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Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem

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ABSTRACT

In this study, the effect of limited tillage versus traditional tillage, residue retention versus removal and crop rotation (maize–wheat) versus monoculture (maize) on the bacterial community structure in soils was investigated by means of 454 pyrosequencing of the 16S rRNA gene. Using taxonomic and phylogenetic information it was found that zero tillage most affected the bacterial communities. The relative abundance of Actinobacteria, Betapreoteobacteria and Gammaproteobacteria was affected by tillage and correlated to the total organic carbon (TOC) and clay content in soil. Residue management had a significant effect on the bacterial community structure when phylogenetic membership and the total enumeration of bacteria were considered. Residue management affected the relative abundance of Bacteroidetes, Betaproteobacteria, Cyanobacteria and Gemmatimonadetes. When no tillage was applied, crop residue management affected the microbial communities more than when conventional tillage was applied. Wheat–maize rotation or crop monoculture did not affect the bacterial community structure. No significant differences in richness, diversity and total abundance of bacteria was found between the treatments. This indicated that even though phylotypes changed, the number and diversity of the bacterial communities were similar.

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1. Introduction

It is well known that bacteria play important roles in several biogeochemical soil processes (Falkowski et al., 2008). The soil is a heterogeneous matrix with a vast diversity of physical and chemical characteristics, which lead to a wide range of different niches that can sustain a large microbial diversity. Agriculture is one of the most impacting anthropogenic activities that affect the soil physical, chemical and biological properties of the soils, and consequently, their functioning. Conservational agriculture practices, i.e. reduced tillage, crop residue retention, and crop rotation, have been proposed as alternative to monoculture, crop removal and tillage as they improve soil structure, increase soil organic matter content and increase yields (Alvear et al., 2005; Madari et al., 2005; Ussiri et al., 2009). Improved soil structure facilitates soil aeration, diffusion of water and nutrients through the soil profile, moderates soil temperatures and reduces erosion (Horn et al., 1994; Doran et al., 1998). These improvements in soil quality and organic matter content can also increase soil microbial diversity, and it is then expected that soils with conventional agricultural practices will contain different bacterial communities in terms of structure, diversity and abundance than those with conservation practices.

Plenty of studies have investigated the effect of reduced tillage, crop residue retention, and crop rotation on the microbial biomass, activity, abundance and composition (Enwall et al., 2007; Esperschuetz et al., 2007; Govaerts et al., 2007, 2008). However, the large majority of these studies is based in indirect techniques, such as fumigation (Jiang et al., 2011; Wang et al., 2012). Fingerprinting techniques used to study the community composition are limited in their phylogenetic resolution and providing little or no taxonomic information (e.g. PCR-denaturing gradient gel electrophoresis (DGGE), or certain group markers of phospholipid fatty acids) and/or focuses on a particular group of cultivable bacteria or fungi resulting in conflicting results (Caesar-TonThat et al., 2010; Schneider et al., 2010; Jiang et al., 2011; Lupwayi et al., 2012). For instance, some authors reported a significant effect on microbial

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biomass or bacterial composition as a consequence of the tillage practice (Kihara et al., 2012; Lupwayi et al., 2012), while others reported no effect (Jangid et al., 2011; Jiang et al., 2011). Additionally, when phylogenetic techniques have been applied to study the bacterial community and diversity in soils under different agricultural practices, they were merely descriptive and do not result in an in depth analysis of the effect of tillage or crop residue on the bacterial structure and composition (Ceja-Navarro et al., 2010). As such, knowledge on the effect of the tillage, crop residue retention and crop rotation on the enumeration, taxonomic distribution and phylogenetic composition of the bacterial communities, and consequently, the correlation between physicochemical soil properties and the bacterial community composition is very limited.

In this study, massive sequencing, and taxonomic and phylogenetic analyses were used to analyse the effect of different agricultural practices on soil bacterial communities. The bacterial community structure was determined in terms of taxonomy distribution and phylogenetic diversity, and compared between samples (beta-diversity) by multivariate analyses at different taxonomy levels (principal component analysis (PCA)) and using based-divergence methods (principal coordinate analyses (PCoA) of weighted and unweighted UniFrac distances). Correlation of the physicochemical factors with the bacterial phyla and the different classes of Proteobacteria was done by canonical correlation analysis (CCA). Additionally, the total bacterial population in the soil samples was measured through quantitative PCR.

2. Materials and methods

2.1. Long-term field experiment at El Batán

The research station El Batán is located in Texcoco (2240 masl; 19.318 N, 98.508 W), in the semiarid, subtropical highlands of central Mexico, with monthly average temperatures between 12.5 and 17.5 °C. Soil used in this study was collected from a field experiment that investigated the effect of crop rotation, residue management and tillage on yields maize (*Zea mays* L.) or wheat (*Triticum* spp.) in monoculture or in rotation. Details of the agricultural practices applied in this field experiment started in 1991 can be found in Govaerts et al. (2005, 2006, 2007, 2008). Five treatments were used in this study. A first treatment combined maize monoculture (MM) with conventional tillage (CT) and crop residue removal (−R), i.e. the traditional agricultural practice in the central highlands of Mexico, while the other four treatments combined a wheat–maize crop rotation (WM) with CT or zero tillage (ZT), and −R or residue retention (+R) (Table 1).

2.2. Soil sampling and characterization

Soil samples were collected from two maize rows ($n = 2$) 3.75 m apart in two plots ($n = 2$) from five treatments. The 0–20 cm layer was sampled 20 times with a 2 cm soil auger during the maize crop cycle (July 27, 2011). The samples taken from each maize row were pooled separately so that 20 different soil samples were obtained (two subplots per two plots and that from five treatments). This field-based replication was maintained in the laboratory study.

The soil samples were analysed for total organic carbon (TOC) and nitrogen (N), electrolytic conductivity (EC), pH, clay content and water holding capacity (WHC) as described by Aguilar-Chávez et al. (2012).

2.3. DNA extraction and PCR amplification of bacterial 16S rRNA genes

Metagenomic DNA was extracted from 3 g soil (6 times from 0.5 g) with a technique described by Ceja-Navarro et al. (2010). Primers designed in this work 8-F (5′-AGA GTT TGA TCI TGG CTC A-3′) and 556-R (5′-TGC CAG IAG CIG CGG TAA-3′), 10 pb multiplexed and containing the Roche 454 pyrosequencing adaptors Lib-L, were used to amplify the region V1–V3 of the 16S rRNA gene from the metagenomic DNA. The PCR mixture (25 μl) contained 1× reaction buffer, 10 mM of each of the four deoxynucleoside triphosphates, 10 pM of each of the primers, 0.7 U Phusion hot start high fidelity DNA polymerase (FINNZYMES) and 20 ng metagenomic DNA as template. The following thermal cycling scheme was used: initial denaturation at 95 °C for 10 min, 25 cycles of denaturation at 95 °C for 45 s, annealing at 53 °C for 45 s, and extension at 72 °C for 45 s followed by a final extension period at 72 °C for 10 min. All samples were amplified in triplicate, pooled in equal amounts, and purified using the DNA clean and concentrator purification kit as recommended by the manufacturer (Zymo Research, Irvine, CA, USA). Quantification of the PCR products was done using the NanoDrop™ 2000 (Thermo Fisher Scientific Inc., Suwanee, GA). Sequencing was done by Macrogen Inc. (DNA Sequencing Service, Seoul, Korea) by using a Roche GS-FLX Titanium 454 pyrosequencer (Roche, Mannheim, Germany) and the instructions of the manufacturer for amplicon sequencing.

2.4. Analysis of pyrosequencing data

The QIIME version 1.5.0 software pipeline was used to analyse the pyrosequencing data (Caporaso et al., 2010b). Sequences were sorted by each barcode and those <200 bp in length, reads with

Table 1
Characteristics and the mean of quantitative PCR of bacterial 16S rRNA genes in the different treatments at El Batán (Texcoco, Mexico).

Management practice	Caption	pH	EC[a] (dS m^{-1})	Total N	TOC	WHC	Clay	Ratio C/N	USDA textural classification	Bacterial 16S rRNA 10^{10} copies g^{-1} FW
				(g kg^{-1} soil)						
Monoculture, conventional tillage, residue removal	MMCT − R	6.65	0.45	1.09	29.2	796	357	27	Sandy clay	5.77[b] (0.11)[c]
		6.60	0.32	1.41	28.7	676	391	20	Clay loam	3.03 (0.08)
Maize–wheat rotation, conventional tillage, residue removal	WMCT − R	6.30	0.33	1.30	30.5	804	381	24	Clay loam	3.98 (3.21)
		6.65	0.35	1.36	27.0	722	376	20	Clay loam	5.18 (0.53)
Maize–wheat rotation, conventional tillage, residue retention	WMCT + R	6.42	0.44	1.34	33.2	794	354	20	Clay loam	10.71 (2.18)
		6.32	0.38	1.76	30.7	764	380	25	Clay loam	8.43 (0.42)
Maize–wheat rotation, zero tillage, residue removal	WMZT − R	6.67	0.40	1.30	31.0	785	350	24	Sandy clay	6.42 (0.88)
		6.55	0.52	1.41	26.5	753	341	25	Sandy clay loam	7.20 (0.92)
Maize–wheat rotation, zero tillage, residue retention	WMZT + R	6.30	0.44	1.69	41.2	795	365	19	Sandy clay	8.50 (2.62)
		6.15	0.36	1.57	35.5	769	330	23	Clay loam	7.47 (0.72)
Minimum significant difference ($P < 0.05$)		0.37	0.18	0.52	5.1	153	38	9		5.85
P value		0.030	0.409	0.161	0.0001	0.887	0.056	0.823		0.0476

[a] EC, TOC, WHC, FW indicate electrolytic conductivity, total organic carbon, water holding capacity and Fresh weight.
[b] Mean of two sub-samples of the number of bacterial copies.
[c] Value between parenthesis is the standard deviation.

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