Efectos de los diferentes métodos de manejo de la siembra en la estructura de la comunidad bacteriana

Effects of Different Planting Management Methods on Bacterial Community Structure

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Resumen
Los principales sistemas de cultivo y las prácticas de manejo pueden afectar significativamente el contenido de nutrientes del suelo y la diversidad bacteriana y la abundancia relativa en el suelo. La flora microbiana es un factor importante en el ciclo de los nutrientes, que puede afectar la fertilidad del suelo, la productividad de las plantas y la sostenibilidad ambiental. Este experimento incluyó los efectos de diferentes métodos de labranza del cultivo de soja sobre la fertilidad del suelo y la comunidad bacteriana. Las diferencias de diversidad microbiana y la abundancia relativa de bacterias del suelo bajo diferentes sistemas de manejo de siembra se analizaron mediante el uso de tecnología de secuenciación de amplificación, y se exploraron los factores reguladores que afectan la estructura de la comunidad bacteriana del suelo. Este estudio encontró que la abundancia relativa de Proteobacteria se correlacionó significativamente de manera positiva con el contenido de P y Cu disponible en el suelo. La abundancia relativa de Bacteroidetes está correlacionada con la P, Cu, PH, Mn efectiva en el suelo, Actinobacteriaactinomycetes se correlacionó negativamente con el OM, P y DTPA disponibles, y correlacionada negativamente con P, Cu. Los contenidos de DTPA Cu, Fe y Mn en el suelo están más relacionados con la estructura y diversidad de especies de bacterias en el suelo. En comparación con el sistema de soja, el sistema de barbecho del suelo y la rotación de cultivos pueden equilibrar mejor la fertilidad del suelo y aumentar la estabilidad del micro-ecosistema del suelo, lo cual es de gran importancia para mantener el desarrollo sostenible de la productividad del suelo.

Palabras clave: Germen; Diversidad comunitaria; Sistema de cultivo; fertilidad del suelo.

Abstract
Major cropping systems and management practices can significantly affect soil nutrient content and bacterial diversity and relative abundance in soil. Microbial flora is an important driving factor in nutrient cycling, which may affect soil fertility, plant productivity and environmental sustainability. This experiment included the effects of different tillage methods of soybean cultivation on soil fertility and bacterial community. The differences of microbial diversity and relative abundance of soil bacteria under different planting management systems were analyzed by using amplification sequencing technology, and the regulatory factors affecting soil bacterial community structure were explored. This study found that the relative abundance of Proteobacteria was significantly positively correlated with the content of available P and Cu in the soil. The relative abundance of Bacteroidetes is correlated with effective P, Cu, PH, Mn in soil, Actinobacteriaactinomycetes was negatively correlated with available OM, P and DTPA Mn, Acidobacteria was positively correlated with OM and negatively correlated with P, Cu. The contents of DTPA Cu, Fe and Mn in soil are most related to the structure and species diversity of bacteria in soil. Compared with soybean system, the system of soil fallow and crop rotation can better balance soil fertility and increase the stability of soil micro-ecosystem, which is of great significance for maintaining the sustainable development of soil productivity.

Key words: Germ; Community diversity; Cropping system; Soil fertility

1. Introduction

Microbial communities in soil promote important nutrient cycling processes, which may affect soil quality, planting productivity and environmental sustainability. Soil microorganisms regulate soil fertility and are vulnerable to management practices (Zhang, 2016). Agricultural management affects chemical, physical and biological processes in soil, and causes many changes in soil community composition (Fierer et al., 2012). Abnormal accumulation or excessive consumption of soil nutrients can lead to rapid propagation of pathogenic
microorganisms, chemical accumulation, imbalance of soil microbial population structure, and decline of crop yield and quality (Xing et al., 2011). Crop diversification and crop rotation promote the rational use of soil nutrients, the maintenance of soil fertility, the improvement of soil physical, chemical and biological characteristics, and the richness and uniformity of microorganisms (Tian et al., Wang et al. 2015). Wang et al. found that traditional agriculture reduced the biodiversity and biomass of soil microorganisms. Reduced tillage is a sustainable plant management system that increases surface microbial activity and biomass, and protects soil, water and air quality and biodiversity. However, crop rotation can also change soil quality and fertility factors, thus affecting microbial community composition (Benitez et al., 2017).

Microbial diversity in rotation soils was higher (Fierer et al., 2012). The crop rotation system of legumes or fibers can significantly affect microbial composition. In plant systems containing soybeans, appropriate crop management techniques are necessary for sustainable production (Fierer et al., 2009). The microbial diversity of bacteria in soil varies greatly in different planting systems, which affects the change of soil biodiversity and the richness of individual species. Proteus, Acidobacterium and actinomycetes are the main phylogenetic units of soybean continuous cropping (SC), Soybean-Maize and maize-soybean rotation systems in all soil samples. With the accumulation of soil organic matter (OM) and nutrients, the relative abundance of actinomycetes decreased, while the relative abundance of Proteus increased (Zeng et al., 2017). Lu et al. (2011) found that increasing leguminous crop rotation could release nutrients faster, resulting in higher N and K content and lower P content. In previous studies, microbial communities were significantly correlated with OM and available N, and the addition of external carbon (C) and N to the system significantly affected microbial composition (Fierer et al., 2012). However, there are few studies on the interaction between different cropping systems and soil fertility and microbial communities, especially the effects of mineral elements on soil microbial communities. Therefore, biological control and potential biological control of crops are of great significance in reducing fertilizer use (Cai et al., 2015).

Microbial communities are affected by physical and chemical properties of soil (Xun et al., 2015; Cai et al., 2017) and species or even genotypes of host plants (Ofek et al., 2014). Heilongjiang Province is the main soybean production area in northern China. Therefore, in this study, the effects of cropping system on microbial community composition and diversity were analyzed, and the relationship between microbial community and soil fertility was studied. The objective is to determine how cropping systems drive changes in soil bacterial microbial composition and to study microbial nutritional preferences. The results will lay a foundation for regulating soil bacterial and microbial community structure, guiding planting system and protecting soil ecology.

2. Materials and methods

2.1 Location description

Samples were taken from Heihe City, Heilongjiang Province, northern China. The experimental site is a continental monsoon climate in the cold temperate zone. Its geographic coordinates are 124°65'50" The average annual temperature is 0.8-1.4 degree C, the average rainfall is 480-512 mm, the winter is cold, long and dry, the lowest temperature is -47.3-43.7 degrees C, and the highest temperature is 33.9-37.4 degrees C in the same season in summer. It's China's major planting areas, and the soil type is black calcareous soil.

2.2 Experimental design

The experiment is a randomized block design of five patterns of soil planting system: uncultivated plot (CK), soybean continuous cropping (SC), fallow-soybean cropping (FS), corn-soybean rotation (CS), wheat-soybean rotation (WS), three repetitions, in a field of 15 plots, each plot (666.7m2). Soil tillage depth is about 20 centimeters. Continuous cropping of soybean is a common way of cultivation in the region. Every year, diammonium phosphate 23.3 kg/mu, urea 13.3 kg/mu, potassium phosphate 13.3 kg/mu. Planting lasted for ten years. Soil samples were collected after autumn harvest of soybean crops in 2016.

2.3 Soil sampling

Soil samples were taken from 7 sampling points in each plot. The depth of soil samples ranges from 0 to 20 cm. Roots, stones and animals are taken by hand and divided into two parts. Some of them are air-dried, and soil fertility is analyzed through a 2mm screen. Other parts are stored at 80 C for DNA extraction.

2.4 Chemical analysis

Soil samples were ground after air-drying at room temperature and screened (0.2 mm). Effective states of trace elements such as Cu, Fe, Mn and Zn were extracted with 0.005mol/L DTPA (diethylenetriamine acetic acid), then determined by atomic absorption spectrophotometer and effective state B by potassium imide colorimetry. Soil pH was determined by soil-water ratio of 2.5:1. Soil organic matter (SOM) was determined by potassium dichromate volumetric method, alkali-hydrolyzed nitrogen by diffusion method, available phosphorus

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by 0.5 mol L⁻¹ NaHCO₃ extraction-molybdenum-antimony colorimetric method, and available potassium by 1 mol L⁻¹ NH₄OAc extraction-flame photometry.

2.5 Amplicon Sequencing analysis technique

Bacterial 16S rDNA amplifier sequencing analysis was carried out in Shanghai Tianhao Biotechnology Co., Ltd. The target region of the genome was amplified by PCR. The target region was amplified and enriched by high-throughput sequencing. Then the sequence was compared with a specific database to confirm the species, and then the bioinformatics analysis was carried out. MiSeq platform was used to sequence the library with a 2 x 250 BP double-ended sequencing strategy, followed by bioinformatics analysis. Then, the effective sequence is optimized, and the operational taxonomic units (OTUs) are formed by clustering analysis. The UPARSE clustering method is used to merge the quality-controlled sequences into an OTU cell with 97% similarity among sequences for further OTU classification annotations. The bacterial species (genera) and microbial colony structure represented by the sequences are determined by referring to RDP database. Analyses by Biotechnology Co., Ltd.

2.6 statistical analysis

R (Tian et al., 2015) was used for regression analysis of the relationship between microorganisms and soil physical and chemical properties. SPSS 17.0 was used for ANOVA and Duncan multirange tests to test the significant level of difference between treatments (p < 0.05).

3. Result

3.1 Microbial community structure

The results of bacterial PCoA showed that there were distinct biological communities in the soil of five systems, which were divided into two groups, CKFS group and the rest group. The bacterial colonies of WS and CS were very similar (Fig. 1). CK, FS bacterial community structure is similar. The colonial structure of SC bacteria is far away.

Table 1 Diversity of soil bacterial communities

<table>
<thead>
<tr>
<th>Soil samples</th>
<th>CK</th>
<th>FS</th>
<th>CS</th>
<th>WS</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteri</td>
<td>Chao1 4798±35b</td>
<td>5541±205a</td>
<td>4740±134b</td>
<td>4592±49b</td>
<td>4504±127b</td>
</tr>
<tr>
<td></td>
<td>ACE 4803±27b</td>
<td>5553±156a</td>
<td>4659±179b</td>
<td>4563±35b</td>
<td>4509±106b</td>
</tr>
<tr>
<td></td>
<td>Shannon 6.47±0.02b</td>
<td>6.76±0.08a</td>
<td>6.54±0.08b</td>
<td>6.57±0.03b</td>
<td>6.45±0.06b</td>
</tr>
</tbody>
</table>

Note: CK: uncultivated plot, SC: soybean continuous cropping, FS: lie fallow soybean planting, CS: corn-soybean rotation, WS: wheat-soybean rotation. The same letters mean no significant difference, the different letters mean significant difference, P < 0.05.

The richness of bacterial flora (Chao1/ACE) and the diversity of bacterial colonies (Shannon) were the best in the soil of microbial FS system (p < 0.05), SC soil had the lowest bacterial flora abundance and diversity. The relative abundance of bacteria in CK was the second highest. The relative abundance and diversity of soil bacteria in CS and WS systems were at medium level (Table 1).
Table 2 Percentage of soil bacteria

<table>
<thead>
<tr>
<th></th>
<th>Bacteria</th>
<th>Archaea</th>
<th>No_Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>92.83 ± 0.06b</td>
<td>0.18 ± 0.03ab</td>
<td>6.99 ± 0.04a</td>
</tr>
<tr>
<td>FS</td>
<td>92.96 ± 0.01b</td>
<td>0.14 ± 0.05bc</td>
<td>6.90 ± 0.06a</td>
</tr>
<tr>
<td>CS</td>
<td>94.09 ± 0.17a</td>
<td>0.19 ± 0.05ab</td>
<td>5.72 ± 0.12b</td>
</tr>
<tr>
<td>WS</td>
<td>94.11 ± 0.21a</td>
<td>0.27 ± 0.03a</td>
<td>5.62 ± 0.19b</td>
</tr>
<tr>
<td>SC</td>
<td>94.14 ± 0.31a</td>
<td>0.04 ± 0.01c</td>
<td>5.82 ± 0.32b</td>
</tr>
</tbody>
</table>

Note: CK: uncultivated plot, SC: soybean continuous cropping, FS: lie fallow soybean cultivation, CS: maize soybean rotation, WS: wheat-soybean rotation. The same letters mean no significant difference, the different letters mean significant difference, P < 0.05.

The relative abundance of No_Rank is the highest in CK system. WS treatment was beneficial to the formation of archaea, and the relative abundance of SC Archaea was significantly lowest (Table 2). Planting system significantly affected the structure and diversity of soil microbial colonies.

![Figure 2: Bacterial species diversity. CK: Untilled plots, SC: soybean continuous cropping, FS: lie fallow soybean cultivation, CS: corn soybean rotation, WS: wheat-soybean rotation. Overlapping areas represent common species. Numbers represent the number of species.](image)

There were 747 unique bacterial species in CK treatment and 535 unique bacterial species in FS treatment, which were much higher than other treatments. The number of bacterial species in soybean continuous cropping soil was 268. CK and FS promoted the formation of soil microbial diversity.

![Fig. 3 Community structure at bacteriophyte level. Note: CK: uncultivated plot, SC: soybean continuous cropping, FS: lie fallow soybean cultivation, CS: corn soybean rotation, WS: wheat-soybean rotation. Different colors represent different phylum classification.](image)

Black soil bacteria are mainly distributed in Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria. Proteobacteria is the most abundant of all treatments, and its relative abundance is over 23%. Proteobacteria Bacteroidetes and Actinobacteria relative abundance were added to SC system. CK adds Planctomycetes gates. WS adds relative abundance of Gemmatimonadetes, candidate_division_WPS-1 and Armaitimonadetes. Bacteroidetes is the lowest in WS. Acidobacteria is higher in CK and WS, and lower in SC. Verrucomicrobia increased significantly in CK, FS, and Chloroflexi bacteria were abundant in CK, FS and WS. The relative abundance of Chloroflexi, Verrucomicrobia, Nitrospirae and Actinobacteria in FS system soils were significantly higher than those in other soils.
3.2 Fertilidad del suelo y su correlación

Under five tillage patterns, the soil samples collected had distinct fertility characteristics (p < 0.05). SC can accumulate soil available nutrients OM, N, K, Zn, Fe, Mn, and cause excessive consumption of P. SC can aggravate the accumulation of soil nutrients. FS, the tillage method reduces the content of Cu, Mn, P, and also causes the loss of available phosphorus. WS can increase soil available P, Cu content, but consume more OM, available N, Fe content, CS consume more available K, Zn content in soil. CK increases the content of OM and K, Mn, and decreases the content of Cu, Zn and Fe. Different tillage patterns result in different soil fertility due to cropping systems and management methods (Table 3).

Table 3 Soil Fertility

<table>
<thead>
<tr>
<th>Soil samples</th>
<th>CK</th>
<th>FS</th>
<th>CS</th>
<th>WS</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter (g/kg)</td>
<td>7.42±0.27a</td>
<td>6.05±0.84ab</td>
<td>5.41±0.14bc</td>
<td>4.60±0.19c</td>
<td>7.40±0.36a</td>
</tr>
<tr>
<td>Available N (ppm)</td>
<td>318±11.86d</td>
<td>364±2.08e</td>
<td>311±7.54e</td>
<td>248±8.39e</td>
<td>387±37.54a</td>
</tr>
<tr>
<td>Available P(ppm)</td>
<td>29.33±0.88c</td>
<td>12.33±0.88e</td>
<td>38.33±1.20b</td>
<td>47.33±0.88a</td>
<td>25.00±0.58d</td>
</tr>
<tr>
<td>Available K (ppm)</td>
<td>313±13.22a</td>
<td>228±0.58b</td>
<td>152±11.32e</td>
<td>231±2.52b</td>
<td>323±1.76a</td>
</tr>
<tr>
<td>pH</td>
<td>6.29±0.04a</td>
<td>6.13±0.05b</td>
<td>6.25±0.01a</td>
<td>6.19±0.04a</td>
<td>6.24±0.01ab</td>
</tr>
<tr>
<td>Available Cu (ppm)</td>
<td>0.84±0.04a</td>
<td>0.65±0.02d</td>
<td>1.33±0.04b</td>
<td>1.84±0.04a</td>
<td>1.29±0.01b</td>
</tr>
<tr>
<td>Available Zn (ppm)</td>
<td>0.19±0.01c</td>
<td>0.46±0.09a</td>
<td>0.14±0.04c</td>
<td>0.50±0.03b</td>
<td>0.69±0.02a</td>
</tr>
<tr>
<td>Available Fe (ppm)</td>
<td>136.86±0.55a</td>
<td>244±4.02b</td>
<td>208±9.10c</td>
<td>196±3.47e</td>
<td>304±6.94a</td>
</tr>
<tr>
<td>Available Mn(ppm)</td>
<td>35.09±0.21a</td>
<td>10.91±0.23e</td>
<td>13.95±0.72b</td>
<td>16.48±0.54c</td>
<td>32.54±0.19b</td>
</tr>
<tr>
<td>Available B(ppm)</td>
<td>0.37±0.03ab</td>
<td>0.30±0.00c</td>
<td>1.02±0.64a</td>
<td>0.96±0.11e</td>
<td>0.70±0.27a</td>
</tr>
</tbody>
</table>

Note: CK: uncultivated plots, SC: soybean continuous cropping, FS:lie fallow soybean cultivation, CS: maize soybean rotation, WS: wheat-soybean rotation. The same letters in each row represent no significant difference, while different letters represent significant difference, P < 0.05.

Table 4 The correlation between bacterial community and soil fertility

<table>
<thead>
<tr>
<th>Environmental factor combination</th>
<th>Size correlation bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>0.78</td>
</tr>
<tr>
<td>Cu</td>
<td>0.81</td>
</tr>
<tr>
<td>Cu Fe Mn</td>
<td>0.85</td>
</tr>
<tr>
<td>K Cu Fe Mn</td>
<td>0.84</td>
</tr>
<tr>
<td>K OM Cu Fe Mn</td>
<td>0.84</td>
</tr>
<tr>
<td>N K OM Cu Fe Mn</td>
<td>0.80</td>
</tr>
<tr>
<td>N K OM pH Cu Fe Mn</td>
<td>0.79</td>
</tr>
<tr>
<td>N K OM pH Cu Zn Fe Mn</td>
<td>0.76</td>
</tr>
<tr>
<td>N P K OM pH Cu Zn Fe Mn</td>
<td>0.72</td>
</tr>
<tr>
<td>N P K OM pH Cu Zn Fe Mn B</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Soil microbial community has a good correlation with soil environment (e.g. Table 4). The correlation between bacterial community and 10 soil fertility factors was analyzed. The correlation coefficient between bacterial microbial community and available Cu-Fe-Mn content in soil was 0.85, followed by K-Cu-Fe-Mn and K-OM-Cu-Fe-Mn with correlation coefficient of 0.84.

4. Summary and discussion

In this study, OM and available N contents in SC soil were the highest, which was consistent with previous results (Lu et al. 2011). In addition, the study also showed that available K, DTPA zinc, Fe, Mn, Zn, Fe in SC soil were significantly higher, which was consistent with previous single cropping results (Cai, 2017). Under FS regime, available P, DTPA, Cu and Mn contents decreased significantly. On the contrary, available P and DTPA Cu contents increased significantly in WS soil, but available OM, N and DTPA Fe contents decreased significantly (Song, 2018). The available K and DTPA zinc contents in soil under CS planting system were significantly lower than those in other planting systems. It was found that CK increased the contents of OM and K, Mn and decreased the contents of Cu, Zn and Fe. Therefore, different tillage systems produce different physical and chemical properties of soil, which is consistent with previous research results (Xun et al., 2015), so the tillage system can adjust the physical and chemical properties of soil.

The abundance and diversity of soil bacteria determine the stability of soil microbial communities and their ability to resist pathogens, which are essential for the function and sustainable development of soil ecosystems.
(Pang et al., 2017). In this study, CK and FS systems increased bacterial microbial community abundance and species diversity, which confirmed that reducing agricultural cultivation could increase soil microbial diversity (Wang et al., 2011). The diversity of soil bacteria in WC and CS planting systems is greater than that in SC planting systems, which is consistent with previous studies (Ai et al., 2015).

Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria are the main bacteria in soil. The results of this study confirm these conclusions. Proteobacteria is the most abundant of all treatments, accounting for more than 23% of the total (Fig. 2A). Proteobacteria associated with symbiotic Rhizobium is more abundant in SC soil. Proteobacteria and Bacteroidetes can inhibit the occurrence of disease (Ai et al., 2015). With the increase of continuous cropping years, the relative abundance of Proteobacteria bacteria increased (Zeng et al., 2017; Freedman and Zak, 2015). Acidobacteria is obviously less in SC, but more abundant in WS. Acidobacteria has the ability to decompose complex soil and plant polysaccharides (Ward et al., 2009). The content of Bacteroidetes in FS was significantly higher. Actinobacteria were significantly higher in SC, but significantly lower in FS. With the accumulation of soil organic matter (OM) and nutrients, the relative abundance of actinomycetes decreased (Zeng et al., 2017). Janssen (2006) found that the content of Gemmatimonadetes in soil bacterial communities was 2% (Janssen, 2006). In this study, Gemmatimonadetes were significantly more abundant in WS. We also found that the number of Verrucomicrobia increased significantly in CK and FS. Bergman (2011) studied undisturbed soils and found that 23% of the bacterial sequences belonged to Verrucomicrobia microorganisms (Bergman et al., 2011). In this study, Chloroflexibacteria were abundant in CK, FS and WS, but significantly low in SC. These microorganisms can undergo anaerobic photosynthesis and extract electrons from hydrogen sulfide (Eisen et al., 2002).

Increased OM and N in soil can promote bacterial diversity (Zeng et al., 2017; Li et al., 2016). Increased OM and N in soil can promote bacterial diversity (Zeng et al., 2017; Li et al., 2016). In this experiment, the contents of OM and N in CK, SC and FS systems were significantly increased, but the bacterial abundance and diversity were significantly different, which may be another kind of energy, that is, bacterial diversity was more related to other environmental factors in soil. These results were inconsistent with Cai et al. (2017) and pang et al. (2017), this is due to differences in soil type, sample size and related indicators of the study. Soil and environmental factors affect community structure (Pang et al., 2017; Xun et al., 2015). In this study, we found that the available contents of DTPA Cu, Fe and Mn are closely related to microbial communities. In previous studies, bacterial communities were most correlated with P, DTPA Mn and P, DTPA Cu. Mn (Song, 2018). Cultivation system can improve soil fertility and affect bacterial community structure (Ofek et al., 2014). Different farming systems form different soil environments. FS increased bacterial diversity and relative abundance. Bacterial diversity was most beneficial to soil productivity and microbial community stability (Yuan et al., 2015).

5. Conclusion

Planting system affects microbial community structure. FS planting system can increase the number and diversity of bacteria. CK can increase the number of unique bacterial strains. SC planting system can reduce the number and diversity of bacteria and form its own unique community structure. The correlation between microbial community structure and available DTPA Cu, Fe and Mn in soil. Farming systems change the nature of the soil and the diversity of microorganisms. Planting system, microbial community structure and soil fertility are coordinated and unified. The results of this study will provide data support for the establishment of a good soil bacterial microbial community structure, the regulation of soil fertility and the guidance of planting patterns, and will be of great significance for the establishment of a sustainable agricultural microbial ecological environment.

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References


