



Full Length Article

Long-Term Effects of Tillage Practices on Soil Bacterial Community Abundance and Metabolic Diversity of Black Soil from Northeast China

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Abstract

As a more sustainable farming system, conservation tillage will play an important role on agricultural sustainable development in the future. But studies have scarcely paid attention on the long-term effects of conservation tillage on soil microbial composition and metabolic activity. Our work adopted the high-throughput sequencing technique of the 16S rDNA gene and the Biolog EcoPlates method to investigate the microbial communities and metabolic activity of black soil under three tillage practices (no-tillage, ridge tillage, and conventional tillage abbreviated correspondingly to NT, RT and CRT) as well as the effects of the long-term conservational tillage spanning 15 years, which may improve our understandings of tillage practices and soil quality. The results indicate that the bacterial communities keep similar regardless of tillage practices; the straw returned management only affects the soil microbial community abundance and functional diversity rather than the community structure. The metagenome functional prediction results suggest that the organic material returned management can exert effects on the bacterial community abundance, FDA enzymes activity and C-metabolic activity, which are critical for agricultural sustainability. Ridge tillage of soybean straw mulching may be a better farming practice for soil microbial activity in Northeast China. © 2018 Friends Science Publishers

Keywords: Tillage practices; Soil microbial community; Metabolic activity; Metagenome functional prediction; Black soil

Introduction

Tillage plays an important role in agriculture and has significant effects on soil physical, chemical properties and biological characters. But it has been a major reason for land degradation. Excessive or inappropriate tillage has given rise to negative effects on the properties of agricultural soils, including the reduction of soil organic matter, extensive soil erosion and reduction of biodiversity (Abdullah, 2014; Mbuthia *et al.*, 2015; Kabiri *et al.*, 2016; Rincon-Florez *et al.*, 2016). The conservation agriculture (CA for short, with a minimum of soil disturbance, no-till and crop rotation as characteristics), which aims to remove the negative effects of conventional tillage, is a more sustainable and environmental-friendly crop cultivation and management system (Hobbs *et al.*, 2008; Olson *et al.*, 2013; Abdullah, 2014; Farooq *et al.*, 2011). In many parts of the world, the agriculture protection is more and more popular, and plays an important role in the sustainable development of agriculture, and affects many the physical, chemical and microbial activities related with soil quality (Abdullah, 2014; Mbuthia *et al.*, 2015; Nugis *et al.*, 2016).

Soil biogeochemical cycle includes decomposition, mineralization and retention of soil nutrients and is an

important component of sustainable agricultural ecosystem. Soil microorganisms are the major driver of biogeochemical processes and are critical for agricultural sustainability (Yendi *et al.*, 2013; Sun *et al.*, 2016a). The effect of tillage methods on soil is determined by microbial metabolic activity, which can change soil physical structure and the residual distribution of plants (Nugis *et al.*, 2016). And the metabolic diversity patterns of soil microorganisms are attributed to the soil microbial community composition (Rogers and Tate, 2001; Juan *et al.*, 2008; Yao *et al.*, 2017). The soil microorganisms hence are considered suitable indicators for assessing soil quality under tillage systems due to their rapid responses to environmental changes including soil disturbance (Kabiri *et al.*, 2016; Rincon-Florez *et al.*, 2016). Extensive publications have covered the impact of conservation tillage on the microbial properties (Juan *et al.*, 2008; Guo *et al.*, 2014; Mbuthia *et al.*, 2015; Kabiri *et al.*, 2016; Nugis *et al.*, 2016; Rincon-Florez *et al.*, 2016; Sun *et al.*, 2016a, b; Lian *et al.*, 2017). But, so far, debate about the possible effects of tillage methods on the microbial composition still exists, which can be achieved by different soil types, climate and agricultural practices to explain historical research. Relatively less attention has been paid to the effects of long-term conservation practices on the

microbial composition.

Therefore, in this study, we aimed to explore the potential influences of long-term tillage practices (including disturbance and crop rotation) on bacterial community composition and to reveal the relationship between community composition, Carbon source metabolic functional diversity and physicochemical properties of black soil from a long-term view. The Illumina HiSeq sequencing technique of the 16S rDNA genes was employed to examine the effect of long-term different tillage practices on microbial communities, and the Biolog™ EcoPlates method to measure the community-level physiological profiles.

Materials and Methods

The Experimental Site

The experimental site, soil, experimental design and management practices had been described in more detail in our previous study (Liang *et al.*, 2010). The long-term tillage experiments started in 2001 in Dehui county, Jilin province, China (44°12'N, 125°33'E, and the elevation of 177 m) was used to evaluate the effects of different tillage methods on the physical, chemical properties and soil biological characters including the diversity and metabolic functional activity of microbial communities in soil. The tillage experiments, including conventional tillage (CT), ridge tillage (RT), no tillage (NT) and mouldboard plough (MP), adopted the split-plot design with four replicates. Except the plots under CT practices, in other plots under the treatments of NT and RT, the crop residues were returned and covered on the soil surface after harvest. Each year, the nitrogen (N) of 100 kg/ha was applied to maize as the starter fertilizer and that of 50 kg/ha as the top dressing at the V-6 stage (the 6-leaf collar stage). In addition, the P₂O₅ of 45.5 kg/ha and the K₂O of 78 kg/ha were also applied to maize as starter fertilizers. The starter fertilizers for all plots were applied concurrently with planting using the banding attachment on the no-till planter. For soybean, N, P and K fertilizers were applied at 40 kg/ha, 60 kg/ha and 80 kg/ha as starter fertilizers, respectively. Three tillage treatments were applied at the sub-plot level with both crops present in each year in each whole plot.

Properties of Soil Samples under Different Tillage Practices

The soil samples of this study were collected from ten randomly selected sites of each plot in late September 2015 before harvest. All the samples were divided into three subsamples and stored at -80°C, 4°C and air-dried conditions, respectively. Some physicochemical properties of the soil applied with different tillage practices for 15 years are presented in Table 1, in which the soil pH was determined by a glass electrode meter in the suspension liquid with the soil/water ratio (w/v) of 1:1; the soil moisture

content was estimated by the oven-dry method; the soil organic matter (OM) was measured by the UV spectrophotometric method and total nitrogen (TN) was measured using semi-micro Kjeldahl nitrogen method.

FDA Enzyme Activity

The hydrolysis of fluorescein diacetate (FDA) was tested by incubating soil samples for 2h (37°C), and the released fluorescein was determined according to the references (Schnürer and Rosswall, 1982; Green *et al.*, 2006).

Carbon Source Metabolic Functional Diversity

Carbon source metabolic functional diversity of soil microbial communities were determined in virtue of Biolog EcoPlates (BIOLOG Inc., Hayward, CA) (Flores-Renteria *et al.*, 2016). The system has been recognized as a useful method for comparing microbial communities, since it can detect metabolic activity changes in microbial communities under different tillage practices (Gomez *et al.*, 2004).

The Biolog EcoPlate was operated according to the description of Chen *et al.* (2008) and Sun *et al.* (2016a). Microbial populations inhaled the carbon sources, and released NADH, which can change the well colors, the absorbance reflecting color changes measured daily in 7 days at the wavelength of 590 nm to determine the Average Well Color Development (AWCD). All carbon sources of Biolog EcoPlate were divided into 6 major categories (Polymers, Carbohydrates, Phenolic compounds, Carboxylic acids, Amines and Amino acids). The metabolic functional diversity of the soil microbes was determined by the amount and uniformity of carbon substrates, representing the Shannon-Weaver diversity index and evenness, respectively (Ma *et al.*, 2016; Rincon-Florez *et al.*, 2016).

Microbial Community Composition

The High-throughput pyrosequencing (Hiseq) of the 16S rDNA gene was conducted to determine the soil microbial community composition as the references (Cao *et al.*, 2015; Zarronaindia *et al.*, 2015; Xu *et al.*, 2017). The total genomic DNA was extracted out of the soil sample of 0.25 g by the PowerSoil® DNA isolation kit (manufactured by MoBio Laboratories, CA, USA) according to the instructions, and subsequently the DNA concentration was determined by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). The V3-V4 of the 16S rDNA gene was amplified with reference to the standard protocols (Chen *et al.*, 2016; Xu *et al.*, 2017; Yao *et al.*, 2017; Zhang *et al.*, 2017). The sequencing was carried out on an Illumina Hiseq, committed to the Personal bio Company (Biomarker Technologies Co., LTD., Beijing, China).

Predictive Metagenome Analysis

Based on the 16S rDNA sequences, the online PICRUSt program was used to predict the metagenome functions of

the bacterial communities according to the references (Langille *et al.*, 2013; Chen *et al.*, 2016), which is more informative than a purely taxonomic community structure approach.

Data Analysis

The Analysis of Variance (ANOVA) based on the Generalized Linear Mixed Model (GLIMMIX) was adopted to analyze the data. The *p* value for the significant differences in the means of three replicates among the field treatments were calculated to be smaller than 0.05 using Fisher's protected LSD in SAS (SAS Institute, Cary, NC, 2012). The paired-end sequencing was performed on the HiSeq platform. The sequencing results were clustered based on the similarity of 97% by UCLUST in the software of QIIME (version 1.8.0) to obtain OTUs (Operational Taxonomic Units), and the OTUs were classified as the Silva (Bacteria) taxonomy database Annotations. A variety of analyses including Shannon, ACE and Chao1 indices were performed using the Mothur software (version 1.31.2). The heatmap.2 of the R software package (version 3.1.1) (Zhang *et al.*, 2017) was used to conduct the clustering analysis. The CANOCO 4.5 was used to carry out the canonical correspondence analyses (CCA), so as to compare the influences of soil characteristics on the bacterial community structure and functional metabolic diversity.

Results

Soil Microbial Activity and Functional Diversity

In terms of the soil disturbance, among all the practices, the MP and the no-tillage are of the maximum and minimum levels, respectively. In the rotation system, it is the soybean stalks left in last planting seasons that are returned to the field. In contrast, the continuous cropping system of maize adopts the maize own stalks. Due to the lower value of C:N in comparison to the maize straws, the soybean straws are easier to be decomposed, resulting in the higher soil organic matter contents in the straw returned system than the

conventional tillage without straws returned. The corn straws of high values of C:N are difficult to be degraded, hence the residual organic matter after several planting seasons being high.

As shown in Table 1, soil disturbance levels affect greatly the soil organic matter contents. With low soil disturbance levels, the practices of no-tillage (NT.MS and NT.MM) and ridge tillage (RT) result in the organic matter contents of 46.03, 62.16 and 43.33 g/kg, respectively, higher than the content of the conventional tillage practice (14.48 g/kg, CRT). But the practice of MP in the organic matter content is higher than the CRT, being 34.05 and 36.27 g/kg. The effect of long-term tillage on soil enzyme activity was determined (Fig. 1). The result also indicate that soil disturbance levels and the type of straw returned were two important factor affecting soil FDA enzyme activity, conservation tillage practices improve FDA enzyme activity.

The Biolog EcoPlates™ is a well-known method for assessing the soil carbon sources utilization by soil microbial communities under different environments. The source utilization profiles of different tillage practices are shown in Fig. 2. The utilization of specific types of carbon sources by the soil microbial communities under different tillage practices is reflected by the AWCD values of various carbon sources. As shown in Fig. 2, the soil microbial communities under the conventional tillage practice (without straws returned) metabolized much less carbon sources than those under other tillage practices (with straws returned). Besides, the type of straw returned (the soybean or the corn) is significant to the utilization of carbon sources. The utilization levels of Phenolic compounds, Carboxylic acids, Amines, Polymers, Carbohydrates and Amino acids vary with the type of straws. Comparatively, the soybean stalks are more favorable to use the specific types of carbon sources. As for the Carboxylic acids, Amines, and Amino acids, these specific types of carbon sources are utilized more by the tillage practices of NT and RT than the tillage practice of MP. As the Principal Coordinate Analysis (PCoA) shown (Fig. 3), it is the type of straws that dominates the carbon source utilization and carbon metabolism activity of soil microbes.

Table 1: Properties of soil samples under different tillage practices

Treatments		pH	WAT (%)	OM (g/kg)	TN (g/kg)
Crop rotation (Maize-Soybean)	NT.MS	5.37 (±0.02) ^c	12.67 (±0.02) ^a	46.03 (±1.87) ^b	2.15 (±1.87) ^b
	RT.MS	5.42 (±0.03) ^b	11.85 (±0.99) ^a	43.33 (±2.41) ^b	1.93 (±0.37) ^{bc}
	MP.MS	5.67 (±0.01) ^a	9.94 (±1.20) ^c	34.05 (±1.71) ^c	1.76 (±0.40) ^{bc}
Continuous cropping (Maize)	NT.MM	4.97 (±0.01) ^e	8.70 (±0.02) ^{bc}	62.16 (±2.44) ^a	2.73 (±0.36) ^a
	MP.MM	5.23 (±0.02) ^d	7.99 (±0.53) ^c	36.27 (±3.29) ^c	1.50 (±0.04) ^c
	CRT.MM	5.34 (±0.01) ^c	8.46 (±0.41) ^c	14.48 (±0.63) ^d	1.93 (±0.06) ^{bc}

1:NT.MS, No tillage for corn-soybean rotation system with soybean straw returned; RT.MS, ridge tillage for corn-soybean rotation system with soybean straw returned; MP.MS, Moldboard plough for corn-soybean rotation system with soybean straw returned; NT.MM, No tillage for corn continuous cropping system with corn straw returned; MP.MM, Moldboard plough for corn continuous cropping system with corn straw returned; CRT.MM, Conventional tillage (Ridge tillage for corn continuous cropping system without straw returned)

2: Values are means ± standard deviation (n=3). Values within the same column followed by the different letters indicate significant differences (*P* < 0.05). Properties: pH (pH 1:1 H₂O), WAT (Soil water content), OM (Organic material), TN (Total Nitrogen), FDA (FDA hydrolase)

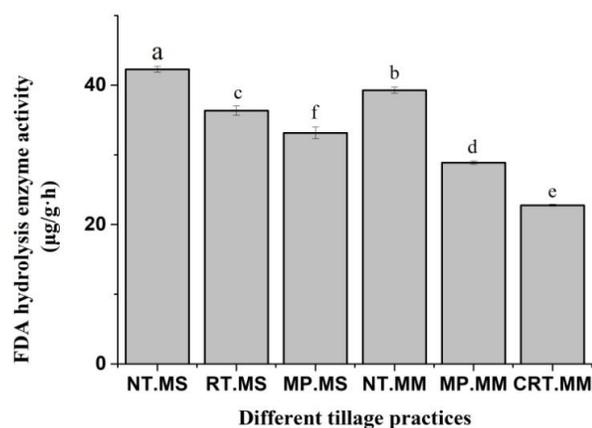


Fig. 1: FDA hydrolysis enzyme activity under different tillage practices

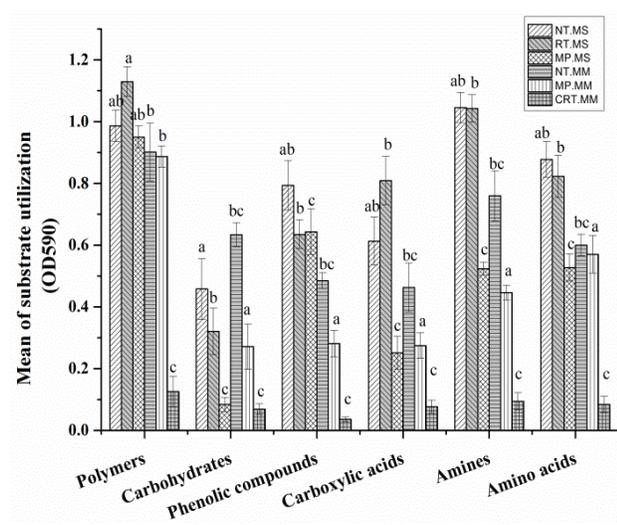


Fig. 2: Comparison of the utilization of specific carbon sources under different tillage practices in Biolog Eco-Plates. The lower case letters of 'a', 'b', 'c' and 'd' indicate the significant differences ($P < 0.05$) among different treatments. Each sample was measured in triplicate

Results of High-throughput Sequencing and Bacterial α -Diversity

In this work, HiSeq high-throughput sequencing method based on 16S rDNA genes was used to study the influence of long-term different tillage methods on the community composition and diversity of soil microbial in black soil. In the analysis of bacterial communities from 6 soil samples, 296,487 high quality sequences (59.46%, total 295,706) were obtained. The Good's coverage value is between 83.48 and 85.74% indicating that the current sequence reads enough to capture the diversity of bacterial in the soils. Based on the Mothur clustering, the numbers of OTUs in these samples is between 1495 and 1616, and there are three

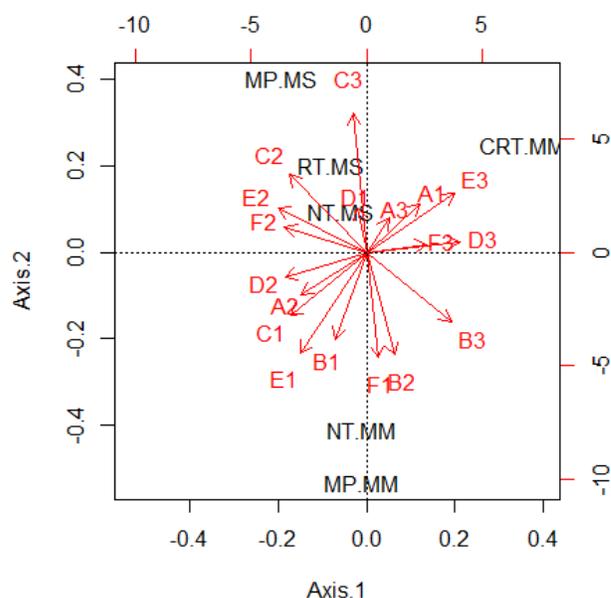


Fig. 3: Principal coordinate analysis (PCoA) based on the weighted UniFrac distance matrix showing the relationship between the utilization of carbon sources with tillage practices. (P -value < 0.05)

different tillage methods with corn-soybean rotation (Table 2). The practice of NT in the corn-soybean rotation system acquired the highest number of total OTUs, and the lowest number occurred with the practice of MP in the corn-continuous cropping system.

Under different tillage practices, the microbial richness and diversity of Chao1, Shannon, Simpson and goods coverage measures were significantly different (Table 2). The Chao1 index of the RT.MS was the highest compared to other tillage practices. It is suggested that the degree of soil disturbance greatly affects the Chao1 index. For example, the treatments of NT and RT, resulting in less disturbance degrees than MP, obtained the higher abundance of soil microbial communities. Because the decomposition rate of the corn straw (with a high C:N ratio) is slower than that of the soybean straw (with a low C:N ratio), the soil organic matter content under the corn continuous cropping is lower than that under the soybean straw returning. Therefore, the soil bacterial community abundance under the soybean straw returned tillage management is higher than that of the maize straw returned soil. The soil disturbance degree affects the soil microbial community diversity index to a large extent (reflected by Shannon and Simpson indexes); the larger the disturbance degrees, the less the community diversity. Regardless of the rotation system or the continuous cropping system, the diversity of soil microbial communities under the MP tillage is the lowest. The soil microbial Alpha diversity index suggests that the soil bacterial community abundance and diversity can be improved by the NT and RT with rotation

Table 2: Summary statistics and alpha diversity estimates of 16S rDNA gene sequences from agricultural soil under different tillage practices

Treatments		Tillage practice	Clean reads	Effective sequence OTUs (percent)	Richness			Diversity		Goods coverage
					ACE	Chao1	Simpson	Shannon	(%)	
Straw returned	Corn planting of corn - soybean rotation system	NT.MS	47,869	47,755 (83.48%)	1,616	1,666.01	1,671.07	0.0044	6.3225	99.57
		RT.MS	50,610	50,484 (83.53%)	1,612	1,674.59	1,702.21	0.004	6.3256	99.48
	Continuous cultivation of corn	MP.MS	51,326	51,197 (84.3%)	1,529	1,606.31	1,612.51	0.0073	6.0529	99.49
		NT.M.M	49,822	49,696 (84.27%)	1,526	1,613.60	1,646.40	0.0045	6.1699	99.42
		MP.M.M	47,391	47,253 (85.04%)	1,495	1,568.60	1,586.59	0.0072	6.0012	99.46
No straw returned		CRT.M.M	49,469	49,321 (85.02%)	1,587	1,640.49	1,644.29	0.0045	6.2617	99.56

Table 3: Spearman correlations between alpha diversity and soil properties

Soil properties	Chao1	Shannon	Simpson
pH	0.543	0.257	-0.207
WAT	0.829*	0.771	0.414
OM	0.086	0.314	0.414
TN	0.406	0.638	0.840*
FDA	0.371	0.600	0.621

* $P < 0.05$

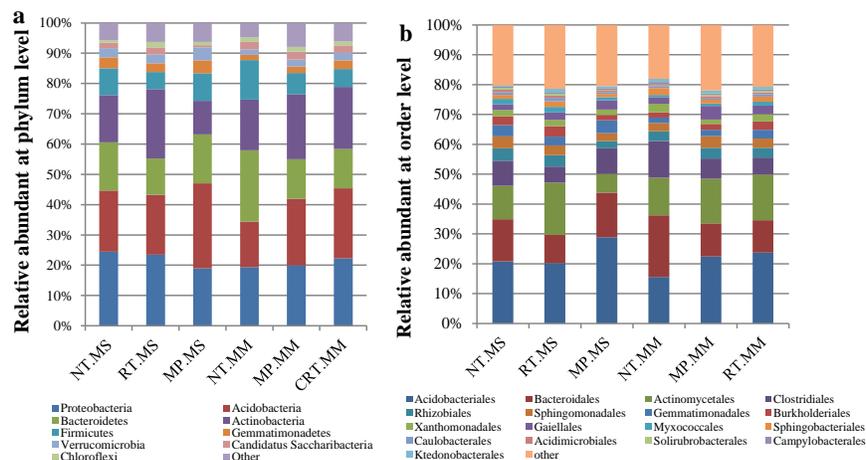


Fig. 4: Relative abundance of soil bacteria under different tillage practices combined with rotation cropping or continuous cropping at two levels. a, phylum level; b, order level. The relative abundance is represented as a proportion of 16S rDNA gene reads of the total number of reads. The bacteria phyla with relative abundance >1.0% are shown

tillage management.

The relationships between the α -diversity and soil properties are shown in Table 3. Based on the Spearman correlations of the alpha diversity with soil properties, the Chao1 indices are closely relevant with the soil water content ($r=0.829$, $P < 0.05$). And the Simpson indices are mainly influenced by the concentration of soil total nitrogen but are negatively associated with the soil pH value.

Composition and Relative Abundance of Soil Bacterial Taxa

Based on the Illumina platform analysis, the sequences are classified into 23 different bacterial phyla, 48 classes, 79 orders, 132 families, and 187 genera for all treatments. The

bacterial phyla are known to be abundant and ubiquitous in soil. As shown in Fig. 4a, the bacteria composition under the six practices at the phylum level is similar, whereas the relative abundances of phyla are different. There are nine phyla whose relative abundances are more than 1%. The soil bacterial composition is dominated by Proteobacteria (18.95–24.47%), Actinobacteria (11.16–22.92%), Bacteroidetes (12.00–23.54%), Acidobacteria (15.04–28.06%), and Firmicutes (5.62–13.06%), of which the proportions are all larger than 5%. But the relative abundance of the main phyla (>1%) is strongly linked with the tillage practices. For the practices of NT.MS and RT.MS, under which the soil is less disturbed, it is the Proteobacteria phylum that ranks the highest proportion among bacterial sequences, the relative abundance reaching

24.47 and 23.46%, respectively. However, for the practices of MP and the control groups, the Acidobacteria phylum is recognized to be maximum in the proportion, the relative abundance being 28.06, 21.98 and 23.12%, respectively.

Considering the Oral taxonomic ranks, tillage practices affect the relative abundance of the bacterial community yet not the bacterial community structure, as shown in Fig. 4b. The relative abundance of the GP1 under the MP tillage is highest among all tillage practices.

The relative abundance heatmap involving all genus for different tillage practices is shown in Fig. 5 (for the relative abundance > 0.1%). No significant structure differences between tillage practices are found at the genus level. Notably, the relative abundance of Gp1 varies with tillage practices, which is highest for all MP soil samples (Fig. 5), reaching 27.14% (MP.MS) and 21.66% (MP.MM), respectively. These results indicate that the community functional diversity is reflected more by the bacteria community composition than the community structure.

Relationships between Bacterial Communities and Environmental Variables

According to the CCA plots, the tillage practices with different retained management are closely correlated with bacterial community structures and metabolic activity.

The CCA plots drawn on the CANOCO software are targeted to compare the bacterial community compositions among all soil samples and to identify the major environmental variables that affect the community structures (Fig. 6a, b). According to the results of CCA, there are strong links between soil parameters and bacterial community structures. The variation of bacterial communities among samples can be totally explained by the soil properties. The two parameters of OM and pH have large variance inflation factors (VIF), which imply that their variables are redundant with other variables in the soil environmental factors. The first two axes of CCA account for 82.45 and 10.05% (92.50% in total) of the total variation in the data.

The effects of tillage practices on the abundance of bacterial communities under all conservational treatments (NT.MS, NT.MM, RT.MS, RT.MM, MP.MS and MP.MM) and the conventional treatment (CRT.MM) have been evaluated. Firstly, the long-term tillage practice, the conservational tillage or the conventional tillage, affects the bacterial communities. Secondly, under a same tillage practice, the crop straw returned management has a detectable impact on bacterial communities and metabolic activity. In addition, for the practices of NT and RT, water can change the metabolic activity of bacterial communities (Fig. 6c).

These results stress that the type of straws returned is a key factor for the distinctions on the bacterial communities and metabolic activity under same tillage practices.

16S Genes Functional Prediction

Although various functional similarities of these samples can be seen, significant differences regarding the functional gene abundances also exist (Fig. 7a). The reads of the metabolism of amino acid, carbohydrate, Glycan, Lipid, Terpenoids and Polyketides-related metabolism are significantly higher compared with those of other metabolism pathways, which indicated the particular importance of these metabolism pathways. Notably, the practice of soybean straw returning, compared with the conventional practice, acquires much higher reads of Xenobiotics metabolism and membrane transport-related reads. The degradation of soybean straws returned to soil is closely related to soil microbial activities, which can explain the presence of the above-mentioned degrading bacteria in this soybean straw returned management. Polymer degrading bacteria can transform microbial products from soybean straws into organic carbon sources that can meanwhile enhance the bacterial relative abundance and carbon-metabolic activity in these tillage practices management.

The type of straw affects the abundance of microbial metabolic genes, these genes being functional for the straw degradation (Fig. 7b). The soybean straws are easier to be decomposed by soil microorganisms than the corn straws, consequently the soybean straws and corn straws separately obtaining the higher reads of Xenobiotics metabolism and Glycan metabolism. The result indicates the differences and changes of metabolic pathways for functional genes, and shows that the predictive gene abundance can well explain the FDA enzyme activity and C-metabolic activity in soil under the soybean straw returned management.

Discussion

Soil enzymes play a fundamental role in nutrient cycling and their activities reflect some degree of soil function and functional diversity, but they are very sensitive to the changes of soil physical-chemical properties, all of which may be affected by different tillage methods (Bissett *et al.*, 2013). The hydrolysis of FDA has been suggested to be available to measure the microbial activity in soil because the ubiquitous enzymes activities of lipase, protease, and esterase are involved in the hydrolysis of FDA (Green *et al.*, 2006). Several studies have shown that the activity of FDA hydrolase in the soil has a very significant positive correlation with soil microbial biomass, and the activity of FDA hydrolase is usually used to characterize the soil microbial biomass. Therefore, the hydrolysis of FDA can reflect the activity of soil microorganism well (Green *et al.*, 2006; Bissett *et al.*, 2013). It can be used as an important parameter to evaluate the soil microbial biomass and activity (Bissett *et al.*, 2013). In this study, the effects of tillage and nutrient addition to the potential of soil functional were evaluated through the activity analysis of

the FDA hydrolase. As shown in Fig. 1, the FDA activities of the conservational tillage (NT.MS, NT.MM, RT.MS, RT.MM, MP.MS and MP.MM) were higher than the conventional tillage (CRT.MM), which proves the effects of long-term tillage practices. In addition, the soybean straw returned management has a detectable impact on the FDA activity. This observation indicates that less disturbance and straws returned favor the FDA activity, which further scales up the soil microbial metabolic activity and increases the content of organic matter in soil (Bissett *et al.*, 2013).

In this study, the change of metabolism of soil microbial community diversity seems to be a response to the straw, rather than a response to the tillage methods, indicating the straws returned and the type of straws dominate the carbon source utilization and carbon metabolism activity of soil microbes. Comparatively, the soybean stalks are more favorable to use the specific types of carbon sources. All the analyses of FDA activity (Fig. 1) and specific carbon source utilization (Fig. 2) indicate that enzyme activity and less soil disturbance and straws returned can enhance the metabolic activity of soil microbial populations. Similar findings have also been reported by Sun *et al.* (2016a).

Soil microbes play an important role in degradation and nutrient cycling. SOM as the most important substrate for soil microbial survival and growth, the positive correlation between soil organic matter and microbial activity and soil enzyme activity is prevalent in the soil (Bissett *et al.*, 2013; Jia *et al.*, 2015). The effects of agricultural management regimes on soil microbial community composition and metabolic capacity have been reported in literatures (Patricia *et al.*, 2012; Sengupta and Dick, 2015; Sun *et al.*, 2016a). In these agricultural management regimes, the crop rotation and different tillage practices are critical for the agricultural fields. To date, many studies have adopted the HiSeq to study the effects of different tillage methods and the effects of straw returned management on soil microbial diversity and metabolic activity (Dorr de Quadros *et al.*, 2012; Lienhard *et al.*, 2013; Yendi *et al.*, 2013; Sengupta and Dick, 2015; Degrunne *et al.*, 2016). Although some authors have observed that no tillage (NT) practice has been shown to increase microbial biomass and microbial relative diversity in topsoil because the soil surface is covered with residues and in less disturbing ways (Dorr de Quadros *et al.*, 2012; Lienhard *et al.*, 2013; Yendi *et al.*, 2013; Azevedo *et al.*, 2015; Sengupta and Dick, 2015), there is an inconsistent understanding of the possible effects of tillage on the microbial community composition comparing conventional tillage practice, many studies have revealed that there is no difference or decrease in the relative abundance of the microbial community diversity (Degrunne *et al.*, 2016). The studies, however, is the focus of the soil and is characterized by very specific environmental conditions, such as different soil types, climates and organic ingredients, makes it difficult to put them the result of comparing with our results.

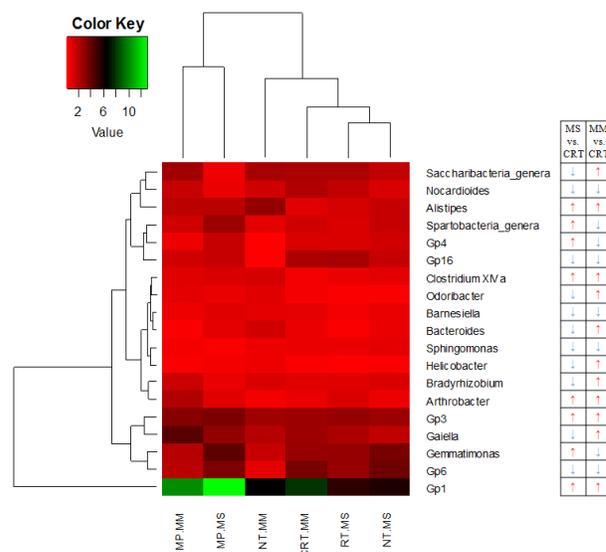


Fig. 5: The main identifiable phylotypes and their responses to different tillage practices. The heat map shows the visualized distribution of the main classes with the total relative abundance > 1.0% for the bacterial communities. The arrows of red and blue indicate separately the increase and decrease of the relative abundance of the MS (soybean straw returned) and MM (corn straw returned) groups relative to the conventional tillage (CRT.MM) group, which is based on the Turkey's HSD test ($P < 0.01$)

As mentioned above, our results indicated that the dynamic of organic matter is closely related to microbial activity. The effect of straw returned on soil microbial composition is greater than that of farming methods. In our study, only the soil microbial community abundance and functional diversity were affected, not community structure under different tillage practices. In no tillage practice, the soil is not physically disturbed, and because of this, there is less aeration than in other tillage practices. However, this feature does not change the structure of soil microbial communities but changes their abundance and metabolic diversity under different tillage methods. This distinction about the phyla of Proteobacteria and Acidobacteria shall be connected closely with the soil disturbance. In the events of the low soil disturbance and no-tillage, the Proteobacteria phylum shall be the most abundant; but the Acidobacteria phylum shall be the most in the MP and CRT, under which the soil is more disturbing, and the straws may also not be returned. The similar observation and explanation can be found in Yendi *et al.* (2013). RT showed different results with NT and MP, because RT not only had reduced soil interference, but also included crop residues and aeration in the soil. The higher soil bacterial community abundance might attribute to the higher soil microbial metabolic activity, which showed that the soybean straw returning farmland soil microbial activity may be a better way of tillage for

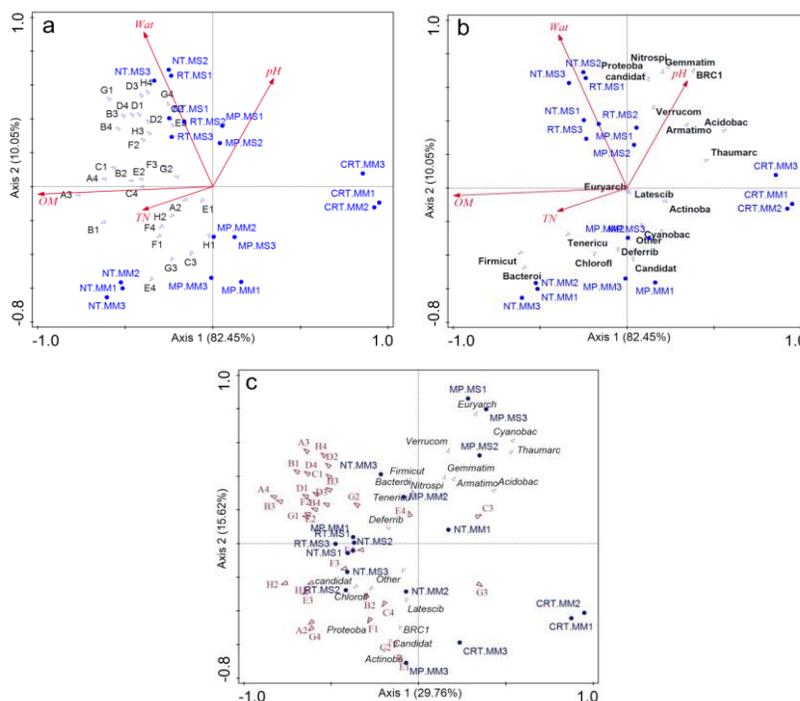


Fig. 6: Canonical correspondence analysis (CCA) of changes of bacterial functions and communities with environmental variables

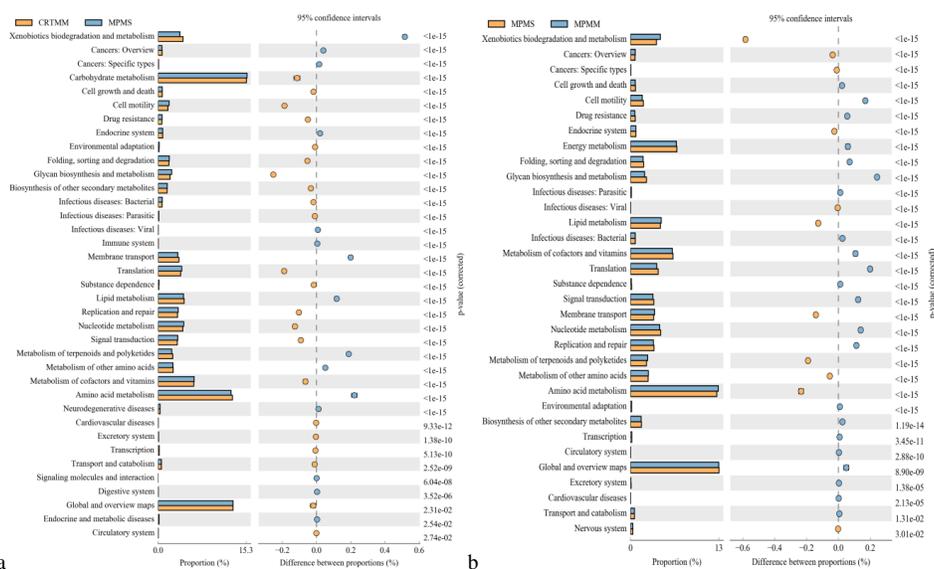


Fig. 7: Functional genes of metagenomes predicted. a: The comparison of functional genes in metagenomes under the practices of conventional tillage and corn straw returning with those of other tillage practices, b: Comparison of functional genes in metagenomes under the practices of corn straw and soybean straw returning with those of other tillage practices. The figure only displays significant differences after data screening. The *P* value of lower than 0.05 indicates significant differences

providing better ecological conditions for soil microbial activity in Northeast China.

PCoA and CCA analyses summarized the analyses of

microbial community structure and metabolic diversity, and RT and NT systems in this study, as well as, the crop rotations clustered with different group of bacteria and the

soil carbon sources, showing that the physical disturbance caused by tillage may be a crucial factor in determining soil species community diversity. The straw returning to the field and cropping system significantly influenced the microbial composition and carbon metabolic functional diversity. Different straw return methods affect the diversity of soil microbial communities and the diversity of metabolic functions by affecting soil moisture and soil aeration conditions. The method of different tillage practices influenced to a more degree. The CRT practice clustered with D-galactosidase, L-hydroxybutyrate, Itaconic acid and 4-hydroxy benzoic acid, suggested their preference for low organism environments. The soybean straw returned to field (NT.MS, RT.MS and MP.MS) clustered with influenced 2-hydroxy benzoic acid, Trehalose, N-acetyl-D-Glucosamine, D-Aminoglucose, Tween 80, suggested their preference for lower C:N organism environments. The continuous cultivation of corn clustered with the metabolism of Glycogen, Xylose and Galactonic acid, suggested their preference for high C:N organism environments.

Together, in this work, the tillage system had a greater impact than soil physicochemical properties on microbial abundance and carbon-metabolic functional diversity. RT practice may create relatively stable environments, which results in more aeration in soil and higher nutrient turnover.

Conclusion

As indicated by our work, firstly the tillage practices and straw returned management have impacts on the bacterial communities and metabolic activity; the soil bacterial community structure is less affected, but the relative abundance of the soil bacterial communities of all taxonomic levels differs, and varies with the tillage practices. Secondly, the type of straw returned under the conservation tillage is a key factor for community composition and metabolic activity of soil bacteria. Compared with corn stalks, the soybean straws returned more organic matter in soil, and encourage the stronger metabolic activity in the microbial community and the activity of FDA hydrolase. Besides, soil organic matter, pH and water also affect the soil microbial community diversity and metabolic activity. The metabolic activity keeps at a low level under the conventional tillage without straw returned. The predictive metagenome analysis shows that the type of straw controls the predicted abundances of KOs assigned to the metabolism of the amino acid, carbohydrate, Glycan, Lipid, Terpenoids and Polyketides, and soybean straws are more beneficial to getting the higher abundances than corn stalks. In summary, the tillage practices did not change the soil microbial community structure component but changed the soil microbial community abundance and metabolic functional diversity. Ridge tillage with soybean straw returned might be a better tillage practice for providing a better condition for soil microbial activity in Northeast China.

Acknowledgements

The authors gratefully acknowledge a financial grant received from the Natural Science Foundation of Jilin province, China (Subject to guide project, No. 20170101004JC), the Natural Science Foundation of Jilin Province, China (No. 20170101077JC), the Jilin province science and technology development plan item (20160307006NY), and National Key Technology R&D Program of China (No. 2016YFD0300807).

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(Received 08 January 2018; Accepted 13 July 2018)