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## Bacterial Diversity under Different Tillage and Crop Rotation Systems

**Adriana Pereira da Silva<sup>(1)</sup>; Letícia Carlos Babujia<sup>(2)</sup>; Leopoldo Sussumu Matsumoto<sup>(3)</sup>;  
Maria de Fátima Guimarães<sup>(4)</sup>; Mariangela Hungria<sup>(5)</sup>**

<sup>(1)</sup>Estudante, CCA/Universidade Estadual de Londrina, CEP 86051-980, Londrina, PR, [drikapera@yahoo.com.br](mailto:drikapera@yahoo.com.br); <sup>(2)</sup> Estudante, CCE/Universidade Estadual de Maringá, CEP 87020-900, Maringá, PR, [leticia\\_cb@hotmail.com](mailto:leticia_cb@hotmail.com); <sup>(3)</sup>Professor, CCB/Universidade Estadual do Norte do Paraná Campus Luiz Meneghel, CEP 86360-000, Bandeirantes, PR, [leopoldo@uenp.edu.br](mailto:leopoldo@uenp.edu.br); <sup>(4)</sup> Professor, CCA/Universidade Estadual de Londrina, CEP 86051-980, Londrina, PR, [mfatima@uel.br](mailto:mfatima@uel.br); <sup>(5)</sup>Pesquisador, Embrapa soja, CEP 86001-970, Londrina, PR, [hungria@cnpso.embrapa.br](mailto:hungria@cnpso.embrapa.br)

**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. Bacterial diversity was examined in the 0-10 cm layer in two field experiments by analyzing soil DNA using 16S rDNA PCR-DGGE profiles. Experiment 1 was a 26-year trial consisting of 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 2 was a 10-year trial consisting of 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 the 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, but the results could be related to a previous fallow period. The results suggest that the DGGE profiles are sensitive bioindicators of the changes in bacterial diversity caused by soil tillage.

**Keywords:** Soil management, crop management, microbial diversity, PCR-DGGE

**INTRODUCTION** - Soil microorganisms play a key role in the maintenance, functioning and sustainability of agroecosystems (Yin et al., 2010), mainly by regulating carbon (C) and nitrogen (N) cycling, with direct implications on soil fertility and plant nutrition (Gu et al., 2009). The responsiveness of microorganisms to disturbances caused by crop and soil management may lead to changes in the diversity and activity of soil biota (Ndour et al., 2008). Soil microbial communities with high diversity should have greater resilience to stress

(Zhou et al., 2002), and greater functional diversity should be a key element in sustainability.

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 (Carrera et al., 2007).

However, we are far from understanding how soil microbial communities are affected by agricultural practices, as there are still very few studies available covering only a restricted number of ecosystems. 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 bacterial community in two experiments, one long and the other of short-term set up in southern Brazil.

**MATERIALS AND METHODS** - The experiments were carried out at the experimental station of Embrapa Soja in Londrina, Paraná. The soil (Latosolo Vermelho Eutroférico, Brazilian Soil Classification System; Typic Rhodic Eutradox, USA Soil Taxonomy) presented the following physical composition: 710 g kg<sup>-1</sup> of clay, 82 g kg<sup>-1</sup> of silt and 208 g kg<sup>-1</sup> of sand. Two experimental areas were analyzed.

**Experiment 1— 26-year trial** - The experiment consisted of four soil tillage system as treatments: (1) no-tillage (NT); (2) conventional tillage (CT) with disc plow (DP); (3) conventional tillage with field cultivation (FC) and (4) conventional tillage (CT) with light disc harrowing, each with four replications per treatment. The soybean was sown in the summer of 2006/2007.

**Experiment 2— 10-year trial** - The experiment consisted of two types of soil tillage as treatments: (1) NT and (2) CT with disc plow (DP) in the summer and disc harrowing (DH) in the winter and three crop rotations (CR), each with four replications per treatment. Crop rotations are shown in Table 1. In the summer of 2006/2007, both CRs were planted with maize (*Zea mays* L.).

**Soil sampling** - A soil sample of approximately 150 g was then taken from the middle of the square using a shovel. The procedure was repeated eight times in four

replications in the field, at points spatially distributed as representative of the whole area. Samples were homogenized and combined to form two composite samples.

**Soil DNA extraction and PCR amplification of 16S rDNA** - The microbial DNA was extracted from the composite soil samples using the Ultraclean™ Soil DNA Kit, according to the manufacturer's instructions. Two successive amplifications were carried out. The soil DNA was amplified with universal primers fD1(AGAGTTTGATCTGGCTCAG) and rD1(AAGGAGGTGATCCAGCC), as described by Weisburg et al. (1991). The second amplification was performed using the products of the first reaction as a template. The F-968 primers (5'-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCA CGGGGGGAACGCGAAGAACCTTAC- 3') (Nubel et al., 1996), with a GC-clamp (underlined) and L-1401 (5'-GCGTGTGTACAAGACCC- 3') (Muyzer et al., 1993) were used to amplify the 16S rDNA region of approximately 433 bp, corresponding to the V3 hypervariable region.

**DGGE analysis of the bacterial community** - 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 at 60°C and a constant voltage of 100 V for 16 h.

**Statistical analysis of DGGE fingerprints** - The DGGE gels were analysed using Bionumerics software. Similarities between fingerprints were analysed statistically using the unweighted pair-group method with arithmetic averages (UPGMA) and the Jaccard (J) coefficient (Sneath and Sokal, 1973), with a tolerance of 5% to create a distance matrix. The Shannon diversity index (H), evenness index (E) and richness index were analyzed using SPADE software (Species Prediction and Diversity Estimation) (Chao and Shen, 2009).

**RESULTS** - In the first experiment, after 26 years, when compared to the other soil tillage systems, NT showed three distinct non-dominant communities (fainter bands), and one dominant community (more intense band). 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).

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% (Figure 1). Group A included treatments with tillage disturbance, while group B consisted of plots under NT. The Shannon diversity index (H) was higher for NT than all other treatments. In addition, diversity in the FC treatment was higher than for DP, but not DH, and no differences were detected between DH and DP (Table 2). In terms of the evenness index (E), the highest value was observed under NT (0.979) and the lowest under DH (0.962).

In the second experiment, a ten-year trial under two soil tillage management systems (NT and DP) and three crop rotation (CR) systems [CR 1, CR 2, and CR 3 (Table 1)], there was greater diversity of bacterial communities under NT, as follows: NT CR 2 > NT CR 1 > NT CR 3. Under conventional tillage (CT), bacterial diversity was as follows: CT CR 3 > CT CR 2 > CT CR 1. NT CR 3 and DP CR 1, CR 2 and CR 3 exhibited two non-dominant communities, that were absent in the NT CR 1 and CR 2.

Two main clusters (A and B) were observed in the second experiment, joined at a final level of similarity of 86.4% (Figure 2). Treatments with and without soil disturbance were grouped in the same cluster A.

In the analysis of the second experiment, the diversity index (H) and the evenness index (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 both experiments (26 and 10 years), there was no difference in the richness index (ACE) among the different treatments, taking into account the standard error (Table 2).

**DISCUSSION** - 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 as a result of conventional soil management (Peixoto et al., 2006). It is well known that the accumulation of soil organic C favors soil aggregation, representing a major source of energy and nutrients that stimulate 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 factors such as soil pH and types of organic compounds (Yin et al., 2010).

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. On the other hand, agronomic management systems could exert selective negative pressure on bacterial diversity and activity (Wakelin et al., 2008).

However, different conclusions have been drawn from other studies, demonstrating the complexity of different crop rotation arrangements (Yin et al., 2010), 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.

Figueiredo et al. (2007) reported that, in systems under conventional tillage, the incorporation of residues resulted in the dominance of bacteria, while under NT, filamentous fungi were more abundant. In our study, the results confirm that NT also favors bacterial diversity. A better understanding of how tillage systems affect the soil microbial community will help in the development of more productive and sustainable systems.

**CONCLUSIONS** - In both the 26-year and the 10-year field experiments, differences in soil tillage management systems were the main factor 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 also affect soil functionality. Although different crop rotations have only minor effects, further studies should be conducted, since the previous fallow period could have minimized the effects. It is also worth noting that the results of our study confirmed the potential use of DGGE profiles as sensitive bioindicator of changes in soil bacterial communities as a result of soil tillage.

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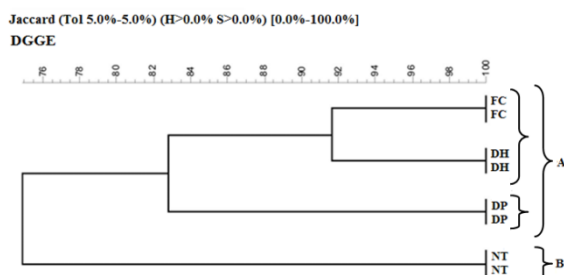
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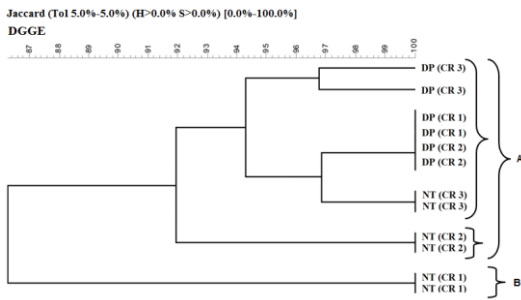
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**Figure 1** - Similarity dendrogram (0 to 10 cm depth) for two replicates of 16S rDNA DGGE profiles of bacterial communities. Soil tillage: NT- no tillage; DP- disc plow; DH- disc harrow; FC- field cultivation. Crop succession with summer soybean and winter wheat. *Experiment 1— 26-year trial.*



**Figure 2** - Similarity dendrogram (0 to 10 cm depth) for two replicates of 16S rDNA DGGE profiles of bacterial communities. Soil tillage: NT- no tillage; DP- disc plow. Crop management: CR- crop rotation as described in Table 1. *Experiment 2— 10-year trial.*

**Table 1** - Crop rotation schemes adopted from 2003 to 2007 in Experiment 2.

Crop management	Summer 02/03	Winter 03	Summer 03/04	Winter 04	Summer 04/05	Winter 05	Summer 05/06	Winter 06	Summer 06/07
CR 1	S <sup>1</sup>	W	S	L	M	O	S	F	M
CR 2	M	W	S	O	S	L	M	F	M
CR 3	M	W	M	O	M	O	M	F	M

<sup>1</sup>S, soybean; M, maize; W, wheat; O, black oat; L, lupine; F, fallow

**Table 2** - Bacterial community diversity indices<sup>1</sup> under different soil tillage management and crop rotation systems.

Bacterial diversity	GRADIENT OF SOIL DISTURBANCE						
	Greater diversity				Lower diversity		
<i>Experiment 1— 26-year trial</i>							
	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			
<i>Experiment 2— 10-year trial</i>							
	NT CR 1 <sup>2</sup>	NT CR 2	NT CR 3	DP <sup>3</sup> 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	
<i>Mean values</i>							
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

<sup>1</sup>Values found ± standard error of mean

<sup>2</sup>CR, crop rotation as described in Table 1.

<sup>3</sup>DP, disc plow

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