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Microbial diversity in an Oxisol under no-tillage and conventional tillage in southern Brazil[1]

Diversidade microbiana em um Latossolo Vermelho sob plantio direto e convencional no sul do Brasil

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ABSTRACT - The no-tillage (NT) system of soil management is recognized as more sustainable than conventional tillage (CT), with an important role played by soil microorganisms. The objective of this study was to estimate differences in soil microbial diversity under NT and CT at different soil depths. For that, bacterial (16S rDNA) and fungal (18S rDNA) communities were evaluated by denaturing gradient gel electrophoresis (DGGE) in a 20-year field experiment established on an Oxisol in southern Brazil on which soybean has been grown in the summer and wheat in the winter. Soil samples were collected at the depths of 0-5, 5-10, 10-20 and 20-30 cm, and submitted to DGGE analyses. The results revealed lower similarity (28%) between bacterial communities in the NT and the CT systems at the 0-5 cm layer. The Shannon index (H) confirmed higher bacterial diversity with NT at all depths, when compared to CT. In relation to fungal communities, higher diversity was detected with CT, especially at the 0–5-cm depth. The results indicate that fungal communities can be more tolerant to environmental stresses related to soil disturbance than bacteria. More emphasis should be given for understanding processes affecting the diversity of microorganisms in agricultural soils, with particular emphasis on tillage systems.

Key words: Bacterial diversity. Fungal diversity. PCR-DGGE. Soil tillage.

RESUMO - O sistema de plantio direto (PD) é reconhecido como mais sustentável que o plantio convencional (PC), desempenhando um importante papel na atividade dos microrganismos do solo. O objetivo deste estudo foi estimar diferenças na diversidade microbiana do solo sob PD e PC, em diferentes profundidades. Para isso, foram avaliadas as comunidades bacteriana (16S rDNA) e fúngica (18S rDNA) através da eletroforese em gel com gradiente desnaturante (DGGE), em um experimento de campo estabelecido há 20 anos em um Latossolo Vermelho no sul do Brasil, com a soja cultivada no verão e trigo no inverno. As amostras de solo foram coletadas nas profundidades de 0-5; 5-10; 10-20 e 20-30 cm, e foram submetidas a análises de DGGE. Os resultados revelaram baixa similaridade (28%) na profundidade de 0-5 cm para as comunidades bacterianas entre o sistema de PD e PC. O índice de Shannon (H) confirmou diversidade bacteriana superior no PD em todas as profundidades, quando comparado ao PC. Em relação às comunidades fúngicas, a maior diversidade foi detectada no sistema de PC, especialmente na profundidade de 0-5 cm. Os resultados indicam que as comunidades fúngicas podem ser mais tolerantes a estresses ambientais relacionados com a perturbação do solo que as bactérias. Maior ênfase deve ser dada para compreender os processos que afetam a diversidade dos microrganismos em solos agrícolas, com particular destaque para os sistemas de preparo do solo.

Palavras-chave: Diversidade bacteriana. Diversidade fúngica. PCR-DGGE. Manejo do Solo

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INTRODUCTION

The adoption of the no-tillage (NT) over the conventional tillage (CT) system has significantly increased worldwide. Brazil is probably the best example of the widespread adoption of NT at over 30 million hectares, accounting for about 70% of grain production (FEBRAPDP, 2012). Numerous studies have reported improvements in soil-chemical and physical properties due to NT, with an emphasis on erosion control (LAL, 2007), as well as benefits to soil microorganisms, reflecting in higher crop productivity and improved soil quality (KASCHUK; ALBERTON; HUNGRIA, 2010; SILVA *et al*., 2010). The great majority of these studies have focused on soil microbial biomass (KASCHUK; ALBERTON; HUNGRIA, 2010), specific microorganisms (HANSEL *et al*., 2008; HUNGRIA *et al*., 2001), or microbial enzyme activities (BALOTA *et al*., 2013). However, the combined analyses of several microbial parameters provide better information about the quality and quantity of microbial communities (JOYNT *et al*., 2006).

Few studies have reported the effects of tillage on the diversity of microbial communities, whereas it is important to understand responsiveness and resilience of microorganisms to management practices and how changes in the composition of soil microorganisms may affect soil quality (WARDLE *et al*., 1999). Furthermore, recent studies suggest that evaluations of soil microorganisms can show changes in soil quality prior to alterations in physical or chemical parameters (BABUJIA *et al*., 2010; HUNGRIA *et al*., 2009; KASCHUK; ALBERTON; HUNGRIA, 2010).

The development of methods for assessing microbial diversity has contributed to our understanding of the structure and functioning of microbial communities in soil. Denaturing gradient gel electrophoresis (DGGE) is increasingly used in studies of microbial ecology, although it has some limitations, e.g. with DGGE-PCR, more-abundant communities sometimes prevail (SMIT *et al*., 1999), and some fungi or bacteria may generate multiple bands (MUYZER *et al*., 1993). Therefore, DGGE may be more suitable for comparative studies (MUYZER *et al*., 1993).

In a previous study of our group, we described quantitative differences between CT and NT at several soil depths, evaluated by the microbial biomass of carbon and nitrogen and microbial activity (basal respiration and microbial quotient, *q*Mic) (BABUJIA *et al*., 2010). The objective of this study was to expand our findings by evaluating changes in bacterial and fungal diversity with depth under NT and CT in the same 20-year-old trial. The information contributes to our still-poor knowledge of microbial diversity in soils in Brazil as affected by soil management.

MATERIAL AND METHODS

Experimental design and soil management

The experiment was conducted in a Rhodic Eutrudox (Latossolo Vermelho Eutroférrico, Brazilian classification), established in the summer of 1988/89 in Londrina, Paraná state, southern Brazil, located at latitude 23°11' S and longitude 51°11' W, and at an altitude of 620 m. The climate is subtropical humid, with average annual temperature of 21 °C; the average maximum temperature is of 28.5 °C in February and the minimum of 13.3 °C in July, respectively. The average annual rainfall is 1,651 mm, with January being the wettest month (217 mm) and August the driest (60 mm).

The experiment consists of plots of 7.5 m wide x 30.0 m long (225 m^2), with four replicates per treatment, arranged in a randomized block design. For this study we used the treatments under two soil management practices: (1) no-tillage (NT), with sown directly through the residues of the previous crop, with the opening of only a narrow channel line for sowing [ranging from 1.5 cm to 2 cm for the wheat (*Triticum aestivum* L.) and 3 cm for the soybean (*Glycine max* (L.) Merr.)]; (2) conventional tillage (CT), where the soil is prepared annually with a disc plow (plowing varies between 20 and 25 cm) and harrow (15 cm range). The sequence of soybean in summer and wheat in the winter has always been applied for both NT and CT.

Soil sampling

At the time of sampling, the experiment was 20 years old. Samples were taken when soybean was at full bloom (R2). Samples were collected at four different depths: 0-5, 5-10, 10-20 and 20-30 cm in the central part of each plot (four replicates per treatment). A trench of 20 cm wide x 50 cm in length x 60 cm depth was opened, from which the soil samples were collected with a spatula, from the midpoint of each layer and at the four sides of the trench. Subsamples were used to compose a sample of bulk soil (approximately 1.0 kg). At the laboratory the samples of each treatment were mixed and sieved (<4 mm, 5 mesh) and then stored in plastic bags at -20 ºC until the analyses.

DNA extraction from soil

The DNA was extracted from soil samples (0.25 g) using the soil UltraClean™ DNA kit (MoBio Laboratories, Inc., California, USA) following the manufacturer's protocol. The concentration of DNA was analyzed in 1% (w/v) agarose gel in 1X TBE, to verify the quantity, the purity and the

molecular size, using standard DNA lightweight Mass™ (Invitrogen Life Technologies). The amount of DNA was visually assessed by electrophoresis on agarose gels stained with 0.00005% ethidium bromide.

Specific PCR conditions for bacterial and fungal communities

Two successive amplifications were performed for the V3 hypervariable region encoding the 16S rRNA gene. First, the soil DNA was amplified with primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3'), as described by Weisburg *et al*. (1991), which amplify almost the entire region of the DNA encoding the 16S rDNA (approximately 1,500 bp).

The first PCR reaction consisted of 36.6 µL deionized water; 3.0 µL dNTPs (deoxynucleotides) 1.5 mmol L^{-1}; 1.5 µL $MgCl_2$ 50.0 mmol L^{-1}; 5.0 µL 10X buffer (20.0 mmol L^{-1} Tris-HCl, pH 8.4); 1.5 µL of each primer (fD1 e rD1) 10.0 pmols; 0.2 µL 5U of Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA) and 1.0 µL of extracted soil DNA (~30 ng) in a total volume of 50.0 µL. The PCR program consisted of: an initial denaturation at 95 ºC for 2 min; 15 cycles of denaturation at 94 ºC for 15 s; 93 ºC for 45 s; primer annealing at 55 ºC for 45 s, and extension at 72 ºC for 2 min; holding at 4 ºC until removal. The amplification was performed in a thermocycler MJ Research, Inc., model PTC-200.

The second amplification was performed using 1.0 µL (~20 ng) of the products of the first reaction as template. The primers F-968, (5´-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGACGGGGAACGCGAAGAACCTTAC-3´) with a GC-clamp (underlined) and R-1401 (5´-GCGTGTGTACAAGACCC-3´) (NÜBEL *et al*., 1996) were used to amplify the 16S rDNA region of approximately 430 bp, corresponding to the V3 hypervariable region. PCR mixtures were prepared with: 5.0 µL dNTPs 1.5 mmol L^{-1}; 1.3 µL $MgCl_2$ 50.0 mmol L^{-1}; 2.5 µL buffer 10X [20.0 mmol L^{-1} Tris- HCl (pH 8.4)]; 1.0 µL of each primer (F-968 and R-1401) 10.0 pmoles; 0.2 µL 5 U of Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA); 1.0 µL of the PCR product of the first reaction with fD1 and rD1 primers (~10.0 ng); sterile ultrapure water to complete a final volume of 25.0 µL. The following amplification cycles were used: one initial denaturation cycle at 94 ºC for 2 min; 2 cycles at 94 ºC for 1 min, at 60 ºC for 2 min, and at 72 ºC for 2 min; 2 cycles at 94 ºC for 1 min, at 59 ºC for 2 min, and at 72 ºC for 2 min, 94 ºC for 1 min, 58 ºC for 2 min, 72 ºC for 2 min (2 cycles); 94 ºC for 1 min, 57 ºC for 2 min; 72 ºC for 2 min (2 cycles); 94 ºC for 1 min, 56 ºC for 2 min, 72 ºC for 2 min (2 cycles); 94 ºC for 1 min, 55 ºC for 2 min, 72 ºC for 2 min; and for 10 min at 72 °C; holding at 4 ºC until removal. Amplification was confirmed by running 2.0 µL of PCR product in a 1% (w/v) agarose gel in 1X TBE, staining with ethidium bromide (0.3 µg mL^{-1}) and visualizing under UV light. The products of the amplification were subjected to the DGGE analysis as described by Silva *et al*. (2013).

The 18S rDNA was amplified with the primers EF4f (5'-GGAAGGGATGTATTTATTAG-3') and EF3r (5'-TCCTCTAAATGACCAGTTTG-3'), as previously described by Smit *et al*. (1999), generating fragments of approximately 1,500 bp. The reactions were carried out with 29.65 µL of deionized water; 6.6 µL dNTPs 1.5 mmol L^{-1}; 3.75 µL $MgCl_2$ 50.0 mmol L^{-1}; 10.0 µL 10X buffer (20.0 mmol L^{-1} Tris-HCl, pH 8.4); 1.0 µL of primer EF4f (10 pmol); 1.0 µL of primer EF3r (10 pmol); 0.6 µL of Taq DNA polymerase (5U) and 2.0 µL of DNA (approximately 30.0 ng). The reactions were performed in a thermocycler MJ Research, Inc., model PTC-200, as follows: 94 °C for 3 min (1 cycle); 94 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min (25 cycles); 72 °C for 10 min (1 cycle); holding at 4 ºC until removal.

For the DGGE analysis, the product of the first PCR reaction was diluted 1:2000 and used as template for a second PCR with primers based on the fungal EF4f and NS3r (5´-CGCCCGCCGCGCCCCGCGCCCGGCCCGCGCCCCCGCCCCGGCTGCTGGCACCAGACTTG-3') with a GC-clamp (underlined) (WHITE *et al*., 1990) to produce fragments of 530 bp. The dilution was necessary to ensure the formation of more specific bands. The PCR was performed with 29.65 µL of deionized water; 6.6 µL dNTPs 1.5 mmol L^{-1}; 3.75 µL $MgCl_2$ 50 mmol L^{-1}; 5.0 µL 10X buffer (20.0 mmol L^{-1} Tris-HCl, pH 8.4); 1.0 µL of primer EF4f (10.0 pmol); 1.0 µL of primer NS3r (10.0 pmol); 1.0 µL of Taq DNA polymerase (5U) and 2.0 µL of the first PCR product diluted. The program used for the second PCR was as follows: initial denaturation at 94 °C for 4 min (1 cycle); 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min (10 cycles); 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min (15 cycles) and 72 °C for 5 min (1 cycle); holding at 4 ºC until removal. PCR products were verified by electrophoresis on agarose gel (1.5%).

Bacterial and fungal analysis using electrophoresis in denaturing gradient gel (DGGE)

The fragments of 16S rDNA were separated in a 6% (w/v) polyacrylamide gel (ratio of acrylamide: bisacrylamide, 37.5:1 w/w) denaturing gradient gels containing 35% to 60% of urea (100% denaturant corresponding to 7 mol L^{-1} urea and 40% formamide). The gel received 20.0 µL of PCR products of the second

16S rDNA amplification and 10.0 µL of loading buffer (2% cyanol bromophenol blue xylene 2% glycerol and 100% deionized water). Electrophoresis was performed in a vertical DCode (BioRad) at 0.5 X TAE buffer (10.0 mmol L^{-1} Tris-acetate and 0.5 mmol L^{-1} EDTA, pH 8.3), with the voltage of 200 V at 60 °C for 16 h.

The fragments of 18S rDNA were separated in an 8% (w/v) polyacrylamide gel (acrylamide: bisacrylamide, 37.5:1 (w/w) containing 30% to 55% of urea (100% denaturant corresponding 7 mol L^{-1} urea and 40% formamide). Twenty µL of the PCR product and 10.0 µL of loading buffer were applied to the gel and then subjected to electrophoresis in the DCode system, as described for the bacterial community, with 1 X TAE buffer, and the voltage of 85 V at 55 °C for 17 h.

After electrophoresis, the gels were stained with ethidium bromide solution for 3 min and visualized under UV light.

Data Analysis

The PCR-DGGE profiles were analyzed using the Bionumerics software (Applied Mathematics, Kortrijk, Belgium, v.4.6). The similarities between the fingerprints were statistically analyzed using the UPGMA algorithm with the Jaccard coefficient (SNEATH; SOKAL, 1973) and the tolerance index of 5%.

The profiles obtained at each depth and soil management system were also analyzed with the program Spade ("Prediction of species and diversity of estimation") (CHAO; SHEN, 2013) with a sample size of 100 and a cut-off of 4.0. The Shannon-Weaver index of diversity (H) was used to compare changes in diversity of microbial community structure in soils, and calculated using the equation proposed by Shannon and Weaver (1963), where N_i is the height of a peak and N is the sum of all peak heights of the curve.

$$H = -\sum_{i=1}^{s} P_i \ln P_i = -\sum_{i=1}^{s} (N_I / N) \ln (N_I / N) \quad (1)$$

$$E_{II} = H / H_{\max} = H / \ln S \quad (2)$$

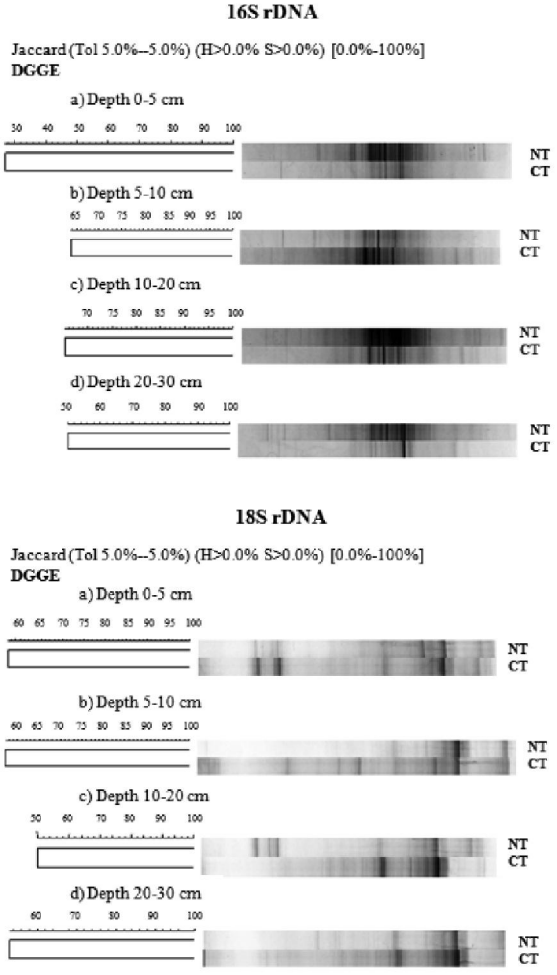
For each community the ACE index (abundance-based coverage estimator), a non-parametric index proposed by Chao and Lee (1992) was also estimated.

RESULTS AND DISCUSSION

The PCR-DGGE profiles of the 16S rDNA communities showed that some bands were common to all depths, irrespective of the soil-tillage system. In addition, the 0-5 cm layer of the NT soil had four dominant bands (more intense bands) that were absent in the CT system. The analysis of the profiles based on band position indicated lower similarity (28%) between bacterial communities of the NT and the CT systems in the 0-5 cm layer (Figure 1). For the 5-10 and 10-20 cm layers, the similarity between NT and CT was 65% and in the 20-30 cm layer it was 50% (Figure 1).

The DGGE profiles of the soil-bacterial communities showed greater diversity, when estimated by the Shannon diversity index, at all depths with NT in comparison to CT (Table 1). For the bacterial

Figure 1 - Similarity dendrogram using the Jaccard coefficient with tolerance of 5% and the unweighted pair-group method with arithmetic averages (UPGMA) for the 16S rDNA and 18S rDNA-DGGE profiles of soil bacterial and fungal communities, respectively, at different soil depths, under no-tillage (NT) or conventional tillage (CT). Profiles for each system and depth are representative of all replicates



communities, the richness indices (ACE) showed no differences between NT and CT. In relation to the evenness index (E), the values can be considered high in both tillage systems and at all depths; however, the highest value was observed under NT and the lowest under CT, both in the 0-5 cm layer (Table 1).

The PCR-DGGE fingerprints of the 18S rDNA region in the 0-5 cm layer showed two distinct dominant communities (more intense bands), and one non-dominant community (fainter bands) in the CT system that were absent in with NT. The fungal communities were similar (60%) between NT and CT at the depths of 0-5 cm and 5-10 cm (Figure 1). For the 10-20 and 20-30 cm layers, the similarities were 50% and 54%, respectively.

The diversity index for the fungal community was higher in the CT than in the NT system, except for the 5-10 cm layer, and the highest diversity was found in the 0-5 cm layer (Table 2). For the NT, no differences in diversity were found at the different depths. The richness indices were also higher in the CT system in the 0-5 and 10-20 cm layers (Table 2). As for bacteria, the evenness indices were high in all treatments, and

Table 1 - Number of DGGE bands and Shannon's diversity index[1] (H) of soil bacterial community (16S rDNA) as influenced by soil management and depth

Bacterial diversity	No-Tillage (NT)			
	0-5 cm	5-10 cm	10-20 cm	20-30 cm
Shannon Index (H)	3.288 ± 0.055	3.099 ± 0.079	3.340 ± 0.057	3.232 ± 0.074
Richness Index (ACE)	38.2 ± 4.9	44.8 ± 12.4	41.9 ± 5.5	61.0 ± 19.3
Total bands	29	25	31	28
Evenness (E)	0.975	0.963	0.973	0.970
Bacterial diversity	Conventional Tillage (CT)			
	0-5 cm	5-10 cm	10-20 cm	20-30 cm
Shannon Index (H)	2.649 ± 0.077	2.811 ± 0.079	2.936 ± 0.085	2.966 ± 0.088
Richness Index (ACE)	30.0 ± 9.9	31.2 ± 8.0	40.1 ± 12.9	40.3 ± 12.3
Total bands	20	18	21	22
Evenness (E)	0.884	0.972	0.964	0.959

[1]Values ± standard error of mean; SPADE settings: m=100 (sample size) and K=4 (cut-off value)

Table 2 - Number of DGGE bands and Shannon's diversity index[1] (H) of soil fungal community (18S rDNA) as influenced by soil management and depth

Fungal diversity	No-Tillage (NT)			
	0-5 cm	5-10 cm	10-20 cm	20-30 cm
Shannon Index (H)	2.427 ± 0.068	2.558 ± 0.08	2.306 ± 0.096	2.539 ± 0.095
Richness Index (ACE)	13.8 ± 1.9	22.6 ± 6.0	14.9 ± 3.3	22.0 ± 5.6
Total bands	12	14	11	14
Evenness (E)	0.977	0.969	0.962	0.962
Fungal diversity	Conventional Tillage (CT)			
	0-5 cm	5-10 cm	10-20 cm	20-30 cm
Shannon Index (H)	2.807 ± 0.065	2.546 ± 0.150	2.654 ± 0.092	2.558 ± 0.076
Richness Index (ACE)	21.7 ± 2.8	18.4 ± 3.4	24.3 ± 6.8	17.3 ± 2.8
Total bands	18	14	16	14
Evenness (E)	0.971	0.965	0.957	0.969

[1]Values ± standard error of mean; SPADE settings: m=100 (sample size) and K=4 (cut-off value)

the highest values were found in both tillage systems at the 0-5 cm depth (Table 2). Although the evenness index showed small variations among treatments (Table 1 and 2), the uniformity of profiles of bacterial and fungal communities implies dominance of a few communities, regardless of soil-tillage system.

Previous studies have shown that microbiological parameters are more sensitive to disturbance than soil chemical and physical parameters and also that NT can substantially increase microbial activity, showing that microorganisms can be used as bioindicators of soil quality (BABUJIA *et al*., 2010; HUNGRIA *et al*., 2009; NAKATANI *et al*., 2011). In one of these experiments, evaluations were performed on the same treatments as in our present study, which detected differences in microbial biomass and activity, as well as in the stocks of soil C and N in NT versus CT, and also with depth (BABUJIA *et al*., 2010). Considering the whole soil profile of 0-60 cm, the NT system resulted in considerably higher microbial biomass of C (35%) and of N (23%) than the CT (BABUJIA *et al*., 2010). Therefore, it was important to investigate whether increased microbial biomass under NT is associated or not with higher microbial diversity. Our study has now shown clear differences between the structures of the bacterial and fungal communities at different depths under NT and CT.

To develop cropping practices that ensure optimal use and protection of soil biodiversity, the main challenge is to predict impacts of tillage systems on soil organisms. As shown in our study, the bacterial diversity was more sensitive than the fungal community to soil disturbances. In the most superficial layer, of 0-5 cm, NT showed greater bacterial diversity than CT (Table 1). In our previous study, at this same layer, microbial biomass-C was 82% higher in the NT system in comparison to the CT and also presented a lower value of qCO_2, indicating higher microbial metabolic efficiency (BABUJIA *et al*., 2010). Therefore, the combined results of both studies indicate that, at the 0-5 cm layer, the NT system has greater bacterial diversity, as well as greater microbial biomass and metabolic efficiency. Altogether, this might contribute to decreases in CO_2 emissions (BOND-LAMBERTY; WANG; GOWER, 2004) and increases in soil organic C (BABUJIA *et al*., 2010).

The lower bacterial diversity in the 0-5 cm layer under CT (Table 1) might be related to the intense soil disturbance, reducing the macroaggregates that represent important niches for protection and preservation of soil organic matter and microorganisms (LÓPEZ- GUARRIDO *et al*., 2012). In addition, the redistribution of the residues within the profile with CT appears to impoverish the superficial layer in C sources, which might be reflected in decreased diversity of specific groups of microorganisms. For example, it has been reported that the diversity of proteolytic bacteria in an agricultural soil was higher near the surface because of the greater abundance and variety of substrates (FUKA *et al*., 2009). On the other hand, the lower bacterial diversity with CT does not necessarily mean that soil function will be affected because there may be physiological redundancy (KENNEDY, 2003); however, our results truly show that bacteria are more sensitive than fungi to soil-tillage practices.

Studies indicate that soil type and management system affect the structure of bacterial communities (LÓPEZ- GUARRIDO *et al*., 2012). In addition, soil depth is a key factor determining the dynamics of soil microorganisms (BABUJIA *et al*., 2010; WANG; CUI; WANG, 2009); also, diversity of functional groups may be limited with soil depth, as a result of changes in physical and chemical attributes (FRANZLUEBBERS, 2002).

Previous studies show that fungi are more abundant with NT than with CT (WANG *et al*., 2010). Lower fungal diversity with CT may be explained in terms of the impact of disc plowing, i.e. breaking soil aggregates and fungal hyphae (CORNEJO, YEAR; RUBIO, YEAR; BORIE, 2009; KHIARA *et al*., 2012). However, our results, especially for the 0-5 cm layer do not agree with this observation (Table 2). Our findings of higher fungal diversity under CT might be explained by the lower pH of the NT soil, as previously shown (BABUJIA *et al*., 2010), and, indeed, Wakalin *et al*. (2008) suggested that pH is the strongest factor linked to soil catabolic function and biological community structure. It is well known that fungi have optimal growth in the 2.0 to 7.0 pH range, whereas, for bacteria, the best range is between pH 5.0 and 9.0 (SMITH and DORAN, 1996). Our results are also consistent with a metagenomic analysis in a similar area at the same experimental station, showing greater abundance of fungi with CT than with NT, and the authors suggested that it may be related to higher tolerance of fungi to environmental stresses (SOUZA *et al*., 2013).

The results from our study confirm that evaluation of microbial communities can provide valuable ecological indicators of soil health, as they are particularly sensitive to external influences and prematurely reflect changes related to disturbance (KASCHUK; ALBERTON; HUNGRIA, 2010; POWLSON, 1994). Our results also emphasize the importance of evaluating not only quantitative but also qualitative microbial parameters to gain better understanding of the effects management practices have on soil quality.

CONCLUSION

In a long-term field experiment performed in an Oxisol of southern Brazil, we have shown that bacterial diversity was higher with NT than with CT, especially in the 0-5 cm layer. In contrast, the diversity of fungi was higher in the CT than in the NT system, mainly at the top soil layer.

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