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Original article

Effects of tillage and residue incorporation on composition and abundance of microbial communities of a fluvo-aquic soil

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ABSTRACT

This article studied the effects of tillage and no tillage and their combinations with different residue incorporation levels (0%, 50% and 100%) on soil properties and microbial communities in a fluvo-aquic soil. The purpose was to find a promising farming practice for improving the soil fertility and microbial communities of the Huang-Huai-Hai Plain of central China. Surface soils were sampled from a long-term field experimental site. The soil organic carbon (SOC), microbial biomass carbon (MBC), total nitrogen (TN), and bacterial and fungal abundance and diversity were analyzed by chemical and molecular methods. No-tillage treatment had positive effects on MBC, MBC/SOC, bacterial abundance and soil bacterial and fungal diversity and inhibited the pathogens such as *Cochliobolus lunatus* as compared with the conventional tillage. 100% residue incorporation had positive effects on SOC, C/N ratio, pH, bacterial abundance and soil bacterial and fungal diversity, and benefited *Filobasidium floriforme*. The interaction of no tillage and 100% residue incorporation could increase bacterial abundance and diversity and fungal diversity. The treatment of no tillage with 100% residue incorporation may be a promising farming practice for improving the soil fertility and microbial communities of the fluvo-aquic soil in Huang-Huai-Hai Plain of central China.

1. Introduction

Conservation tillage, including no-tillage or reduced tillage and crop residue incorporation, is a promising agricultural practice for improving soil quality. This farming practice has the following advantages: increasing soil organic matter content, preserving soil moisture, reducing erosion, buffering soil temperature fluctuation, promoting soil aggregate stability [1,2], improving soil chemical and biological properties, improving crop yield and reducing greenhouse gas emissions [3–5]. However, the effects of conservation tillage on soil bacterial, especially on fungal communities, are not well understood. Soil bacteria and fungi generally comprise 90% of the total soil microorganisms. They play essential roles in agroecosystem, such as promoting soil nutrient cycling, providing nutrition to plants, and inhibiting soil-borne plant diseases [6–8]. In many cases, bacteria and fungi were more abundant under no tillage than under conventional tillage. Some reports also claimed that no tillage would lead to a shift to fungal dominance. It was hypothesized that the presence of surface residues in no-tillage favored fungal growth because fungi, unlike bacteria, can bridge the soil-residue interface and simultaneously utilize the spatially separated carbon and nitrogen resources [9]. Crop residue incorporation stimulated microbial activity and microbial biomass, yet with effect depending on placement (surface residues in no-tillage vs. incorporated residues in conventional tillage), quantity and quality of residue. These variables could also affect the composition of bacterial and fungal populations [3,10–12]. However, the effects of no-tillage and its interaction with different residue incorporation levels on soil bacterial and fungal communities need to be studied.

Huang-Huai-Hai Plain is the most important wheat and corn production area in China [13]. Under the traditional farming practices, including tillage and crop residue removal, soil fertility and crop yields are declining. This study aims to explore the effects of no tillage with different levels of crop residue on the abundance and diversity of soil bacterial and fungal communities determined using molecular methods in this soil. The purpose was to find a promising farming practice for improving soil fertility and microbial communities.

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2. Materials and methods

2.1. Study area and sampling

The field experimental site was established in 2007 at the Fengqiu State Key Agro-ecological Experimental Station (35°01′N, 114°32′E) in Henan Province in central China. The research site belongs to temperate monsoon climate with a mean annual temperature of 13.9 °C and an annual precipitation of 615.1 mm. The soil type is fluvo-aquic soil, with 11.13 g/kg organic matter, 1.39 g/kg total nitrogen and bulk density 1.16 g/cm^2 in 0–20 cm soil layer. The annual planting system is a rotation of winter wheat (from early October to mid-May) and maize (from early June to mid-September) [14]. The mixed fertilizers (N: P_2O_5: K_2O = 15: 15: 15) (~220 kg/ha) and urea (~150 kg/ha) for wheat and maize were used as base fertilizer and topdressing, respectively. The study was designed as a split-plot experiment, with tillage as the main treatment and residue management as split treatment. Tillage treatments included conventional tillage (CT) and no tillage (NT). Residue management treatments included no residue, 50% residue incorporation, and full residue incorporation. The 6 treatments were conventional tillage + no residue (CT 0), conventional tillage + 50% crop residue incorporation (CT 50), conventional tillage + 100% crop residue incorporation (CT 100), no tillage + 0% residue (NT 0), no tillage + 50% crop residue incorporation (NT 50), and no tillage + 100% crop residue incorporation (NT 100). Each treatment had four replicates, and each treatment plot (4 m × 100 m) was randomly arranged [14].

Surface soils (0–20 cm) were randomly sampled from 10 points in each treatment plot with a 2.5 cm diameter auger after wheat harvest on June 2010, and thoroughly mixed into one composite sample. A total of 24 composite samples (6 treatments × 4 replicates = 24 samples) were collected. The samples were sieved (<2 mm), and then stored at 4 °C (for soil characterization) or −80 °C (for soil DNA isolation) until analysis. For molecular analysis, 3 out of 4 replicates of each treatment were randomly chosen.

2.2. Soil characterizations

Soil organic carbon (SOC) and total nitrogen (TN) were determined according to Ryan, Estefan and Rashid [15]. Microbial biomass carbon (MBC) was measured by the chloroform fumigation and extraction method [16]. The pH of soils was determined in a 1:2.5 soil-to-water suspension [17].

2.3. DNA extractions

DNA was extracted from 0.5 g of frozen soil using a FastDNA SPIN soil DNA isolation kit (MP Biomedicals, Santa Ana, CA) followed by purification on a Qiagen column (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. The quantity and purity of DNA was detected by NanoDrop (NanoDrop-1000, Thermo Scientific, USA). Then the DNA was stored at −20 °C before use.

2.4. Real-time PCR assay

The copy numbers of bacterial 16S rRNA gene and fungal 18S rRNA gene were quantified with real-time PCR assay. The PCR amplification of soil samples was performed on an ABI prism 7000 (Applied Biosystems, Foster City, CA) and SYBR Green as the detection system. The 25 μL reaction mixture consisted of 12.5 μL of 2× SYBR Premix Ex Taq II (TaKaRa, Dalian, China), 1 μL of forward and reverse primers each (primer pair 341F-758R for bacteria and FF390-FR1 for fungi were used) [18,19], 0.5 μL of 50× ROX Reference Dye II (TaKaRa, Dalian, China), 2 μL of template DNA (~50 ng/μL, $A_{260/280}$ = ~1.9, $A_{260/230}$ = ~2.0) and 8 μL ddH_2O. The amplification was a three-step PCR for both bacterial 16S rRNA gene and fungal 18S rRNA gene, i.e., 40 cycles with denaturation at 95 °C for 10 s, primer annealing at 55 °C (bacteria)/50 °C (fungi) for 30 s, and extension at 72 °C for 30 s.

Standards for real-time PCR assays were prepared as described previously [20,21]. Briefly, the bacterial 16S rRNA gene and fungal 18S rRNA gene were PCR-amplified from extracted DNA with the primers 341F/758R and FF390/FR1, respectively, and the PCR products were cloned into the pMD19-T Simple Vector (TaKaRa, Dalian, China). Plasmids used as the standards for quantitative analyses were extracted from the correct insert clones of each target gene using the Axygen Plasmid Miniprep Kit (Axygen, Hangzhou, China). The concentration of plasmid DNA was determined on a Nanodrop® ND-1000 UV–Vis Spectrophotometer (NanoDrop-1000, Thermo Scientific, USA), and the copy numbers of target genes were calculated directly from the concentration of the extracted plasmid DNA. Ten-fold serial dilutions of a known copy number of the plasmid DNA were subject to real-time PCR assay in triplicate to generate an external standard curve.

2.5. Denaturing gradient gel electrophoresis analysis

Denaturing gradient gel electrophoresis analysis of bacterial and fungal PCR products was performed with the DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, USA). For DGGE analysis, the primers 341F GC-758R for bacteria and FF390-FR1 GC for fungi were used. Polymerase chain reaction products of bacterial and fungal genes were loaded onto polyacrylamide gradient gels with a denaturing gradient of 30–60% and 30–50% (100% denaturant containing 7 M urea and 40% formamide), respectively. Electrophoreses were both run at 180 V for 6 h. The gels were stained with 1:10,000 GeneFinder (Bio-V Inc., China) for 30 min according to the manufacturer's instructions, scanned by a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, USA) and analyzed using the software Quantity One (Bio-Rad Laboratories).

2.6. Cloning and sequencing

The bands in the DGGE gel were excised and suspended in 50 μL of TE buffer (pH 8.0) for 12 h, and amplified with the primers 341F/758R and FF390/FR1. The purified PCR products were ligated into the pMD19-T Simple Vector (TaKaRa, Dalian, China). The resulting ligation mix was transformed into *Escherichia coli* DH5α competent cells following the instructions of the manufacturer. The positive clones were amplified using the above primers with GC clamp, and then verified by DGGE. The correct one was selected for sequencing.

2.7. Phylogenetic analyses

The sequences were aligned with BLAST search program, and phylogenetic tree was constructed using MEGA version 5.0 by neighbor-joining method with the bootstrap value of 1000 replicates [22]. UPGMA algorithms were used to cluster the DGGE patterns. The Shannon diversity index H and Evenness index E were used to calculate the diversity of bacteria and fungi communities based on the equations:

$$H = -\sum_{i=1}^{S} p_i \ln p_i = -\sum_{i=1}^{S} (N_i/N)\ln(N_i/N)$$

$$E = H/H_{\max} = H/\ln S$$