

Bacterial Diversity Under Different Tillage and Crop Rotation Systems in an Oxisol of Southern Brazil

A.P. Silva^{a,b}, L.C. Babujia^{a,c}, L.S. Matsumoto^{a,d}, M.F. Guimarães^b and M. Hungria^{a,*}

^aEmbrapa Soja, Caixa Postal 231, 86001-970, Londrina, PR, Brazil

^bUniversidade Estadual de Londrina, Dept. Agronomia, Caixa Postal 6001, 86051-980, Londrina, PR, Brazil

^cUniversidade Estadual de Maringá, Dept. Química, 87020-900, Maringá, PR, Brazil

^dUniversidade Estadual do Norte do Paraná- Campus Luiz Meneghel, Dept. Biologia, Caixa Postal 261, 86360-000, Bandeirantes, PR, Brazil

Abstract: Microbial diversity can be used to assess the impact of agricultural practices on the long-term sustainability of cropping systems. The aim of this study was to investigate changes in soil bacterial diversity as a result of the impact of different soil tillage and crop rotation systems in an oxisol of southern Brazil. Bacterial diversity was examined in the 0-10 cm layer in two field experiments by analyzing soil DNA using 16S rDNA-DGGE profiles. Experiment one consisted of a long-term 26-year trial with four soil tillage management systems: (1) no-tillage (NT), (2) disc plow (DP), (3) field cultivator (FC), and (4) heavy-disc harrow (DH), all under soybean (summer)/wheat (winter) crop succession. Experiment two consisted of a short-term 10-year trial with DP and NT and three crop rotations (CR) including grasses, legumes and green manures. Cluster analysis of the 16S rDNA sequences revealed that the main effect on clustering was attributed to differences in soil tillage management systems. The Shannon index confirmed greater bacterial diversity under NT, followed by the FC, DH and DP. Therefore, diversity decreased as tillage practices intensified. The evenness index demonstrated uniformity of the profiles of the bacterial communities, with dominance of a few communities, regardless of soil tillage and crop rotation. Different crop rotations had only minor effects on bacterial diversity, what could be related to a previous fallow period. The results suggest that bacterial diversity analyzed by DGGE may be useful as bioindicator of the changes caused by soil tillage.

Keywords: Soil tillage, Crop rotation, Crop succession, Microbial diversity, PCR-DGGE.

INTRODUCTION

Soil microorganisms play a key role in the maintenance, functioning and sustainability of agroecosystems [1], mainly by regulating carbon (C) and nitrogen (N) cycling, with direct implications on soil fertility and plant nutrition [2]. The responsiveness of microorganisms to disturbances caused by crop and soil management may lead to changes in the diversity and activity of soil biota [3, 4]. Soil microbial communities with high diversity should have greater resilience to stress [5], and greater functional diversity should be a key element in sustainability [6, 7].

Recent studies have focused on the effects of agricultural practices on the diversity of soil microorganisms [1, 2, 8]. The no-tillage (NT) management system—also called zero-tillage or direct-seeded system—interferes with soil's physical properties and thus potentially affects the habitat of soil microorganisms [9-11]. Nowadays, Brazil is a reference worldwide in NT, with more than 26.6 million ha under NT

grain production in 2008 [12], and estimates of over 30 million ha today. In addition, soil microorganisms should also be greatly affected by crop management, as different plant species affect nutrient cycling and, consequently, the structure and functioning of the soil microbial community [13].

Currently, a variety of molecular tools are being used to describe the diversity and composition of the soil microbial community. They include denaturing gradient gel electrophoresis (DGGE) carried out on 16S and 18S rDNA [14] to highlight microorganism genera/species in the bands produced. DGGE-based analysis has proved to be particularly useful for producing unique fingerprints that can be used to identify changes or shifts in the populations of the predominant community members [3, 15].

Understanding how major changes in land management affect the structure of the soil's microbial community could provide an important index for assessing the relative ability of the soils to respond to future disturbance [11, 16]. However, we are far from understanding how soil microbial communities are affected by agricultural practices, and results available still do not cover the great majority of the ecosystems. Long-term experiments, especially when compared to medium and/or short-term experiments can generate

*Address correspondence to this author at the Embrapa Soja, Cx. Postal 231, 86001-970 Londrina, Paraná, Brazil, Tel: (+55)4333716206; Fax: (+55)4333716100; E-mails: hungria@pq.cnpq.br; mariangela.hungria@embrapa.br

Table 1. Crop Rotation Schemes Adopted from 1999 to 2007 in Experiment Short-Term Set Up— 10 Years.

Crop Management	¹ Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.	Win.	Sum.
	98/99	99	99/00	00	00/01	01	01/02	02	02/03	03	03/04	04	04/05	05	05/06	06	06/07
CR1	² S	W	S	W	M	O	S	O	S	W	S	L	M	O	S	F	M
CR2	M	O	S	W	S	L	M	L	M	W	S	O	S	L	M	F	M
CR3	S	L	M	O	M	W	M	O	M	W	M	O	M	O	M	F	M

¹ Sum, summer; Win, Winter.

² S, soybean (*Glycine max* (L.) Merr.); M, maize (*Zea mays* L.); W, wheat (*Triticum aestivum* L.); O, black oat (*Avena strigosa* Schreb); L, lupine (*Lupinus albus* L.); F, fallow

important information to predict the dynamics of the soil microbial community with time. In this context, the aim of our study was to evaluate the effects of soil tillage systems and crop rotations on the diversity of the soil's bacterial community in two experiments, one long and the other of short-term set up in southern Brazil.

MATERIALS AND METHODOLOGY

Geographic Location and General Description of the Field Plots

The experiments were carried out at the experimental station of Embrapa Soja in Londrina, State of Paraná, Brazil, latitude 23°11'S, longitude 51°11'W and elevation 620 m. The average annual temperature in Londrina is 21°C, with an average high of 28.5 °C in February and an average low of 13.3°C in July. Average annual rainfall is of 1,651 mm, with 123 days of rainfall per year [17]. Maximum rainfall occurs in the summer (January–March) and the minimum in winter (June–August). According to Köppen's classification, the climate in Londrina is subtropical humid (*Cfa*: humid, subtropical, with hot summers). The soil (Latossolo Vermelho Eutrófico, Brazilian Soil Classification System; Typic Rhodic Eutradox, USA Soil Taxonomy) presented the following physical composition: 710 g kg⁻¹ of clay, 82 g kg⁻¹ of silt and 208 g kg⁻¹ of sand. Previously, we have analyzed microbial biomass of carbon and nitrogen in four short to long-term experiments in our experimental station [10]. Two out of the four experiments were analyzed in this study for bacterial diversity.

Experiments Description

Experiment 1 - Long-Term 26-Year Trial

The experiment was set up in the summer of 1981/1982, with a crop succession (CS) of soybean (*Glycine max* L. Merr.)/summer and wheat (*Triticum aestivum* L.)/winter every year. The experiment consisted of plots, 8.0 m in width x 50.0 m in length (four replicates per treatment), distributed in a completely randomized block design comparing four soil tillage systems as treatments: (1) no-tillage (NT) (sowing directly through the residue of the previous crop, opening a narrow groove from 1.5 to 4 cm deep in the row); (2) conventional tillage (CT) with disc plow (DP) (the soil is mobilized approximately 20 to 25 cm, followed by light-disc harrowing); (3) conventional tillage with field cultivation (FC) (scarified to a depth of 0–20 cm followed by light-disc harrowing); and (4) conventional tillage with heavy disc harrowing (DH) that mobilizes the soil to a depth of approxi-

mately 15 cm. Soil preparation of the winter crop in the DP and FC treatments was accomplished by DH, followed by light-disc harrowing. The soybean was sown in the summer of 2006/2007. In both experiments, this one and the other described below, herbicides were applied after the grain harvest to dry out crop residues. For the NT system, crop residues were left on the soil surface, whereas in the other soil management systems, the residues were incorporated into the soil.

Experiment 2 - Short-Term 10-Year Trial

This site had been farmed under conventional tillage (CT) with disc plow for six years, cropped with soybean in the summer and with wheat in the winter. Then, a new experiment was set up in the summer of 1997/1998. Field plots were 15.0 m in length x 8.0 m in width, with a completely randomized block design in a factorial scheme, with two types of soil tillage as treatments: (1) NT, and (2) CT with disc plow (DP) in the summer and disc harrowing in the winter—note that this CT was slightly different than that in Experiment 1, as in the view of the benefits accruing from NT, the farmers began to reduce the soil-management operations even where CT was normally used—and three crop rotations (CRs), each with four replications per treatment. Crop rotations are shown in Table 1. In the summer of 2006/2007, all CRs were cropped with maize (*Zea mays* L.).

Soil Sampling

The soil was sampled in the summer, when the soybean and the maize were at full flowering stage. For soil sampling, crop residues were carefully removed from surface and procedure was as described before [10]. Each treatment consisted of four replicates. Basically, an area of 0.4 m² was delimited in each plot with a metal square. A soil sample of approximately 150 g was then taken from the middle of the square using a shovel, at the 0-10 cm layer. The samples were then placed in previously labeled bags suitable for 150 g of soil. The procedure was repeated eight times for each replicate in the field, at points spatially distributed as representative of the replicate. After collecting all samples, the eight discrete soil samples of each replicate were homogenized and combined. Each treatment had four composite samples of approximately 1.2 kg. The samples were then placed in plastic bags, and taken to the laboratory.

Before beginning the laboratory analysis, the samples were homogenized again, and plant residues removed. Approximately 200 g of soil from four composite samples were again mixed yielding two replicates per treatment. The samples

were then passed through a 4 mm (5 mesh) sieve, and kept in plastic bags in a ultra low freezer at -80°C, prior to molecular analysis, that took less than four months to be completed.

Soil- DNA Extraction and PCR Amplification of 16S rDNA

The microbial DNA was extracted taken 0.25 g of each of the two replicates per treatment, using the Ultraclean™ Soil DNA Kit (MoBio Laboratories, Inc. California, USA), according to the manufacturer's instructions. Aliquots of DNA were analyzed on 1% (w/v) agarose gels in 1X TBE to check the amount, purity and molecular size, using Low DNA Mass™ (Invitrogen-Life Technologies) as a standard weight.

Two successive amplifications were carried out for each DNA sample. First, soil DNA was amplified with universal primers fD1(5'-AGAGTTTGATCCTGGCTCAG-3') and rD1(5'-AAGGAGGTGATCCAGCC-3'), which amplify nearly the entire region of the DNA coding for 16S rDNA (~1,500 bp), as previously described [18]. The PCR reaction consisted of: 3.0 µL deoxynucleotides (dNTPs) 1.5 mM; 1.5 µL MgCl₂ 50.0 mM; 5.0 µL buffer 10X [20 mM Tris- HCl (pH 8.4)]; 1.5 µL of each primer (fD1 and rD1) 10 pmols; 0.2 µL 5 U of Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA); 1 µL of soil DNA (30 ng); sterile Milli-Q water to complete a final volume of 50 µL. The PCR program consisted of: an initial denaturation at 95°C for 2 min; 15 cycles of denaturation at 94°C for 15 s; 93°C for 45 s; primer annealing at 55°C for 45 s, and extension at 72°C for 2 min; the reaction was finalized by holding at 4°C.

The second amplification was performed using 1 µL (~20 ng) of the products of the first reaction as a template. The F-968 (5'-CGCCCGGGCGCGCCCGGGCGGGGCGGG-GGCACGGGGGAACGCGAAGAACCTTAC-3'), with a GC-clamp (underlined) and R-1401 (5'-GCGTGTGTAC-AAGACCC-3') [19] were used to amplify the 16S rDNA region of approximately 430 bp, corresponding to the V3 hypervariable region. PCR mixtures were prepared as: 5.0 µL dNTPs 1.5 mM; 1.3 µL MgCl₂ 50.0 mM; 2.5 µL buffer 10X [20 mM Tris- HCl (pH 8.4)]; 1.0 µL of each primer (F-968 and R-1401) 10 pmols; 0.2 µL 5 U of Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA); 1 µL of the PCR product of the first reaction with fD1 and rD1 primers (~10 ng); sterile Milli-Q water to complete a final volume of 25 µL. The following amplification cycles were used: one initial denaturation cycle at 94°C for 2 min; 2 cycles at 94°C for 1 min, at 60°C for 2 min, and at 72°C for 2 min; 2 cycles at 94°C for 1 min, at 59°C for 2 min, and at 72°C for 2 min, 94°C for 1 min, 58°C for 2 min, 72°C for 2 min (2 cycles); 94°C for 1 min, 57°C for 2 min; 72°C for 2 min (2 cycles); 94°C for 1 min, 56°C for 2 min, 72°C for 2 min (2 cycles); 94°C for 1 min, 55°C for 2 min, 72°C for 2 min; and for 10 min at 72°C. Amplification was confirmed by running 2 µL of PCR product on a 1% (w/v) agarose gel in 1X TBE, using as a marker for the MW the Low DNA Mass™ (Invitrogen-Life Technologies). Gels were then stained with ethidium bromide (0.3 µg mL⁻¹) and visualized under UV light.

DGGE Analysis of the Bacterial Community

Denaturing gradient gel electrophoresis (DGGE) was carried out using a D-Code System (Bio-Rad, Hercules, CA,

USA), by loading 25 µL from the last PCR product for each replicate of each treatment. The 6% (w/v) polyacrylamide gels were made up with a denaturing gradient ranging from 35 to 55%, using a mixture of 100% denaturing solution [7 M urea and 40% (v/v) formamide] and 0% solution (no urea and formamide added). The electrophoresis was run in 0.5X TAE buffer [10 mM Tris-acetate and 0.5 mM disodium EDTA (pH 8.3)], first with a pre-running at 60°C and 100 V for 1 h and then at a constant voltage of 100 V for 16 h. After electrophoresis, the gels were stained for 4 min with ethidium bromide and photographed under UV light.

Statistical Analysis of DGGE Fingerprints

The DGGE gels were analyzed using Bionumerics software (Applied Mathematics, Kortrijk, Belgium, v.4.6). The standard mix of soil bacteria was prepared in the laboratory and consisted of equal proportions of *Burkholderia*, *Bradyrhizobium*, *Methylobacterium*, *Azorhizobium* and *Rhizobium*. The mixture of bacteria was loaded in two lanes of each gel. Each gel image was normalized by identifying bands of mixture of bacteria in the reference lanes, which separated distinctly, spanning the gradient range, and then marking each band relative to the reference positions. Similarities between fingerprints were analyzed statistically using the unweighted pair-group method with arithmetic averages (UPGMA) and the Jaccard (J) coefficient [20], with a tolerance of 5% to create a distance matrix.

The Shannon diversity index (H), evenness index (E) and richness index (ACE) were analyzed for each replicate using SPADE software (Species Prediction and Diversity Estimation) [21], taking a sample size of 100 and a cut-off of 4.0. Richness was calculated with ACE (Abundance-based Coverage Estimator), a nonparametric estimator proposed by [22], based on the separation of observed species into rare or abundant groups with only the rare groups used to estimate the number of missing species.

RESULTS

Bacterial diversity was compared in two experiments, the first a long-term 26-year trial with different soil tillage management systems, and the second a short-term 10-year trial with two soil tillage managements under three crop rotations. The analysis of the DGGE profiles of the 16S rDNA region of the soil bacterial community, considering the relative band intensity and the band position indicated that the replicates of each treatment were highly similar, with up to 100% similarity, using the standard parameters of the Bionumerics software. To facilitate interpretation but at the same time to reinforce similarity between replicates, Figs. (1 to 3) display the results of two sampling replicates of each treatment. The complexity of bacterial diversity was observed in both experiments, with the profiles consisting of some dominant bands vs. a background of several fainter bands, suggesting numerous groups of less dominant bacterial communities. The analyses also revealed that some bands were common to all treatments, irrespective of the soil tillage or the crop rotation system.

In the long-term experiment, the DNA profiles representative of each treatment showed differences in soil bacterial diversity, with greater diversity under NT (Fig. 1). Overall, when compared to the other soil tillage systems, NT showed

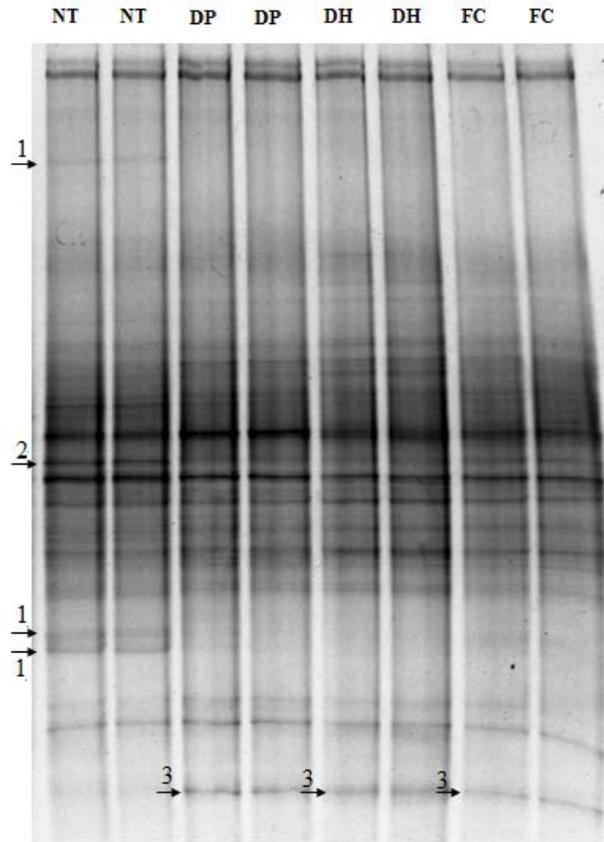


Fig. (1). 16S rDNA-DGGE profiles of soil samples (0-10 cm depth) under different soil tillage systems: NT-no tillage; DP-disc plow; DH-disc harrow; FC-field cultivation. Two out of the four field replicates are used to represent bacterial diversity and homogeneity between replicates. Experiment 1 - Long-term 26-year trial.

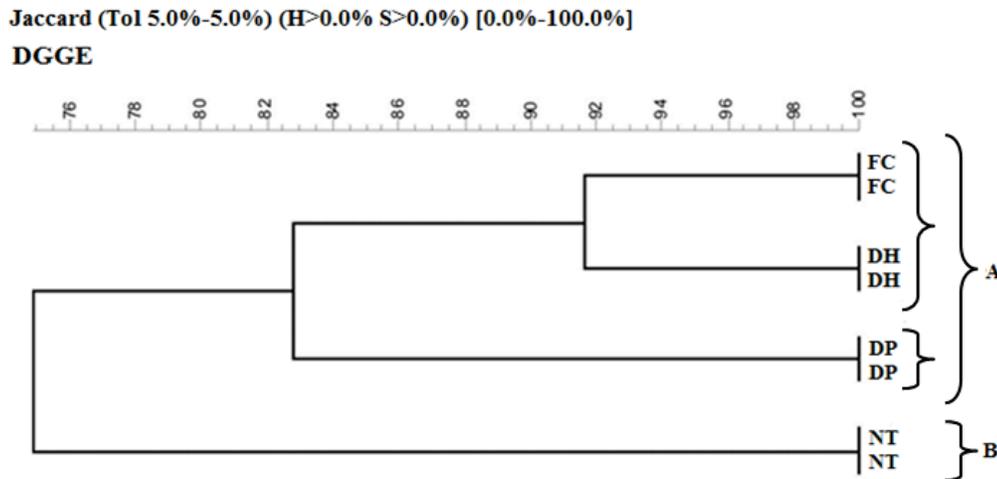


Fig. (2). Similarity dendrogram using the Jaccard coefficient with tolerance of 5% and the unweighted pair-group method with arithmetic averages (UPGMA) for the 16S rDNA-DGGE profiles of soil bacterial communities (0-10 cm layer). Soil tillage: NT-no tillage; DP-disc plow; DH-disc harrow; FC-field cultivation. Crop succession with summer soybean and winter wheat. Two out of the four field replicates are used to represent bacterial diversity and homogeneity between replicates. Experiment 1 - Long-term 26-year trial.

three distinct non-dominant communities (fainter bands), indicated by arrow 1, and one dominant community (more intense band), indicated by arrow 2 (Fig. 1). The DNA fingerprints of treatments with higher soil disturbance (DP, DH and FC) showed similar banding profiles, but differed by exhibiting a non-dominant community (faint band), indicated by arrow 3 (Fig. 1).

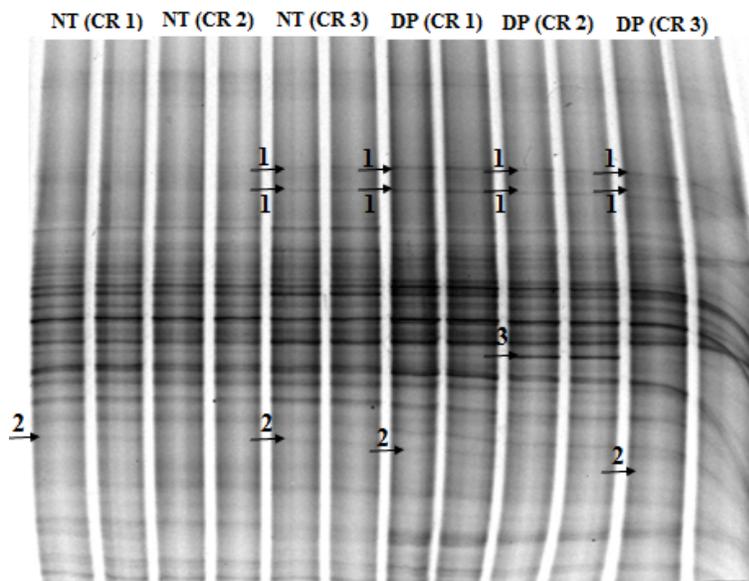
In this first experiment, the 16S DNA profiles from soils under different tillage management systems were split in two main clusters (A and B), joined at a final level of similarity of 75% (Fig. 2). Group A included treatments with tillage disturbance, while group B consisted of plots under NT. It is worth noting that in group A two subclusters were observed, one grouping DH and FC characterized by decreased soil

Table 2. Bacterial Community Diversity Indices¹ Under Different Soil Tillage Management and Crop Rotation Systems

	Greater Diversity		Gradient Of Soil Disturbance		Lower Diversity					
	→									
Bacterial Diversity	Experiment 1— 26-year trial									
	No-Tillage (NT)		Field Cultivation (FC)		Disc Harrow (DH)		Disc Plow (DP)			
Shannon index (H)	3.341 ± 0.077		3.180 ± 0.083		3.099 ± 0.086		3.026 ± 0.089			
Richness index (ACE)	155.9 ± 79.1		77.6 ± 30.7		66.6 ± 26.0		67.6 ± 29.3			
Total bands	31		27		25		23			
Evenness (E)	0.979		0.964		0.962		0.965			
Bacterial diversity	Experiment 2— 10-year trial									
	NT CR 1 ²		NT CR 2		NT CR 3		DP ³ CR 1	DP CR 2	DP CR 3	
Shannon index (H)	3.382 ± 0.064		3.445 ± 0.064		3.326 ± 0.074		3.114 ± 0.069		3.189 ± 0.073	3.226 ± 0.072
Richness index (ACE)	64.0 ± 14.7		86.6 ± 28.5		75.6 ± 24.9		36.2 ± 6.1		46.5 ± 11.8	49.8 ± 12.9
Total bands	32		34		31		25		27	28
Evenness (E)	0.975		0.976		0.968		0.967		0.967	0.968
Mean values										
Systems	NT		CT				CR 1	CR 2	CR 3	
Shannon index (H)	3.384 ± 0.067		3.176 ± 0.071				3.248 ± 0.066	3.317 ± 0.068	3.276 ± 0.072	

¹Values found ± standard error of mean²CR, crop rotation as described in Table 1.³DP, disc plow

SPADE settings: m= 100 (sample size) and K= 4 (cut-off value)

**Fig. (3).** 16S rDNA-DGGE profiles of soil samples (0- 10 cm depth) under two soil tillage and three crop rotation systems: NT-no tillage; DP-disc plow; CR-crop rotation as described in Table 1. Two out of the four field replicates are used to represent bacterial diversity and homogeneity between replicates. Experiment 2 - Short-term 10-year trial.

disturbance and the other consisting of the DP treatment, with the highest level of soil disturbance.

In this long-term 26-year trial, the H was higher for NT than for all other treatments (Table 2). In addition, diversity in the FC treatment was higher than for DP, but not than for DH, and no differences were detected between DH and DP.

In terms of the E, the highest value was observed under NT and the lowest under DH, but they can all be considered high (Table 2).

In the short-term experiment, there was greater diversity of bacterial communities under NT, as follows: NT CR 2 > NT CR 1 > NT CR 3 (Fig. 3). Under bacterial diversity was

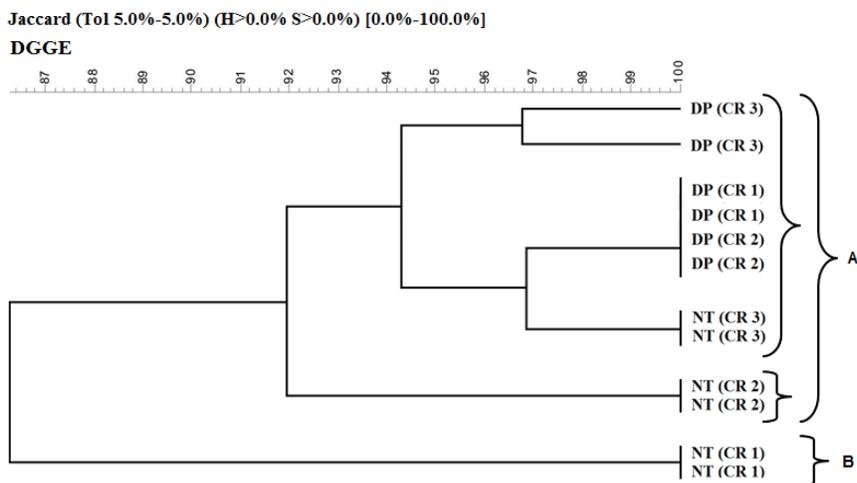


Fig. (4). Similarity dendrogram using the Jaccard coefficient with tolerance of 5% and the unweighted pair-group method with arithmetic averages (UPGMA) for the 16S rDNA-DGGE profiles of soil bacterial communities (0-10 cm layer). Soil tillage: NT-no tillage; DP-disc plow; CR-crop rotation as described in Table 1. Two out of the four field replicates are used to represent bacterial diversity and homogeneity between replicates. Experiment 2 - Short-term 10-year trial.

as follows: CT CR 3 > CT CR 2 > CT CR 1 (Fig. 3). NT CR 3 and DP CR 1, CR 2 and CR 3 exhibited two non-dominant communities, indicated by arrow 1, that were absent in the NT CR 1 and CR 2 (Fig. 3). CR 1 and CR 3 exhibited one non-dominant community, indicated by arrow 2, absent in NT CR 2 and DP CR 2. Finally, one dominant community was observed in DP CR 2 (Fig. 3).

Two main clusters (A and B) were observed in the analysis of the DGGE profiles of the short term experiment, joined at a final level of similarity of 86.4% (Fig. 4). Treatments with and without soil disturbance were grouped in the same cluster A, that was further split in two main subclusters. The first subcluster included DP soil management with all three CRs and NT with the rotation CR 3. DP CR 1 and DP CR 2 exhibited identical profiles and two distinct non-dominant communities were present. The second subcluster of cluster A included NT CR 2. Finally, group B consisted exclusively of NT CR 1 (Fig. 4).

In this short-term 10-year trial, the H and the E indicated that for the NT system the highest values were obtained for CR2, while for the DP system, the values were higher for CR3 (Table 2). However, when the means of the diversity indices of the three crop rotations were considered, fewer effects were observed in CR 2. In contrast, when the means of the tillage treatments were considered, a greater diversity was observed under NT in comparison to DP (Table 2). In both experiments (26 and 10 years), there was no difference in the ACE among the different treatments, taking into account the standard error (Table 2).

DISCUSSION

Analysis of microbial communities in soils under different tillage and crop rotation systems offers an important opportunity for exploring the relationships between soil biotic and abiotic factors. Different soil and crop management systems can result in different substrate availabilities that will ultimately determine by promoting or inhibiting them the establishment of different microbial groups [8, 23]. Our study has shown that the impact caused by soil tillage seems to be more relevant than the effects of different cropping sys-

tems in determining changes in soil bacterial diversity. Furthermore, the qualitative differences in bacterial diversity observed in our study as a function of different tillage systems are in agreement with previous observations of quantitative differences evaluated in terms of the microbial biomass carbon (MB-C) and nitrogen (MB-N) [9, 24, 25]. Most important, they are in agreement with MB-C and MB-N analyses from the same experiments; considering the average MB-C and MB-N values of summer and winter samplings, NT was 49% and 81% higher, respectively, than the CT system [10].

In the first experiment, cluster analysis of the DNA profiles and estimates of the H and E indices have shown that bacterial diversity was greater after 26 years under NT, in comparison to the treatments with different levels of tillage (DP, DH and FC) (Fig. 2, Table 2). The superiority of NT over DP was also confirmed in the second experiment, conducted over a 10-year period (Table 2). Although the E shows a small variation between the different treatments, this demonstrates the uniformity of the profiles of bacterial communities, with dominance of a few communities, regardless of soil tillage and crop rotation. It has been suggested that soil structural improvement under NT favors the environmental conditions needed for re-establishing native microbial genotypes repressed by soil degradation resulting from conventional soil management [3]. It is well known that the accumulation of soil organic C favors soil aggregation, representing a major source of energy and nutrients that stimulates the growth and activity of microorganisms. Indeed, it has been suggested that the degree of soil aggregation could have a higher impact on microbial diversity and community structure than other factors such as soil pH and types of organic compounds [1, 15, 26, 27]. In terms of soil structure, macroaggregates appear to be more sensitive to farming practices, and as they are closely linked to soil organic matter, they should play a key role in the promotion of the soil microbial diversity [3, 4]. The lower soil disturbance in NT systems could also protect its microbial habitats by increasing soil moisture content and by decreasing temperature swings, and both might benefit biodiversity [28]. On the other hand, agronomic management systems could exert se-

lective negative pressure on bacterial diversity and activity [29, 30, 15]. Our study has shown that even tillage systems considered less aggressive to the soil, such as the FC, result in decreased bacterial diversity when compared to the NT system. This means that intensifying soil tillage can eliminate several groups of microorganisms, affecting soil quality.

In the second experiment, when the mean values of the crop rotation systems were considered, only minor effects were observed, the treatment with lupine crop before the sequence maize-fallow-maize being slightly superior (CR 2) (Table 2). First we may suppose that the lack of significant differences between CRs could be attributed to the fallow period in the previous winter (Table 1), as fallow periods may have a negative impact on the soil's microbial community [31]. Another possibility for the lack of differences between the CRs could be related to previous observations [32], in which in a comparison of soil bacterial communities under diverse agricultural land management systems, although soils exhibited similar bacterial diversity, they differed in genetic composition, detected in the sequencing of DNA fragments of the same size.

Contrary to our results, there are studies reporting higher variability in microbial communities in response to different crop rotations [33, 34]. The effects of CR could be attributed to differences in organic matter composition and changes during the decomposition process, modifying the availability of substrates, and consequently microbial diversity [35]. However, different conclusions have been drawn from other studies, demonstrating the complexity of different crop rotation arrangements [1, 16, 36], related mainly to differences in the quality and quantity of the residues added to the soil. Furthermore, in some studies crop rotation has been found to have a lesser effect on diversity than other factors such as soil type, soil tillage, climate and farming practices [37, 38].

The lack of response to CRs in our study is in agreement with our previous quantitative evaluations of soil microbial biomass [9, 24], including MB-C and MB-N analyses in the same field experiment [10], and might be related to the complex composition of the microbial community as a function of a broad range of effects, including soil and environmental factors, such as organic matter content, total N, crop type, soil type and texture, all identified as having a strong impact on microbial community composition and diversity [34].

A single gram of soil has been estimated to contain several thousand species of bacteria [39, 40], and it is questionable whether increases in the biodiversity would enhance or decrease the dominance of certain species. However, a DNA fingerprint evaluated by DGGE does not necessarily provide information about changes in the abundance or activity of organisms, but rather shows the influence of soil tillage on bacterial communities over a longer time [16]. For example, in a study to evaluate the effect of microbial diversity in suppressing soil diseases the authors observed that the dominant microbial community remained mostly intact after rigorous soil treatments, such as fumigation and flooding, although the soil suppressiveness was lost [41]. In this case, disease suppression is likely to depend on more specific interactions between certain groups of microorganisms, which are not necessarily dominant. Consequently, the greater

microbial diversity in the soil is not necessarily associated with better functionality in terms of crop needs.

The main purpose of determining dominant bacterial communities is to provide information that describes general changes in soil, e.g., due to organic amendments or stress factors [42]. In the CT systems, the incorporation of residues into the soil profile results in the dominance of bacteria, while under NT, filamentous fungi are more abundant [43]. Our results confirm that NT also favors bacterial diversity, and that finding can be even more important in the light of recent results from our group showing, by using a metagenomic approach, that bacteria domain plays a far more important role in soil diversity than the other domains [44]. A better understanding about how tillage systems affect the soil microbial community will help in the development of more productive and sustainable systems.

CONCLUSION

In both, the long-term 26-year and the short-term 10-year field trials, differences in soil tillage management systems were the main factors affecting bacterial diversity. The NT system always resulted in significantly greater diversity than the other more disturbing tillage treatments. Intensive soil tillage can therefore eliminate groups of bacteria—as observed in our study—and might compromise soil functionality. Although different crop rotations had only minor effects in bacterial diversity, further studies should be conducted, since the previous fallow period could have minimized the effects. It is also worth mentioning that the results of our study highlight that bacterial diversity analyzed by DGGE may be useful as bioindicator of the changes caused by soil tillage.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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