Comparation of Bacterial and Archaea Diversity in Uncultivated and Cultivated Typical Black Soil in Northeast China

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Abstract

Understanding the effects of tillage on the community composition and diversity of bacteria and archaea is useful for the long-term sustainable utilization of black soil. In this study, non-disturbed virgin black soil (NDBS) in the past 60 years, and the adjacent black soil under the arable land farming (DFBS) and paddy rice (PBS) for 3 years were chosen to investigate the microbial characteristics and their relationship with physical and chemical properties. The microbial diversity was investigated using 16S rRNA genes high-throughput sequencing technique and its relationship with soil physico-chemical properties was analyzed by redundancy analysis. The diversity of archaea decreased significantly after arable land farming. After conversion from arable land farming to rice paddy, the diversity of bacteria increased significantly, while the diversity of archaea increased significantly. In DFBS, the available phosphorus increased significantly, yet pH, organic matter and available potassium decreased significantly. However, the pH and available phosphorus increased significantly, organic matter and total nitrogen decreased significantly in PBS. The soil pH had the closest correlation with the microbial diversity. These indicated that tillage disturbance changed the diversity of bacteria and archaea as well as physical and chemical properties in black soil.

Introduction

Microbial community is an important part of the soil ecosystem and it plays a key role in the biogeochemical processes such as the formation and transformation of soil organic matter, cycling of nutrients (Copley 2000; Wu et al. 2020). Approximately 80-90% of the transformation processes in soil are mediated by microorganisms, including soil structure maintenance, organic matter dynamics, dinitrogen fixation (Sengupta and Dick 2015; Gu and McGill 2017; Cai et al. 2020). According to the previous studies, soil properties, including pH, nutrient availability, organic matter, moisture contents and oxygen all affect microbial community structure and composition (Wakelin et al. 2008). However, more than 99% of microorganisms in soil are not known and new biochemical functions are still to be discovered. Currently, molecular ecological analysis methods, culture independent ones, are effective to reveal the diversity of soil microbial community and function. Earlier studies reported that tillage led to the change of soil microbial community structure, especially the ecological function related to the biogeochemical cycle of soil nutrients (Zhu et al. 2016) to enhance soil fertility status and increase crop yield.

Soil ecosystems are influenced by intensive anthropogenic activities and disturbance. Therefore, understanding the effect of physical disturbances is essential to gain knowledge on microbial community and function so that justified action can be taken to protect soil for long-term sustainable agriculture. It has been reported that disturbance is defined somewhat ambiguously in microbial ecology (Plante 2017). Tillage is a common physical disturbance in agricultural practices in arable land farming (Zhang et al. 2018) and paddy rice (Suzuki et al. 2019) to enhance aeration and release of nutrients for crops. Tillage changes soil physical properties significantly (Dick 1992) and affects soil microbial community composition and diversity (Lienhard et al. 2013). The microbial community richness and diversity are indicators of soil quality (Mathew et al. 2012), but tillage effects on soil microorganisms are poorly understood (Wang et al. 2016). Meanwhile, the productivity of black soil has been severely deteriorated...
after a long-term tillage during cultivation (Liu et al. 2005). It is necessary to investigate the soil microbial community and its responses to tillage.

The objectives of this study were to investigate the effect of tillage on the diversity of both bacteria and archaea in the black soil of Northeast China. Combining with soil physical and chemical properties, the relationship between the changes of soil microbial diversity and environmental factors was delineated upon tillage operation. This provides a meaningful scientific evidence for utilization and conservation of black soil in Northeast China.

**Materials And Methods**

2.1 Site description

The sampling site is located in Keshan Farm, Heilongjiang Province, P.R. China (125°07'40"-125°37'30" E, 48°11'15"-48°24'07" N). The terrain is hilly and belongs to the warm and cold climate area. The annual average temperature is 1.3°C, and the annual precipitation is 502.5 mm. The soil is black soil (Chernozems). Three sampling sites were located as adjacent plots. The first sampling site was a non-disturbed virgin black soil (NDBS) as a control treatment, not cultivated for the past 60 years. The second site is arable black soil (DFBS) where wheat-soybean rotation has been conducted for three years. And the third one is rice paddy (PBS) converted from arable land farming to paddy 3 years ago before this sampling.

2.2 Soil sample collection

The 500 g of the NDBS, DFBS and PBS were taken to a depth of 0~20 cm using a five-point sampling method using soil Auger sampler. The samples were stored in the sealed plastic bag at 4°C and then transported back to the laboratory. Air dried and passed through a 2 mm mesh screen before use for the determination of the physical and chemical properties of the soil.

2.3 Measurements of physicochemical properties of the soil

Soil pH values were determined by mixing soil in 0.01 mol L⁻¹ CaCl₂ solution and shaking for 30 min (1:2.5, weight: volume), then measured using a pH meter (HORIBA, Japan) (Hu et al. 2018). The soluble salt (SS) contents in soil were measured by the residue drying method. Soil total nitrogen (TN) (Jones and Willett 2006) and organic matter were measured with an Elemental analyzer (Vario EL III, Elementar, Heraeus, Germany). The available phosphorus (AP) was determined by the NaHCO₃ extraction methods (Olsen et al. 1954). The available potassium (QP) was extracted by acetic acid and ammonium leaching method (Mehlich 1984), and then the extracted QP were quantified using inductively coupled plasma-atomic emission spectrometry (ICPS-7500, Shimadze, Japan).

2.4 Extraction of total DNA from soil and analysis of soil microorganism diversity
The total genomic DNA was extracted by the modified chlorobenzene method (Zhu et al. 1993). The DNA integrity was checked by the electrophoresis with 1% agarose gel. The electrophoresis voltage is 120 V for 15 min, and the concentration is determined by Nanodrop 2000c. Bacteria 16S V4 region primers: 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCCGYCAATTCTMTTTRAGT-3'). Archaea primers: 344F (5'-ACGGGGYGCAGCAGGCGGA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR reaction was conducted for bacteria and archaea respectively. PCR amplification was performed (Initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min) and the product was purified and quantified. Conduct two PCR reactions for each sample, and combine them together after PCR amplification. The total DNA of the submitted qualified samples is sent to China Sichuan Bobet Biotechnology Co., Ltd for high throughput sequencing. The PCR products were detected by electrophoresis using 1% agarose gel; the same amount of samples were mixed according to the PCR product concentration, and the PCR products were detected by 1% agarose gel electrophoresis after thorough mixing. For the target band, use Shenggong Company The gel recovery kit provided (Sangon Biotech, China, Cat# SK8132) recovers the product, and uses Nanodrop to determine the concentration and quality. Use TruSeq® DNA PCR-Free Sample Preparation Kit for library construction. The constructed library is quantified by Qubit and qPCR. After the library is qualified, use the v2 sequencing kit (2×250 bp) and Miseq sequencer to run the library and Sequencing.

2.5 Sequencing data processing

Use FLASh (V1.2.7, http://ccb.jhu.edu/software/FLASH/) to splice the reads of each sample, and the resulting spliced sequence is the original Tags data (Raw Tags). Raw Tags that are spliced need to undergo strict filtering to obtain high-quality tags data (Clean Tags). Refer to the tags quality control process of Qiime (V1.9.0, http://qiime.org/scripts/split_libraries_fastq.html). Truncate Raw Tags from the first low-quality base site where the number of consecutive low-quality values (the default quality threshold is <=3) has reached the set length (the default length is 3). The tags data set obtained after tags is intercepted, and the tags whose continuous high-quality base length is less than 75% of the tag length are further filtered out. Use Usearch software (v8.0, http://drive5.com/uparse/) to detect the chimera sequence, and get the final effective data after removal (Effective Tags).

2.6 OTU clustering and species annotation

Use Qiime software to cluster all Effective Tags of all samples (cd-hit method), cluster the sequences into OTU (Operational Taxonomic Units) with 97% similarity by default, and select representative sequences of OTU. Remove Singleton in OTU. Species annotations for OTU representative sequences, and RDP Classifier software (Version 2.2, http://sourceforge.net/projects/rdp-classifier/) for species annotation analysis (threshold: 0.8). The data of each sample in the OTU table is normalized, and the sample with the least amount of data is used as the standard. The subsequent Alpha diversity analysis and Beta diversity analysis are based on the data after the normalization.

2.7 Statistical analysis
The microbial community diversity and dominant microbe were analyzed and compared using the results of sequencing results. The dominant microbe relationship with soil physic-chemical properties was analyzed by redundancy analysis (RDA). All the experiments were carried out triplicates and One-way analyses of variance (ANOVA) was performed using the OriginPro 2017 software (OriginLab USA) in order to evaluate the statistical significance.

**Results**

3.1 Tillage on physical and chemical properties

The physical and chemical properties of the black soil are the primary factors affecting soil microbial activity and diversity. The contents of soluble salts (SS), pH value, organic matter (OM), total nitrogen (TN), available phosphorus (AP) and available potassium (QP) in DFBS and PBS were monitored. No significant effect of tillage disturbance on the SS in soil was observed over the experimental. Compared with NDBS, the OM decreased significantly and AP increased significantly for both DFBS and PBS ($p < 0.05$). The pH value decreased significantly in DFBS ($p < 0.05$), while increased significantly in PBS ($p < 0.05$). In contrast, tillage led to a significant decrease of the TN in PBS ($p < 0.05$), but not in DFBS. On the contrary, tillage caused the QP significantly decreased in DFBS ($p < 0.05$), but not in PBS.

| Sample | SS/g·kg$^{-1}$ | pH value | OM/g·kg$^{-1}$ | TN/g·kg$^{-1}$ | AP/mg·kg$^{-1}$ | QP/mg·kg$^{-1}$ |
|--------|----------------|-----------|----------------|----------------|----------------|----------------|
| NDBS   | 2.6±1.5 a      | 6.40±0.00 b | 45.43±0.91 a  | 2.36±0.31 a    | 5.37±1.24 c    | 233.67±18.56 a |
| DFBS   | 1.8±0.6 a      | 5.93±0.12 c | 38.67±2.55 b  | 2.36±0.42 a    | 29.50±5.47 b   | 203.33±4.16 b  |
| PBS    | 2.3±0.8 a      | 7.40±0.10 a | 34.17±1.24 c  | 1.64±0.16 b    | 45.40±2.52 a   | 229.67±15.50ab |

a. The same lowercase letters in the table represent no significant difference between different samples at the $P = 0.05$ level.

b. SS: soluble salt, OM: organic matter, TN: total nitrogen, AP: available phosphorus, QP: available potassium.

3.2 Tillage on the diversity of bacteria and archaea

The Chao1 index is an index reflecting the richness of species. The larger the index, the higher the richness of the microbial community. The Shannon index is an indicator reflecting the diversity of the
microbial community, and the higher the index, the higher the diversity of the microbial community. The Chao1 index and Shannon index of bacteria at DFBS were lower than NDBS. The Chao1 index of bacteria at PBS was lower than NDBS, the Shannon index was higher than the NDBS (Table 2). The Chao1 index and Shannon index of archaea disturbed by DFBS were lower than the NDBS, the Chao1 index and Shannon index of archaea at PBS were higher than the NDBS (Table 3). The diversity of archaea decreased significantly after arable land farming \((p < 0.05)\). After conversion from arable land farming to rice paddy, the diversity of bacteria increased significantly, while the diversity and richness of archaea increased significantly \((p < 0.05)\).

Table 2 Alpha diversity index of bacteria

| Samples | Chao1         | Shannon        |
|---------|---------------|----------------|
| NDBS    | 3742.45±190.00 a | 9.61±0.14 ab   |
| DFBS    | 3097.32±113.83 a | 9.42±0.30 b    |
| PBS     | 3453.89±386.54 a | 9.94±0.26 a    |

Table 3 Alpha diversity index of archaea

| Samples | Chao1         | Shannon        |
|---------|---------------|----------------|
| NDBS    | 1209.10±362.00 b | 4.51±2.17 a   |
| DFBS    | 961.14±259.39 b  | 3.91±0.76 b    |
| PBS     | 1855.66±199.43 a | 6.68±0.54 a    |

Based on the data of high-throughput sequencing, the effect of tillage on the bacterial and archaea community were further analyzed with respect to the relative abundance at the phyla and genus levels.

The dominant bacteria phyla included Proteobacteria, Chloroflexi, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Planctomycetes, Actinobacteria, Nitrospirae, Firmicutes and Armatimonadetes accounted for > 87% of the total bacteria species in all sample types, among which half of the phyla were sensitive to the tillage disturbance (Fig. 1). At the phylum levels, the diversity of dominant bacteria was decreased significantly in DFBS \((p < 0.05)\), but not affected in PBS. After soil was disturbed, the relative abundance of Gemmatimonadetes was increased significantly in DFBS \((p < 0.05)\), and the relative abundance of Chloroflexi was increased significantly in PBS \((p < 0.05)\). Compared with NDBS, the relative abundance of
Proteobacteria and Armatimonadetes exhibited significantly increased in DFBS ($p < 0.05$), but Actinobacteria and Nitrospirae significantly decreased in both soil samples ($p < 0.05$).

Similarly, the relative abundance of most bacteria at the genus levels was also dramatically affected by tillage disturbance ($p < 0.05$). At the genus level, *Bacillus, Flavobacterium, Anaerolinea, Bradyrhizobium, Variovorax, Flavisolibacter, Sporosarcina, Rhodoplanes, Kaistobacter* and *Methylibium* were the dominant genera (Fig. 2). The bacterial genus level was analyzed and found the diversity of the dominant ones were increased dramatically in both soil samples after tillage disturbance comparing with the non-disturbed virgin soil ($p < 0.05$). The relative abundance of *Kaistobacter, Flavobacterium* and *Rhodoplanes* were increased significantly at DFBS ($p < 0.05$), yet *Bacillus* and *Sporosarcina* were decreased significantly ($p < 0.05$). Unlike in DFBS, *Anaerolinea, Variovorax* and *Methylibium* abundance were increased significantly in PBS ($p < 0.05$), yet the *Bacillus* was decreased significantly ($p < 0.05$).

The dominant archaea phyla included Crenarchaeota, Euryarchaeota and Parvarchaeota, the sum of the relative abundance of three archaea phyla were more than 74% of the total in all sample types (Fig. 3), among which most phyla were sensitive to the tillage disturbance. The diversity of dominant archaea was increased significantly after tillage at the phylum level ($p < 0.05$). The relative abundance of Euryarchaeota and Parvarchaeota were increased significantly in PBS ($p < 0.05$).

There were four dominant archaea genera, namely *Methanobacterium, Methanosaeta, Candidatus Nitrososphaera* and *Candidatus Methanoregula* (Fig. 4). In PBS, the number of dominant archaea genera increased from 1 to 3, and the affected was considerably ($p < 0.05$), but there was no affected in DFBS. The relative abundance of *Methanobacterium, Methanosaeta* and *Candidatus Methanoregula* were considerably increased in PBS ($p < 0.05$), yet the relative abundance of *Candidatus Nitrososphaera* decreased considerably ($p < 0.05$).

### 3.3 The relationship between bacterial and archaea community and soil properties

The environmental factors with the most considerable correlation with *Variovorax, Bacillus* and *Sporosarcina* were AP, OM and SS respectively, and the relationship was positive (The smaller the angle between the arrows, the larger the correlation) (Fig. 5). However, the changes of *Flavisolibacter* and *Kaistobacter* were most closely related to QP, *Rhodoplanes* was negatively correlated with pH, TN had the most correlation with *Anaerolinea* and *Methylibium*, and the relationship was negative. Ranked the influence of environmental factors on bacterial community structure: pH> AP> OM> TN> QP> SS.

*Methanobacterium* was positively correlated with AP, and *Methanosaeta* was negatively correlated with TN (Fig. 6). In addition, *Candidatus Methanoregula* was positively correlated with pH. On the contrary, *Nitrososphaera* was negatively correlated with pH. Ranked the influence of environmental factors on bacterial community structure: pH> TN> OM> AP> QP> SS.

**Discussion**
Most of the soil physical and chemical properties were significantly affected by tillage disturbance. Previous studies have shown that the reason for the significant decrease in OM is that tillage improves soil aeration and break down soil aggregates to enhance microbial activity for decomposition (He et al. 2019), thus OM degradation was accelerated. Moreover, flooding creates anoxic conditions and enhances Ca\textsubscript{2}-P phosphate and Ca\textsubscript{8}-P phosphate in soil, both of which are essential components of soil AP pool. Meanwhile, the application of phosphate fertilizer may be the cause of the increase of AP in soil. The moisture contents affected the distribution of ions between solid phases and liquid phases and the dissociation of adsorbed ions on colloidal particles, and the dissolution and dissociation of salts in soil, thus affecting the soil pH values (Gao et al. 2011). Additionally, the microorganisms break down organic matter and fresh crop residues to release a large number of cations in soil solution (Butterly et al. 2013; Xiao et al. 2014), resulting in changing of the soil pH values.

The tillage had dramatic effects on soil microbial diversity in our study. In the soil ecosystem, Proteobacteria and Bacteroidetes mainly participate in the degradation of OM and promote the carbon and nitrogen cycles (Lesaulnier et al. 2008; Michaud et al. 2009; Liu et al. 2014). Hence, it dramatically decreased OM after tillage in soil. Acidobacteria and Actinobacteria are mainly involved in the OM decomposition (Eichorst et al. 2007; Eichorst et al. 2011; Ahn et al. 2012), when the OM reduces, Acidobacteria and Actinobacteria growth was inhibited. Mostly Chloroflexi is anaerobes, which are negatively correlated with oxygen content (Grégoire et al. 2011). In PBS, the oxygen content declined rapidly, which was suitable for the growth of Chloroflexi. In addition, the excessive relative abundance of Firmicutes can threaten the health of plants (Berendsen et al. 2012; Zhang et al. 2014). In this paper, the relative abundance of Firmicutes was reduced after tillage disturbance, indicating that black soil quality was healthy. At the genus levels, \textit{Kaistobacter} and \textit{Bradyrhizobium} have nitrogen fixation capability (Chaintreuil et al. 2000), which contributes to the soil nitrogen fixation. \textit{Bradyrhizobium} and \textit{Flavobacterium} can dissolve inorganic phosphorus, which may be one of the reasons for the dramatically improved AP in DFBS. \textit{Rhodoplanes} has denitrification, it can degrade organic compounds and nitrogen-containing compounds. The relative abundance of nitrogen-fixing bacteria and nitrogen-removing bacteria increased simultaneously, which was helpful in maintaining soil nitrogen dynamics. It may be the main reason for the insignificant change of soil TN in DFBS. Moreover, \textit{Anaerolinea}, \textit{Variovorax} and \textit{Methylibium} have the functions of anaerobic degradation of organic compounds and phosphorus removal. The relative abundance of the three increased dramatically in PBS, which was beneficial to the improvement of the soil environment and the stability of the microbial community.

The change of archaea community structure is positively correlated with soil moisture generally (Li et al. 2019), which is consistent with the results of this study. Previous studies have reported that the abundance of methanogens is higher in terrestrial, freshwater sediments (Breidenbach et al. 2015). When OM is decomposed, soil facultative bacteria and anaerobic bacteria will consume a large amount of oxygen and oxidized inorganic compounds in the soil and produce reducing substances, leading to decline in redox potential and providing a necessary environment for methanogenic archaea activities (Kim et al. 2013; Liu et al. 2014). The products of the decomposition of OM can be used as the precursors...
that can be converted to methane by the methanogenesis (Chidthaisong and Conrad 2000). The survival of ammonia-oxidizing archaea also requires NH$_4^+$ as a nitrogen source (Cao et al. 2013), which might be the main reason for the significant reduction of total nitrogen content in PBS. Overall, under the condition that oxygen is scarce, the electron transfer process of microbial metabolism requires more diverse electron receptors to replace oxygen. Consequently, compared with upland soil, flooded soil requires higher microbial diversity to maintain normal metabolic function.

**Conclusions**

The tillage caused changes in microbial community composition and diversity, and physical and chemical properties in black soil. Overall, except for the archaea in DFBS, the diversity of bacteria and archaea changed insignificantly by tillage, but the diversity of dominant members changed dramatically. Moreover, the relative abundance of dominant microbe affected dramatically. After tillage disturbance, the AP increased considerably, but OM decreased considerably both NDBS and PBS. Soil pH decreased considerably in DFBS, which was opposite in PBS. The TN concentration decreased considerably in PBS and QP decreased in DFBS.

**Declarations**

**Author contributions**

Duo Zhao, Dan Wei, Lei Yan and Fuqiang Song: experiment designing, data collection.

Guinan Shen, Yuan Yuan and Yamei Gao: data analysis, and writing.

Duo Zhao and Wei Zhang: sample processing and editing.

Ji-Dong Gu and Weidong Wang: supervision.

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**Compliance with ethical standards**

**Conflict of interests** No conflicts of interest.
Research involving Human Participants and/or Animals

The study is not related to animals or humans.

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