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Effects of tillage managements and maize straw returning on soil microbiome using 16S rDNA sequencing[FA]

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Research Article

Abstract Agricultural practices could affect bacterial diversity and community structure by altering soil physical and chemical properties. Straw returning and tillage practices are widely used in agriculture, however, the effects of these agricultural practices on microbiomes are still unclear. In the present study, we compared the 18 bacterial communities of soil with different straw returning and tillage treatment combinations. The V3–V4 regions of the 16S ribosomal RNA were amplified and analyzed by high-throughput sequencing technology. The results showed that the bacterial communities were consistently dominated by *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, and *Chloroflexi*. Short-term straw returning and tillage practices significantly altered the diversity, relative abundance and functions of the soil microbiome. Soil subjected to rotary tillage and straw returning (RTS) combination possessed the highest bacterial diversity and lowest ratio of G+/G- bacteria, indicating that RTS could be an efficient integrated management system to improve microbiome in the short term. Double verifications based on relative abundance and network analysis, revealed close relationships of *Mycobacterium* and *Methylibium* with RTS, indicating they could serve as biomarkers for RTS. Investigating microbial changes under different agricultural practices will provide valuable foundations for land sustainable utilization and increase crop yields.

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INTRODUCTION

Soil is home to many microorganisms that play important roles in soil nutrient conversion, energy transformation and formation of humus (Hayat et al. 2010). The relationship between soil and bacteria has made the bacterial community an essential indicator of soil quality (Peiffer et al. 2013). The composition and diversity of microbes are influenced by biotic factors such as surface vegetation, as well as abiotic factors, such as soil PH, moisture content and organic matter content (Strom 1985). Previous studies indicated that tillage and straw returning, which are two important technical measures, could increase soil granular structure and the content of water-stable aggregates, ultimately improving the fertilizer and water conservation (Tian et al. 2010; Fierer 2017). Moreover, studies have attempted to establish a correlation of tillage management methods and straw returning with soil bacterial composition and diversity. Based on phospholipid fatty acid (PLFA) analysis and automated ribosomal intergenic spacer analysis (ARISA), a previous study demonstrated that long-term no tillage resulted in higher viable microbial community and nitrogen contents (Mathew et al. 2012). Additionally, a 30-year straw returning experiment conducted in north-central China

confirmed this process changed the microbial community structure and increased the activities of most hydrolytic enzymes (Zhao et al. 2016). However, studies conducted to date have focused on the relationship between the single tillage method and soil microbiome, while ignoring the combined effects of tillage practices and straw returning on microbial communities. Maize is one of the most important crops grown worldwide, as well as the one of the most productive food crop and a crucial component of animal feed and industrial raw materials (Shiferaw et al. 2011). With the decreasing area of available land, it is important to improve the maize yield per unit area (Chen et al. 2011). Many practices such as rational close planting, application of farmyard manure, straw returning and conservation tillage have been shown to effectively increase maize production (Suthar 2012). However, the relationship between soil microbes and these practices have yet to be identified (Wang et al. 2011). Understanding the relationships between microbial communities and human factors will provide valuable information that will facilitate a development of scientific farm management patterns for maize.

One of the primary goals of microbial studies is to increase the resolution of identification of bacteria (Höfle 1988). Previous experimentally based studies have been limited by their technical conditions. For example, plate cultivation methods simply reflected culturable microorganisms, which account for only 1% of the total population (Hahn et al. 2004; Guan et al. 2011). Additionally, PCR-DGGE (Denaturing Gradient Gel Electrophoresis) and PCR-TGGE (Temperature Gradient Gel Electrophoresis) are not suitable for bacterial analysis because of the error associated with PCR and their dependence of high-fidelity polymerase (Heuer et al. 1997; Dahllöf et al. 2000). However, the rapid development of high-throughput sequencing technology and bioinformation analysis has facilitated microbial researches (MacLean et al. 2009). The 16S rRNA of bacteria has a long evolutionary history and can be divided into 10 conservative regions and nine hypervariable regions (Yang et al. 2016). Sequencing and clustering of the hypervariable regions could distinguish different bacterial species, which serve as the molecular basis of diversity analysis of microbial communities. In consideration of the limits of read length and costs, sequencing the v3-v4 regions became popular, and this strategy was proved to be accurate enough to reflect the bacterial diversity (Cheng et al. 2017; Zhang et al. 2017).

To investigate the influence of different tillage methods and straw returning on bacterial communities in maize planting soil, we compared the bacterial community structure in different experimental fields with six combinations of agricultural managements techniques: deep tillage and straw returning (DTS), deep tillage and no straw returning (DTNS), rotary tillage and straw returning (RTS), rotary tillage and no straw returning (RTNS), no tillage and straw returning (NTS), no tillage and no straw returning (NTNS). The objectives of this study were to: (i) elucidate the influences of different types of tillage managements and straw returning on microbial community structure and diversity; (ii) identify some bacteria that may be biomarkers for special management; and (iii) search for the most suitable management combination among different short-term tillage practices and straw returning. The results presented herein will provide foundational guidance for farmland management.

RESULTS

Reads and OTU statistics

Based on sequencing analysis of the V3 and V4 regions of the 16S rRNA gene, a mass of paired sequences with barcode and primer sequences were obtained. After removing the short reads and trimming the low-quality regions, we finally identified 2,767,124 high quality sequences from the 18 soil samples, with an average length of 436 bp (Table 1). Each sample contained more than 3,000 sequence reads that were used in subsequent analyses. Operational taxonomic units (OTUs) were used to organize the fine scale bacterial diversity based on the DNA sequence similarity (97%). In total, 2,046,708 sequences were clustered into 365,795 OTUs and 256,336 OTUs were identified by aligning the sequence to the Greengenes database (v13_5). The coverage index was used to reflect the coverage scale of sample libraries. As shown in Table 1, all of the coverages of the 18 samples were larger than 0.87, indicating that the depths of sequencing could meet the experimental requirement.

Bacterial diversity evaluated by four alpha indexes

We further evaluated the bacterial diversity of all soil samples subjected to different types of tillage

Table 1. Statistics of sample size and OTU number

Sample name	Sample size	OTUs number	OTUs seq	Coverage
A01	170,161	23,266	123,840	0.88
A02	152,627	21,151	109,041	0.88
A03	77,152	13,042	56,213	0.85
A04	125,703	17,686	91,791	0.88
A05	111,257	16,038	81,193	0.88
A06	122,503	17,410	87,589	0.88
A07	118,697	15,694	90,908	0.9
A08	181,659	23,977	136,483	0.89
A09	194,050	24,984	144,874	0.89
A10	255,597	28,855	187,985	0.91
A11	169,009	21,074	124,187	0.89
A12	153,942	20,199	116,747	0.89
A13	181,303	20,309	139,961	0.91
A14	168,600	21,966	125,464	0.89
A15	123,862	19,476	88,280	0.85
A16	187,642	23,460	136,841	0.89
A17	140,904	18,904	106,600	0.89
A18	132,456	18,304	98,711	0.88

management and straw returning based on four usual indexes (Table S1). To investigate the effects of maize straw returning on microbiomes, we compared the pairwise groups and found that those with maize straw returning (DTS, RTS, NTS) had more diversity than those without straw returning (DTNS, RTNS, NTNS) based on the Chao index (Figure 1A). Comparison based on the *t*-test and one-way ANOVA test revealed significant differences between the pairwise groups ($P < 0.05$) (Table S2), which was in accordance with the results of the Ace index (Figure 1C). Finally, the Shannon (Figure 1B) and Simpson (Figure 1D) indexes, indicated that most groups with the maize straw returning were larger than groups without straw except NTS and NTNS in Simpson ($P > 0.05$). To investigate the effects of the three methods of tillage (deep, rotary and no tillage) on soil microbiomes, we compared DTS, RTS and NTS with DTNS, RTNS and NTNS. For the chao1 and ace indexes, groups with the rotary village had the richest microbial diversity, with or without maize straw returning (ANOVA $P < 0.05$). Shannon and Simpson indexes indicated that deep ploughing resulted in the greatest abundance diversity, although this difference was not significant (ANOVA $P > 0.05$). Interestingly, the microbiomes in groups with the combined maize straw returning and ploughing (DTS or RTS) were more abundant than those of groups with no tillage or straw returning (NTS) (Table S1), while the opposite was observed for groups without maize straw returning.

Analysis of between-group variances

To evaluate differences in the composition of the 18 soil samples subjected to different management styles, principal component analysis (PCA) based on the Bray-Curtis matrix (2,000 sequences per sample) was conducted. The results of PCA indicated significant clustering of microbial communities. As shown in Figure 2, PC1 and PC2 explained 49.62% and 18.55% of the global variations, respectively. DTS were clearly distinguished from DTNS, reflecting the significant influence of straw returning on soil bacteria. Similar patterns were identified in the other two comparisons (RTS and RTNS, NTS and NTNS). Interestingly, DTNS, RTNS and NTNS, were also distinguished from each other (Figure 2). However, DTS was clustered together with RTS, which suggested that maize straw returning may have had a greater impact on the microbiome than tillage management. We assessed the similarities to support the PCA analysis using ANOSIM. Among all combinations, differences between groups were greater than intra-group differences (Table S3).

Community structure of bacteria

The taxonomic distributions of microbial communities were evaluated at different levels of classification (Figure 3). As shown in Figure 3A, the relative abundances of the dominant phyla were similar among 18 soil samples with different tillage management methods. *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Gemmatimonadetes*, *Bacteroidetes*, *TM7*, *Nitrospirae*, *Firmicutes and Planctomycetes* were the most abundant phyla in the 18 soil samples and these phyla comprised the majority of all detected microorganisms (approximately 97%). The dominant genera were *Nitrospira*, *Kaistobacter*, *Rhodoplanes*, *Steroidobacter*, *Streptomyces* and *Aeromicrobium*. Otherwise, 28 genera were enriched when applying the maize straw returning (Table S4). For example, the relative abundance of *Mycobacterium* was higher in DTS (4.10%), RTS (4.70%) and NTS (4.42%) than in DTNS (2.37%), RTNS (3.66%) and NTNS (2.67%). A similar pattern was also

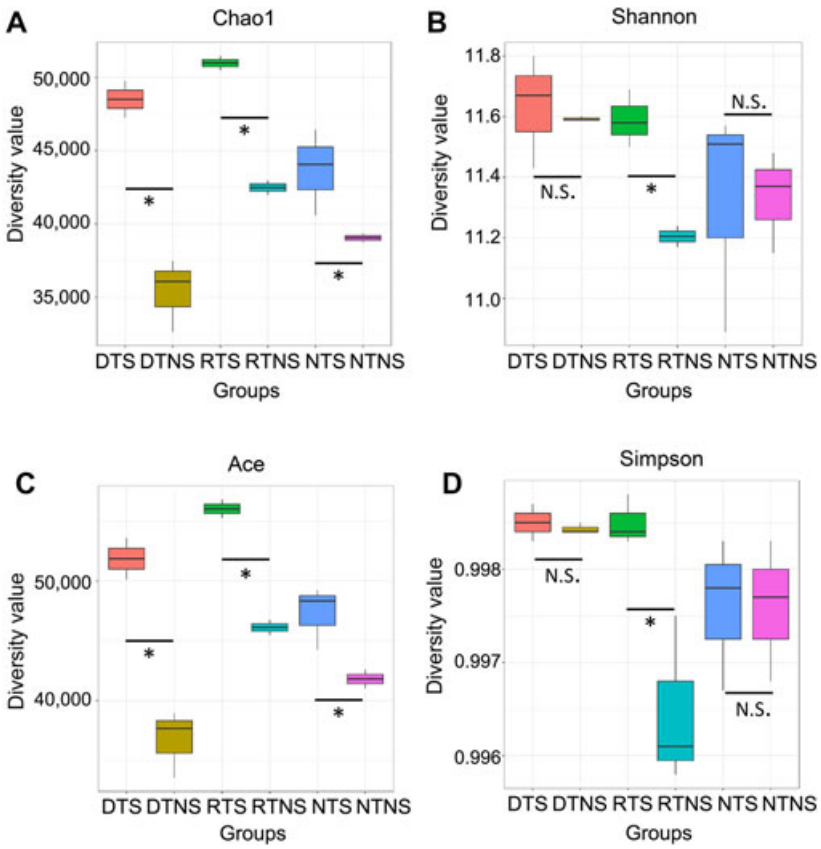

Figure 1. Four alpha diversities comparison between six groups with different management practices
(A) Chao1 index. **(B)** Shannon index. **(C)** Ace index. **(D)** Simpson index. The symbol * represents the significant difference ($P < 0.05$, one-side *t*-test and ANOVA) between two adjacent groups. N.S. represents no significant difference.

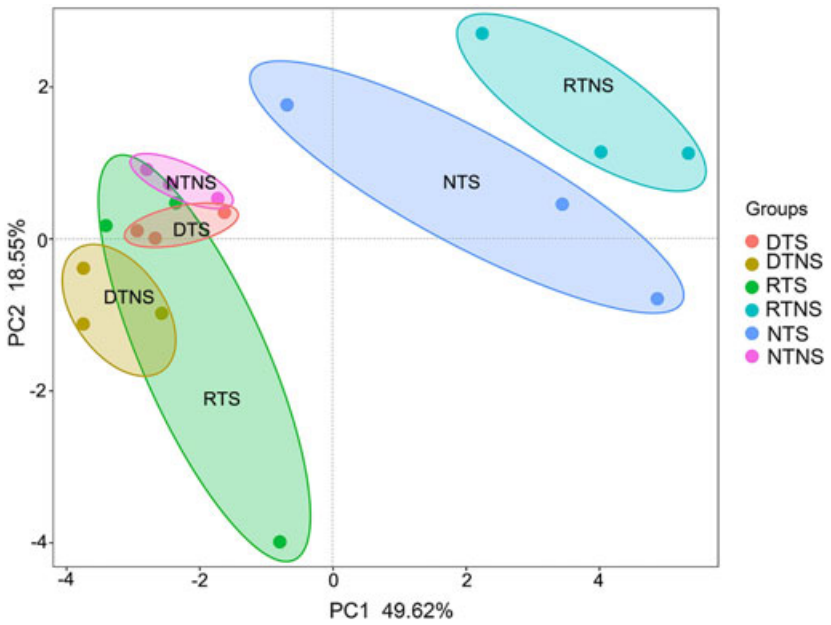

Figure 2. Principle component analysis based on unweighted UniFrac distance between groups with different management practices

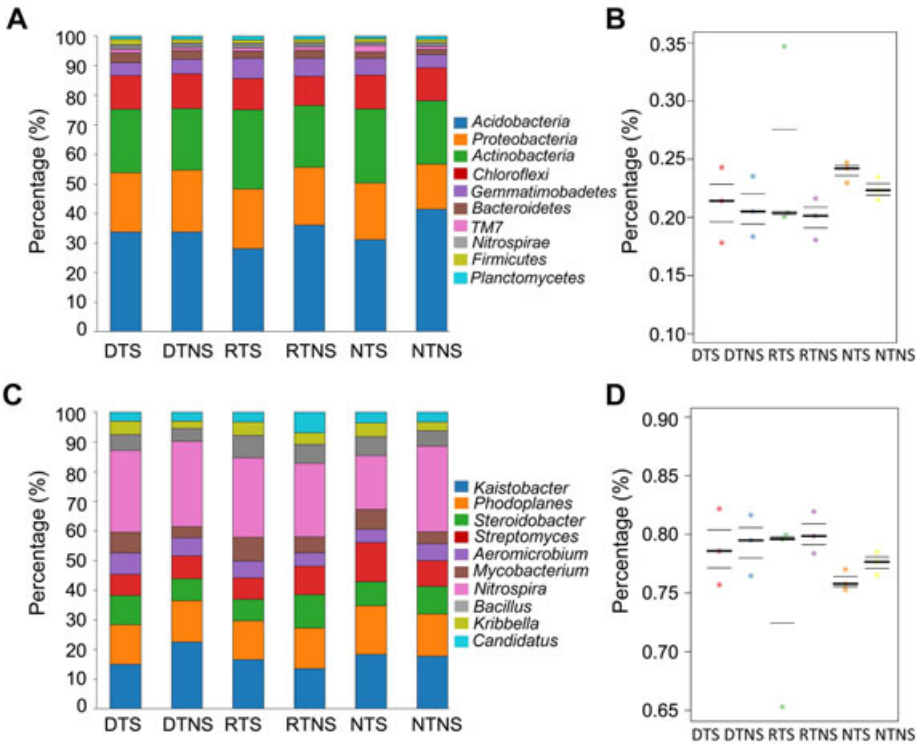

Figure 3. Community structure of the bacteria between six groups with different managements
(A) Phylum distribution of the OTUs. The relative abundance of top 10 phylum were displayed in different colors. **(B)** Percentage of Gram-positive bacteria. **(C)** Genus distribution of the OTUs. **(D)** Percentage of Gram-negative bacteria.

found for *Pilimelia* and *Alicyclobacillus*. However, the relative abundance of 39 genera, including *Nitrospira*, *Candidatus* and *Methylibium*, decreased with maize straw returning. Moreover, some microbes were sensitive to the influence of different tillage methods. Overall, 42 genera, including *Aeromicrobium* and *Lysobacter*, were enriched when employing deep ploughing (Table S5). Similarly, 18 and 24 genera were enriched when employing rotary tillage and no tillage, respectively (Tables S6 and S7).

We next estimated the proportion of Gram-positive (G+) and Gram-negative (G-) bacteria based on the biom-format file and mapfile. As shown in Figure 3 (B and D), there was less G+ bacteria than G- bacteria in every soil sample. When compared with deep ploughing and rotary tillage, no tillage groups (NTS and NTNS) contained more G+ bacteria. Soil with rotary tillage practices possessed the lowest ratio of G+/G- bacteria. Taken together, these results indicated that tillage practices and straw retuning could alter the bacterial community structure and the ratio of G+/G- bacteria.

Network analysis of microbiome

A network composed of 543 nodes and 1,372 edges was constructed to describe the complex relationships between soil microbiomes (Figure 4). All nodes could be split into 93 network groups (ng) with 0.70 average modularity. The average path length was 4.69 and the network diameter was 12.73. All 543 nodes were assigned to 15 bacterial phyla. Five phyla, *Acidobacteria* (29.28%), *Proteobacteria* (24.31%), *Actinobacteria* (21.36%), *Chloroflexi* (8.10%) and *Gemmatimonadetes* (4.60%), accounted for 87.66% of all OTUs. Special nodes that could be influenced by agricultural managements were marked in the network (Figure 4). Among the 67 genera that displayed correlations with straw returning, *Mycobacterium*, *Niastella*, *Prevotella*, *Nitrospira*, *Methylibium*, *Phascolarctobacterium* and *Plesiocystis* were reserved and marked with purple color. For genera related to deep ploughing practices, only *Lysobacter* were found in the network. Five red nodes, corresponding to *Mycobacterium*, *Bacillus*, *Pseudonocardia*, *Virgisporangium* and *Methylibium*, represented the rotary tillage-related genera. Six of the 24 genera

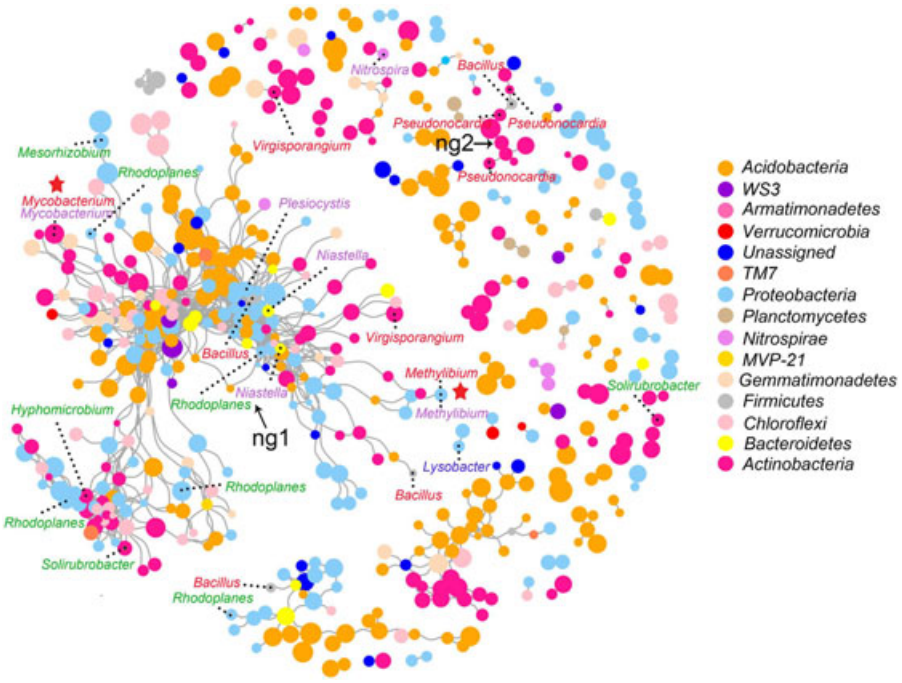

Figure 4. Networks of the bacterial OTUs

Nodes correspond to OTUs and node size corresponds to their relative abundance. OTUs belong to the same phylum were displayed in the same color. Genus sensitive to straw returning were marked in purple. Genus marked in blue, red and green were sensitive to deep ploughing, rotary tillage and no tillage. Genus sensitive to two or more management practices were marked with the red star.

that were sensitive to no tillage practice, *Rhodoplanes*, *Phascolarctobacterium*, *Prevotella*, *Solirubrobacter*, *Mesorhizobium* and *Hyphomicrobium*, remained in the network. Among the 93 ngs, ng1 was the largest subgroup, containing approximately half of all OTUs. In ng1, *Mycobacterium* and *Methylibium* were sensitive to both maize straw returning and rotary tillage. Three OTUs were enriched in ng2, which consisted of the *Peseudonocardia* genus interacting with *Bacillus*.

Effect of management practices on bacterial function

Microbial function was predicted and enumerated by PICRUSt and STAMP. Only KEGG pathways with significant biological differences ($P < 0.05$) were enriched in the soil samples. At level 2 of the subsystem of metabolism, glycan biosynthesis and metabolism, amino acid metabolism, and signal transduction mechanisms pathways were enriched in the groups without straw returning (Figure S1). These findings generally confirmed that soil subjected to straw returning may contain more microbiomes involved in carbohydrate and nitrogenous metabolism. However, the results showed that the group with no straw contained more sequences that were involved in metabolism of macromolecular compounds, such as amino sugars and nucleotide sugars metabolism, as well as those involved in glycerophospholipid metabolism, glycosyltransferases and amino acid metabolism (Figure 5) at level 3. Moreover, most sequences related to C5-Branched dibasic acid metabolism, naphthalene degradation, synthesis and degradation of the ketone pathway were found in soils subjected to maize straw returning. Among tillage practices, there were more functional differences between deep ploughing and no tillage than between rotary tillage and no tillage at both level 2 and level 3 (Figures S2–S5). At level 3, the no tillage group possessed more sequences involved in transpiration and toluene degradation. These findings suggest that deep ploughing breaks the balance of microbiomes and weakens the capacity for terpenoid backbone and fatty acid, stilbenoid, diarylheptanoid and gingerol biosynthesis, cyanoamino acid metabolism, toluene degradation. On the contrary, methane metabolism, arginine and proline metabolism, protein

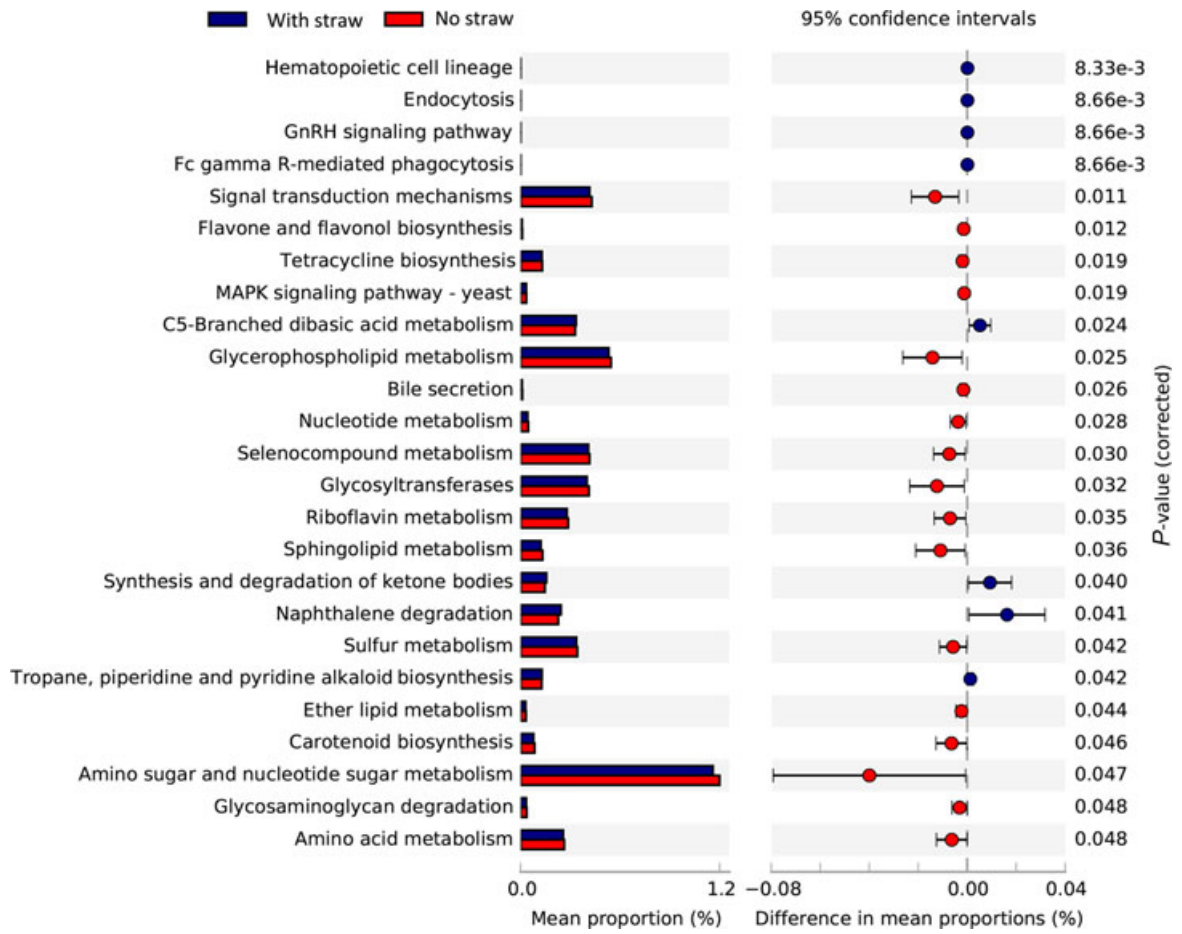

Figure 5. Statistically differences in the functional subsystem between groups with (DTS, RTS and NTS) and without maize straw returning (DTNS, RTNS and NTNS) at level 3

Blue columns represented the groups with maize straw returning and the red one represented no straw group. Only the subsystems with the significant differences and a confidence interval of 95% were showed.

folding and associated processing, *nitrotoluene* degradation were enhanced. Upon comparison of the rotary tillage and no tillage groups, subsystems such as toluene degradation, transcription factors, transcription related proteins and glycosphingolipid biosynthesis differed significantly, similar to the results observed between deep ploughing and no tillage.

DISCUSSION

Evaluation of phylum-level community structure and the four alpha diversity indexes

Despite the differences in tillage methods or maize straw returning, the community structure of the soil at the phylum level was similar among the 18 samples. The top ten major phyla were also detected in previous studies of soil microorganism. Similar bacterial communities were found in five tropical rainforests in Malaysia and one temperate forest in Japan in which *Acidobacteria* and *Proteobacteria* occupied the top two phyla (Miyashita et al. 2013). These similar community compositions were also found in vineyard soil (Burns et al. 2015), riverine wetland (Ligi et al. 2014), copper mine (Rodrigues et al. 2014) and a shallow lake (Song and Wang 2015). These results indicate that maize straw returning and different tillage methods did not transform the taxonomic structure at the phylum level.

Several indexes are used to measure species diversity including the Chao1, Ace, Shannon and Simpson indexes. In this result, these four indexes showed the same tendency indicating that there were significant differences between groups with or without straw returning. Although the Shannon and Simpson

indexes showed the same pattern, there were no remarkable differences among groups. Based on this divergence, we assumed that the Shannon and Simpson indexes essentially reflected the bacterial complexity and evenness, while the Ace and Chao1 indexes were simply used to indicate species richness. Previous research has shown the bias in the Chao1 and ACE indexes when using clone libraries to assess species diversity (Gihring et al. 2012). However, the Shannon and Simpson indexes consider both evenness and abundance, and this feature determined the indistinguishable difference to some extent. Ignoring species abundance made Chao1 and ACE could play a role freedom from sampling time, sampling site and library size.

Candidate biomarkers for different combinations of straw returning and tillage practices

Widespread application of straw recycling could reduce fertilizer use and pollution of the environment (Zhang et al. 2011b). Tillage practices also play an important role in crop growth by influencing soil thermal status and water (Zhang et al. 2011a; Murugan et al. 2014). We attempted to screen out bacteria that showed a close relationship with management practices. Double filtration based on the relative abundance and network analysis, revealed that *Mycobacterium* and *Methylibium* were sensitive to both maize straw returning and rotary tillage. In several environments, *Mycobacterium* were reported to have the ability to degrade polycyclic aromatic hydrocarbons (PAHs), which are one of the main pollutants in soil and water (Bastiaens et al. 2000; Cheung and Kinkle 2001; Chen et al. 2015). Because PAHs are commonly found in contaminated crops, we assumed that the maize straw that was returned to the soil had been polluted by heavy metals to some extent (Bian et al. 2018). In a subtropical acrisol study, the relative abundance of *Mycobacterium* was also reported to be significantly higher in a conventional tillage system (Dorr de Quadros et al. 2012). This increased abundance may have been a result of its ability to degrade carbonyl sulfide (COS) (Hiromi et al. 2008). Soil is the major sink for COS from the atmosphere, and rotary tillage would expose to the soil to more COS than no tillage or deep tillage treatments. It is well known that *Methylibium* possess the ability to degrade hemicellulose, which is abundant in crop straw (Leung et al. 2016). Although few studies have investigated the relationship between *Methylibium* and tillage practices, it is known to be a monoaromatic hydrocarbon degrading bacterium (Salvador et al. 2009). A study of subtropical Brazilian soils showed that tilled soil had an increased ratio of aromatics (Dieckow et al. 2010). Combining the results of previous research with those of the present study indicate that *Mycobacterium* and *Methylibium* played a crucial role in soil subjected to straw returning and rotary tillage and could serve as candidate biomarkers for the combination of these two agricultural management methods.

Optimal solution to improve soil microbiome in the short term

The microbiome is the main body responsible for degradation of organic matter and natural fiber (Paterson et al. 2011). Straw is rich in carbon, nitrogen and phosphorus and other nutrients; therefore, straw returning increases the amount of organic matter in soil which stimulates the growth of soil microorganisms (Miura et al. 2016). To reflect the soil microbiome more objectively, four alpha indexes were adopted in this study. The results indicated that RTS possessed the highest bacterial diversity and all four indexes indicated that straw returning enhanced microbial diversity. These results demonstrated that straw returning could influence the bacterial community, even in the short term. Similar results in response to straw return were also reported in previous reports in tobacco-rice rotation and a rice-wheat cropping system (Lei et al. 2017). It was understandable that straw turning exerted a positive influence on bacteria, but rotary tillage was rarely related with high bacterial diversity. In the present study, three different short-term tillage practices (no tillage, deep ploughing and rotary tillage) investigated to determine the relationship between bacterial diversity change and these practices. Although discordant patterns were observed among the four alpha indexes, no tillage group tended to be disadvantaged, which were proved to be beneficial to soil microbiome. This was not the first time that no tillage had a disadvantage compared with other tillage practices. A previous study found that tillage causes short-term changes in nutrient dynamics and denitrification rates increased after pillaging which implied the increase of denitrifying bacteria (Calderon et al. 2001). In addition, there was little or no detectable increase in soil organic C content in the first 2–5 years from converting conventional tillage to no tillage (Al-Kaisi et al. 2005). Long-term no tillage effectively

enhances the diversity of bacteria by improving the soil microenvironment, but short-term deep ploughing or rotary tillage may build a surrounding suitable for some species. The ratio of G+/G- bacteria could reflect the nutritional states of soil, with a high-percentage of G+ bacteria indicating a poor nutritional state. Groups subjected to rotary tillage (RTS and RTNS) possessed the lowest ratio of G+/G- bacteria. Differences between rotary tillage and deep tillage were that rotary tillage could improve the structure of surface soil rather than the plow pan. Rotary tillage could also increase the combination degree of straw and topsoil and then influence the activity of microbiome. In conclusion, RTS possessed the higher bacterial diversity and lower ratio of G+/G- bacteria which could be an efficient integrated management to improve microbiome over the short term.

MATERIALS AND METHODS

Sampling

The experiment was conducted in the Longshan experimental base (117°32′E, 36°43′N) of the Maize Institute, Shandong Academy of Agricultural Sciences, China. This region belongs to the zone of the continental monsoon climate in which the winter wheat-summer maize double cropping system is the main cropping system. The annual average temperature of this region is about 13.6°C and mean annual precipitation is 693.4 mm. The study site is underlain by brown loamy soil with an organic matter content of 10.6 g/kg.

One experimental field was selected and managed respectively according to goals of the experiments. Fields was divided into six plots and every plot covers an area of 810 m^2 (45m × 18 m). These six plots were managed by different tillage methods (deep ploughing, rotary tillage and no tillage) and different modes of straw application (either with or without maize straw returning field) for one year. Surface soil (5–10 cm) from plots were sampled with three replications on 25 September 2015. Then the roots and stones (>2 mm) were removed from the samples. The sampling tool was a garden trowel, which had been rinsed with alcohol and deionized water. Samples were stored at −80°C until DNA extraction. Details of processing methods and naming after sampling please refer to Table 2.

DNA extraction and sequencing

DNA was extracted from 0.5 g soil of each sample using MOBIO PowerSoil DNA Isolation Kit (MO BIO Laboratories, QIAGEN Inc., USA) following the manufacturer's protocol. Agarose gel electrophoresis was adopted as a rough measurement to assess the qualities of DNA. Then the DNA concentration was quantified using the Qubit2.0 DNA Kit.

Before using, DNA concentrations of 30 samples were diluted to 5 ng/μL. The universal primers (319F: ACTCCTACGGGAGGCAGCAG, 806R: GGACTACHVGGGT WTCTAAT) of the Miseq platform which could amplify the V3–V4 regions of 16S rRNA were used in this PCR reaction. A 20 μl PCR reaction system contained: 4 μL template DNA, 1.5 μL of each primer, 10 μL PCR mix buffer, 0.5 μL DMSO and 2.5 μL nuclease free buffer. Enough dNTPs and high-fidelity enzyme (Phusion High-Fidelity PCR Master Mix with HF Buffer) were mixed in the PCR buffer. PCR amplification was performed with the program: 30S at 98°C, followed by 35 cycles of 15S at 94°C, 15S at 55°C, 15S at 72°C and 1 min at 72°C for final extension. PCR productions were examined by 1% agarose gel electrophoresis. Then the productions were purified with Agencourt AMPure XP 60ml Kit according to matufacturer instructions. After purification, productions were detected by Nanodrop (Thermo, 2000c) and blended equally. AXYGEN Gel Extraction Kit (250) was used to collect the target fragments of DNA. The densities of the collected fragments were detected by Qubit2.0 (Life Tech, Q32866) and quality control was performed with Agilent 2100 Bioanalyzer.

Quantitative PCR (qPCR) was performed to test the efficiency of the adapters. Based on the efficiency, the clone libraries were diluted to a proper concentration

Table 2. Managements and group information of the 18 samples

Sample number	Group	Tillage methods	Straw
A1,A2,A3	DTS	Deep tillage	Maize straw
A4,A5,A6	DTNS	Deep tillage	No straw
A7,A8,A9	RTS	Rotary tillage	Maize straw
A10,A11,A12	RTNS	Rotary tillage	No straw
A13,A14,A15	NTS	No tillage	Maize straw
A16,A17,A18	NTNS	No tillage	No straw

for sequencing. Miseq system (Illumina) were used to accomplish the sequencing of 16S rRNA.

Bioinformatic analysis

Raw data were processed using the QIIME (v1.7.0) pipeline following the standard protocol in http://qiime.org/ (Caporaso et al. 2010). Unique sequences were obtained after low quality reads filtering, trimming and duplication removing. Then OTU clustering were performed based on the 97% unique sequence similarity. USEARCH software (v8.01) were used to generate the OTU table file by aligning the original reads back to the OTUs (Edgar 2010). OTUs which matched chloroplast, mitochondria and nonbacterial sequences were removed. To minimize the anthropogenic influence, singletons (OTU with only one reads) were removed. Taxonomic annotation was performed against the Greengenes database (v13.5). Make_phylogeny.py in QIIME was used to generate the phylogenetic tree file, which was indispensable for the next diversity analysis. Based on the final OTU table file, the α-diversity indexes (chao1, Shannon, ace, Simpson) were calculated using the aloha_deversity.py script. By using SPSS (V13.0), ANOVA and post-hoc Tukey HSD test were performed to study the variances of the four alpha indexes between different groups (Tables S8, S9). For β-diversity analysis, the OTU table needed the standardization firstly with the cumulative sum scaling (CSS) method. Then the weighted and unweight UniFrac distance among the 18 soil samples were calculated based on the standard OTU table. Utilizing the distance matrix, the PCA and PCoA plot was performed using the R package vegan and ggplot2. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt, http://picrust.github.com) was used to predict the functional content based on OTU file (Langille et al. 2013). First, filter_otus_from_otu_table.py was used to pick the closed-reference OTUs against the greengenes database (v13.5). Then OTU matrix was normalized to account for uneven columns using normalize_by_copy_number.py. Based on the normalized OTU table, we predicted the metagenomic functions using predict metagenomes.py. We further proceeded the KEGG analysis using categorize_by_function.py. Bugbase (https://bugbase.cs.umn.edu/) was used to predict the ratio of Gram-positive bacteria/Gram-negative bacteria. Although it only contained 18 soil samples, the network analysis was conducted by igraph, psych, Hmisc and WGCNA packages in R3.5.0 software. The clean data and OTU files are available at http://biodb.sdau.edu.cn/xxyssr/maize_16s.zip.

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AUTHOR CONTRIBUTIONS

L.Y., H.L. and Z.L. designed the study. X.X., P.Z., L.H., X.G., W.L. and Y.Z. analyzed the data. X.X. wrote the paper. All authors approved of its content.

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SUPPORTING INFORMATION

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Figure S1. Statistical differences in the functional subsystem between groups with and without maize straw returning at level 3

Blue columns represented the groups with maize straw returning and the red one represented no straw group. Only the subsystems with the significant differences and a confidence interval of 95% were showed.

Figure S2. Statistically differences in the functional subsystem between groups with deep ploughing and no tillage at level 2

Blue columns represented the groups with deep ploughing and the red one represented no tillage group. Only the subsystems with the significant differences and a confidence interval of 95% were showed.

Figure S3. Statistically differences in the functional subsystem between groups with rotary tillage and no tillage at level 2

Blue columns represented the groups with deep ploughing and the red one represented no tillage group. Only the subsystems with the significant differences and a confidence interval of 95% were showed.

Figure S4. Statistically differences in the functional subsystem between groups with deep ploughing and no tillage at level 3

Blue columns represented the groups with deep ploughing and the red one represented no tillage group. Only the subsystems with the significant differences and a confidence interval of 95% were showed.

Figure S5. Statistically differences in the functional subsystem between groups with rotary tillage and no tillage at level 3

Blue columns represented the groups with deep ploughing and the red one represented no tillage group. Only the subsystems with the significant differences and a confidence interval of 95% were showed.

Table S1. Diversity indexes of 18 soil samples

Table S2. *P*-values of *t*-test analysis between groups with or without maize straw returning

Table S3. One-way analysis of similarity between different groups

Table S4. The relative abundance of the genus which were sensitive to maize straw returning

Table S5. The relative abundance of the genus which were sensitive to deep ploughing

Table S6. The relative abundance of the genus which were sensitive to rotary tillage

Table S7. The relative abundance of the genus which were sensitive to no tillage

Table S8. The ANOVA results of the four alpha indexes

Table S9. The results of post-hoc Tukey HSD test of the four alpha indexes

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