Crop residues serve as carbon source for soil microbiome and lack of tillage reduces the destruction of soil aggregates and subsequent disturbance of soil microbial communities. Many bacterial groups can breakdown recalcitrant carbon source such lignin in crop residues and soil organic matter [36], making them the main decomposition drivers in soil [37]. This may partly explain the increased microbial diversity under the crop residue retention treatment which was in combination with no-tillage.

4.2. Soil Microbial Community Composition

The predominant bacterial phyla in our research indicated that all treatments had common phyla in varying percentage abundance. These dominant groups of bacteria (Acidobacteria, Proteobacteria, Actinobacteria, Gemmatimonadetes, and Planctomycetes) are reported as common and abundant phyla in different tillage systems and ecosystems [38]. At the genus level, *RB41* (phylum Acidobacteria) was associated with no-tillage and tilled treatments [39], but its abundance was increased in bulk soil than rhizosphere soil. *Streptomyces* (class Actinobacteria) occurred at relatively greater abundance in rhizosphere soil than in their bulk soil. *Rhizobium* (class Alphaproteobacteria) occurred in the rhizosphere soil [40] at lower abundance, but it was absent in bulk soil in the present study.

4.3. Association Between Soil Microbial Diversity and Soil Properties

Soil temperature and moisture levels are the most important environmental agent affecting microbial growth and activity that shapes the soil microbiome [41]. Author [42] observed that concentrations of bacteria cell components were greater at higher temperatures and moderate moisture. Soil moisture content also regulate the effects of temperature on soil microorganisms [43]. The top soil Olsen P, TN, TOC, SMBN and SMBC in were significantly increased with conservation tillage treatments in the study. Soil chemical properties are indicators of soil quality, sustainable crop production and affect soil function and microbial processes [44]. Microbial abundance in bulk soil in this was significantly associated with TN, TOC, Olsen P, and SMBC, and occasionally associated with pH. The microbial community in rhizosphere soil was mostly associated with Olsen P. The results suggest that soil bacterial community composition at the study site could be driven by these soil properties and may also be involved in the cycling of the nutrients [45].

5. Conclusion

No-tillage treatments (NTS and NT) increased bacterial 16S rRNA diversity. Tillage significantly influenced bacterial diversity in the 0-10 cm top soil. Soil microbial diversity was mostly associated with TOC, TN, Olsen P and SMBC in this study. The study highlights the benefit of residue retention and no-tillage in improving soil quality for sustainable agriculture.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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