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Members of soil bacterial communities sensitive to tillage and crop rotation

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ABSTRACT

Pyrosequencing was used to study the effect of rotation and tillage on total bacterial communities. We designed primers to the bacterial 16s rDNA and amplified DNA from soil samples from a long-term tillage/rotation trial in Kansas for two seasons. The 2 × 2 factorial trial had two rotation treatments (wheat–wheat and wheat–soybean) and two tillage treatments (conventional and no-till). A total of 20,180 16s rDNA sequences were generated and 2337 operational taxonomic units (OTUs) were assembled using a 97% similarity cut-off. The phylum Proteobacteria represented 38% of 299 identified taxa. The second most abundant phylum was Acidobacteria, making up 20% of the sequences, the majority of which were Acidobacteria Group 1. The phyla Actinobacteria and Gemmatimonadetes comprised 12% and 3.5% of the sequences. Other groups detected included TM7, Nitrospira, Verrucomicrobia, and Bacteroidetes. Some clusters of Acidobacteria Group 1 were more frequent in continuous wheat versus wheat–soybean rotation, some Acidobacteria Group 2 were more frequent in no-till, and some Acidobacteria Group 4 were more frequent in wheat–soybean rotation. These results were validated by quantitative real-time PCR. Pyrosequencing provided taxonomic information about the overall bacterial community, and detected community shifts resulting from different cropping practices.

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1. Introduction

Soil microorganisms play important roles in many soil processes (Abawi and Witmer, 2000; Garbeva et al., 2004; Kennedy, 1999; Van Bruggen and Semenov, 2000; Van Elsas et al., 2002). They regulate carbon and nitrogen cycling and provide nutrition to plants. Bacteria and fungi are critical for the production of soil aggregates and the conversion of plant residue to soil organic matter (SOM). SOM increases aggregate stability, cation exchange capacity, water holding capacity, water infiltration and soil porosity (Nannipieri et al., 2003). Soil microbial communities are also part of the food web that supports populations of invertebrates and protozoans. Furthermore, the abundance of specific antagonistic microbes and the diversity of soil microbial communities are important to the capacity of soil to suppress soilborne plant diseases via antibiosis, competition or stimulation of plant host defenses (Abawi and Witmer, 2000; Cook et al., 1995; Van Bruggen and Semenov, 2000; Van Elsas et al., 2002).

Many studies have been performed to determine the ecological and environmental factors regulating microbial community structure (Baek and Kim, 2009; Högberg et al., 2007; Nagy et al., 2005). Several recent studies have focused on the effects of agricultural practices on the community diversity of soil microorganisms (Buckley and Schmidt, 2003; Clegg et al., 2003; Johnson et al., 2003; Salles et al., 2006; Steenwerth et al., 2002; Vepsäläinen et al., 2004; Yao et al., 2000). No-till or reduced tillage systems can reduce the erosion of soil, which is not a renewable resource and is declining on much of our agricultural land (Kabir, 2005; Papendick, 2004). Reduced tillage can also increase soil organic matter which is generally thought to increase soil quality through better structure, drainage and ion exchange (Dao, 1993; Douglas and Goss, 1982; Drijber et al., 2000; Woods, 1989). Microbial biomass increases have been observed in no-till plots compared to conventional tillage (Drijber et al., 2000). Soils in no-till farming systems, also called direct-seeded systems, where the soil is disrupted only at

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planting, also differ from conventionally tilled soils in several other respects. For example, no-till soils may warm more slowly and remain wetter in the spring. The lack of soil disruption also reduces oxidation and physical disruption of mycelia that affect soil community composition. However, one important weakness of these conservation tillage systems is that certain soilborne plant pathogens can increase to damaging levels (Conway, 1996). Management practices such as crop rotation can sometimes prevent this problem, particularly for pathogens with limited host range.

A thorough analysis of microbial communities requires methodologies for analyzing thousands of microorganisms in a cost-effective and timely manner. Soil microbial diversity has historically been studied by substrate utilization or plating (Hill et al., 2000; Joergensen and Emmerling, 2006). These methods provided an incomplete assessment because they only detect culturable organisms, but recent work suggests that up to 97% of the organisms detected microscopically cannot be cultured (Amann et al., 1995; Pace, 1997). Newer biochemical and molecular analytical tools have recently been used to describe the composition of soil microorganism. These include phospholipid fatty acid (PLFA) or fatty acid methyl ester (FAME) profiles that distinguish broad groups of fungi and bacteria, based on signature fatty acids in cell membranes (Zelles et al., 1992, 1995); denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) which target 16S rDNA and produce electrophoretic bands characteristic of particular groups of microbes (De Oliveira et al., 2006). Although these methods do not rely on culturing of microorganisms, they cannot provide high-resolution taxonomic information. In addition, they detect large-scale population components or activities that are assumed to be important *a priori*, but they miss some small-scale community components that may be also important. Furthermore, DGGE and T-RFLP techniques require extensive cloning and sequencing, which is time consuming and expensive.

Recent advances in DNA sequencing methodology, such as pyrosequencing (Margulies et al., 2005), have made sequence analysis sufficiently affordable to examine population structure in soil (Liu et al., 2007; Sogin et al., 2006). Amplification of a target sequence, such as a fragment of the 16s rDNA, with conserved primers, followed by high-throughput sequencing of amplification products generates taxonomic information for each product, provided that the amplified fragments carry sufficient variation in the sequences. However, no study to date has looked at tillage and rotation effects using this technique. In this study, we examined microbial populations in four cultivation and crop rotation treatments from a long-term field experiment in Kansas. The frequencies of specific sequences generated from each treatment were compared to determine if differences could be detected among the cultural practices. Estimated differences in the abundance of various taxa were validated by quantitative real-time PCR.

2. Materials and methods

2.1. Field plots

Field plots with different cultural and rotation practices were established and cropped annually since 1974 at the Kansas State University Ashland Bottoms Farm near Manhattan. Kansas (McVay et al., 2006). The soil type was a Muir silt loam (fine-silty, mixed, mesic Pachic Haplustoll). Further soil characteristics of these plots were reported by Godsey et al. (2007). All plots were fertilized before planting each year by broadcasting 112 kg N ha^{-1} and 11.3 kg P ha^{-1}. We evaluated a subset of the treatments included in the complete experiment, analyzing the effects of two rotations, continuous wheat (*Triticum aestivum*) and wheat alternated with soybeans (*Glycine max*). Each rotation in our analysis was treated either by no-till, where the seed was drilled directly into the previous year's residue, or by conventional tillage, which consisted of annual cultivations with a chisel plow, disk and field cultivator. The experimental design was a split-plot with four replications at the sub-plot level, with rotation applied in adjacent plots at the whole plot level and tillage as applied to the sub-plot.

2.2. Isolation of DNA from soil and amplification and sequencing of rDNA fragments

In May of 2005 and 2007, when all the plots were sown to wheat, nine soil cores from the top 15 cm of soil were collected from transects within each of the 16 sub-plots sampled in each year. The soil cores from each sub-plot were thoroughly mixed. Ten grams of each bulk sample were then used for DNA extraction. DNA was extracted using the UltraClean™ Mega Soil DNA Isolation Kit (MO BIO Laboratories, Solana Beach, CA) according to the manufacturer's instructions. DNA was visualized by gel electrophoresis and quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham MA).

DNA was amplified from purified soil DNA by PCR using the primers listed in Table 1. Each reaction included U342F-FC-B as the forward primer and one of 16 different reverse primers. Primers were designed to amplify the V3 hyper-variable region of bacterial 16S rDNA. The oligonucleotide design included 454 Life Science's A or B sequencing adapter (shown in bold letters in Table 1) fused to the 5′ end of reverse and forward primers. Unique barcode sequences (underlined) were added between the A sequencing adapter and the reverse 16S primer U529R to differentiate between samples. PCR reactions consisted of 2 ng of soil DNA, 1× reaction buffer, 0.2 mM deoxynucleotides, 0.25 μM of each primer, 5 μl DMSO and 0.125 U of Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA) in a total volume of 25 μL. PCR was performed using the DNA Engine thermocycler (Bio-Rad, CA) using a hot start program: 80 °C 3 min (1 cycle); 94 °C 5 min (1 cycle); 94 °C 30 s, 55–69 °C 30 s, 72 °C 30 s (30 cycles); 72 °C 7 min (1 cycle). Each soil

Table 1
Primers used to amplify bacterial sequences from soil DNA for sequence analysis and real-time amplification to verify population differences.

primer	Sequence (5′–3′)
U341F-FC-B	**GCCTTGCCAGCCCGCTCAG**CCTACGGGRSGCAGCAG
U529R-FC-A3	**GCCTCCCTCGCGCCATCAG**ACTCAACCGCGGCKGCTGGC
U529R-FC-A9	**GCCTCCCTCGCGCCATCAG**AGCAGACCGCGGCKGCTGGC
U529R-FC-A11	**GCCTCCCTCGCGCCATCAG**TATCAACCGCGGCKGCTGGC
U529R-FC-A14	**GCCTCCCTCGCGCCATCAG**AGTATACCGCGGCKGCTGGC
U529R-FC-A16	**GCCTCCCTCGCGCCATCAG**CTACGACCGCGGCKGCTGGC
U529R-FC-A18	**GCCTCCCTCGCGCCATCAG**ACTAGACCGCGGCKGCTGGC
U529R-FC-A20	**GCCTCCCTCGCGCCATCAG**TCTCTACCGCGGCKGCTGGC
U529R-FC-A22	**GCCTCCCTCGCGCCATCAG**ACTCGACCGCGGCKGCTGGC
U529R-FC-A24	**GCCTCCCTCGCGCCATCAG**ACTCTACCGCGGCKGCTGGC
U529R-FC-A25	**GCCTCCCTCGCGCCATCAG**TGTCAACCGCGGCKGCTGGC
U529R-FC-A27	**GCCTCCCTCGCGCCATCAG**CTACTACCGCGGCKGCTGGC
U529R-FC-A29	**GCCTCCCTCGCGCCATCAG**AGCTGACCGCGGCKGCTGGC
U529R-FC-A33	**GCCTCCCTCGCGCCATCAG**TGATGACCGCGGCKGCTGGC
U529R-FC-A35	**GCCTCCCTCGCGCCATCAG**AGCGCACCGCGGCKGCTGGC
U529R-FC-A40	**GCCTCCCTCGCGCCATCAG**TCACTACCGCGGCKGCTGGC
U529R-FC-A42	**GCCTCCCTCGCGCCATCAG**TGTGCACCGCGGCKGCTGGC
RT-64ww-F1	ATCCCGCCGAAGCAGGAGTTT
RT-64ww-R1	GAGGTAACGGCTCACCAAGG
RT-74ww-F2	TTCCACCTGCCTCTACAGTGCTC
RT-74ww-R2	ACGAATGTGCGCTTGAGAGCGT
RT-Gp2-F1	TCAAGCCCTTGCGGGCATTCGT
RT-Gp2-R1	TGATCGGTAGCTGGTCTGAGAGG
RT-GP4-F2	GCATTTCACTGCTACACCGAGA
RT-GP4-R2	AAGTAAGGGTTAATATCTCTTACG