ORIGINAL PAPER

A decade of land use contributes to changes in the chemistry, biochemistry and bacterial community structures of soils in the Cerrado

R. S. Peixoto · G. M. Chaer · N. Franco ·
F. B. Reis Junior · I. C. Mendes · A. S. Rosado

Abstract The bacterial community structures (BCSs) of Cerrado soils cultivated under conventional tillage (CT), no-tillage (NT) and under native Cerrado (NC) vegetation were evaluated using PCR/DGGE of bacterial 16S rRNA (*rrs*) and *rpoB* genes and of *Pseudomonas* group genes. Soil chemical analysis, microbial biomass and the enzyme activities were also evaluated and correlated with the BCS measurements. The multivariate ordinations of DGGE profiles showed differences between the BCS of the NC area and those from cultivated areas. The BCSs of the CT and NT areas also differed in all DGGE fingerprints, including changes in the profile of *Pseudomonas* populations, indicating that agricultural systems can also be responsible for changes within specific microbial niches, although the clearest differences were found in the *rpo*B profiles. The MRPP analysis demonstrated significant differences between the BCSs from different soil layers of NT areas based on all gene fingerprints and those of NC areas based on bacterial 16S rRNA and *rpoB* genes fingerprints. No differences were observed in the microbial fingerprints of CT samples from different depths, indicating that ploughing affected the original BCS stratification. The BCS from NC areas, based on all gene fingerprints, could be related to higher levels of soil acidity and higher amounts of MBC and of phosphatase activity. In contrast, the BCSs from cultivated areas were related to higher levels of Ca + Mg, P and K, likely as a result of a history of chemical fertilisation in these areas. The relationships between *rpo*B and *Pseudomonas* BCSs and all chemical and biochemical properties of soils were significant, according to a Mantel test ($P < 0.05$), indicating that the different changes in soil properties induced by soil use or management may drive the formation of the soil BCS.

Keywords Cerrado · No-tillage · Conventional tillage · Bacterial community structure

R. S. Peixoto (✉) · N. Franco · A. S. Rosado
LEMM—Laboratório de Ecologia Microbiana Molecular, Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
e-mail: raquelpeixoto@micro.ufrj.br

G. M. Chaer
Embrapa Agrobiologia, Seropédica, RJ, Brazil

F. B. Reis Junior · I. C. Mendes
Embrapa Cerrados, Planaltina, DF, Brazil

Introduction

The Cerrado biome (tropical savannah) occupies about 20% of the territory of Brazil and is characterised by high average temperature (22–27°C), rainfall (800–1,600 mm) and solar radiation (475–500 Cal cm^{-2} day^{-1}) (Adámoli et al. 1987; Ratter et al. 1997; Castro et al. 2008). Soils in this biome are highly weathered, which makes them acidic, poor in

nutrients and rich in iron and aluminium oxides. However, the excellent physical structure of these soils, coupled with the technological advances in agriculture that have occurred during the last 30 years, has made the Cerrado the leading grain production area in Brazil (Machado and Silva 2001), converting a great part of this biome into pastures and agricultural fields (Marris 2005). The Cerrado, like the Atlantic Forest biome, is a hotspot of biodiversity in Brazil (Myers et al. 2000). Besides the great ecological and economical importance of this biome, little is currently known about the biodiversity of the Cerrado, especially its soil microbial diversity (Castro et al. 2008).

Driven by the need for sustainable soil management methods, farmers worldwide are reducing or even eliminating the use of tillage (Machado and Silva 2001). The direct seeding method, also called the "no-tillage" (NT) method, has been an important alternative management in Cerrado areas (Mendes et al. 2003; Cook 2006). In 2009, NT had been adopted in 40% of the area cultivated with soybeans (*Glycine max*) and corn (*Zea mays*) in Brazil (Embrapa 2009). The NT system is considered beneficial to the environment due to the remarkable increase in the soil's water infiltration rate, the diminution of erosion and nutrient loss, the increase in organic matter content in the upper soil layer and the greater economic stability of rural communities using this system of farming (Machado and Silva 2001). Studies have also shown that the shift from conventional tillage (CT) management to NT in Cerrado areas has led to changes in microbial biomass and activity (Valpassos et al. 2001; Mendes et al. 2003).

In spite of the profound changes in soil physical, chemical and biological properties caused by the different agricultural management methods adopted in Cerrado soils, very few studies have investigated how these changes affect soil microbial community structure. For instance, Castro et al. (2008) showed a remarkable difference in soil fungal communities among a NC area, a pasture and a soybean plantation, using ribosomal intergenic spacer analysis. Peixoto et al. (2002) described a pioneering study using the universal *rpoB* gene (RNA polymerase *β*-subunit gene), as an alternative to the 16S rRNA gene, to evaluate total bacterial soil communities of Cerrado soils. In a later study, the use of this approach to compare tillage and NT in Cerrado soils was shown to

be efficient in detecting changes in the soil bacterial community structure (BCS) caused by alterations in soil conditions (Peixoto et al. 2005). The authors suggested that it would also be important to evaluate alterations in the profiles of some specific microbial groups of agricultural interest, such as the *Pseudomonas* group, which has a prominent role in several processes important to soil quality and functioning and includes many species of plant growth-promoting rhizobacteria (PGPR) (Timms-Wilson et al. 2004; Bardas et al. 2009; Kumar et al. 2009; Recep et al. 2009; Rosas et al. 2009).

The different agricultural systems adopted in the Cerrado are known to alter physico-chemical and biological soil characteristics, but it is still unclear how these changes affect the soil BCS. The knowledge of how microbial diversity and function are affected by abiotic variations caused by agricultural management is fundamental for conducting sustainable practices in this biodiversity hotspot (Cerrado soils). The aim of this work was to evaluate the BCS using DGGE analysis (evaluating *rpoB* and 16S rRNA genes and the specific *Pseudomonas* 16S rRNA gene) in Cerrado soils under long-term cultivation with either CT or NT management systems comparing these results with those obtained from a nearby NC area. The BCS results where then correlated to several chemical and biochemical soil properties to evaluate the relationships between biotic and abiotic factors and indicate how changes in soil properties induced by soil use or management may drive the formation of the soil BCS.

Materials and methods

Study site and soil sampling

The studied site is located at the experimental station of Embrapa Cerrados, DF, Brazil (15°36′34″ S and 47°44′36″ W). The area has a typical tropical warm humid climate, with an annual mean air temperature of 21°C, ranging from a mean of 17°C in the winter to a mean of 28°C in the spring and summer. Annual rainfall averages 1,400 mm and is concentrated in the period from September to April. The soil at the experimental site was classified as a clayey Typic Haplustox according to Soil Taxonomy (Soil Survey Staff 1999). The area relief is mostly flat.

Soils were sampled from two neighbouring areas of 320 m × 50 m cultivated with annual crops (corn/soybean rotation) under either NT or CT systems since 1992. The CT area was annually prepared by ploughing and harrowing the soil before planting the commercial crop and for incorporation of weeds after harvest, whereas the NT area was managed without ploughing or harrowing. A nearby area of NC (with the same soil classification) was also sampled to serve as a reference for the original soil conditions.

Soils were sampled from each area in January of 2003 during the rainy season (summer), at depths of 0–5 cm and 5–20 cm. For the depth of 0–5 cm, soil samples were collected with a knife after making a small pit. The 5- to 20-cm samples were taken with a soil probe. Samples were taken in triplicates from each sampling area (under NT, CT or NC). Ten sub-samples were taken from each replicate to form a composite sample.

Soil chemical analysis

Soil samples were air-dried and sieved through a 2-mm mesh for chemical analysis using routine methods (Embrapa 1997). Soil pH was measured at a soil: water ratio of 1:2.5 by weight. Ca, Mg and Al were extracted with 1 N KCl and quantified through atomic absorption (Ca and Mg) and titration with NaOH 0.025 M (Al); P and K were extracted using the Mehlich 1 (H_2SO_4 0.0125 M + HCl 0.05 M) method, and quantified through flame spectrophotometry (K), or using the blue-Mo method (P). Soil organic matter (SOM) was determined using the Walkley and Black method.

Soil biochemical analysis

β-Glucosidase (E.C. 3.2.1.21) and acid phosphatase (E.C. 3.1.3.2) activities were determined according to Tabatabai (1994) with toluene omitted from the assays. Microbial biomass carbon (MBC) was determined by the chloroform-fumigation-incubation method (Jenkinson and Powlson 1976), with the adaptations described by Oliveira et al. (2001).

Total DNA extraction

DNA was directly extracted from 0.5-g soil samples using the BIO 101 DNA extraction kit (Q Biogene,

Carlsbad, USA), following the manufacturer's instructions.

PCR and denaturing gradient gel electrophoresis (DGGE)

The *rpo*B amplification was performed with GC1698f and 2041r primers as described by Peixoto et al. (2002). The 16S rRNA amplification was performed with GCU968 and L1401 primers, as described by Heuer and Smalla (1997). PCRs of the *Pseudomonas* group were performed by a nested PCR using specific 16S rRNA primers, Ps*f* and Ps*r*, previously described by Widmer et al. (1998) in the first step, and U968GC (Heuer and Smalla 1997) and Ps*r* in the second step, as described by Evans et al. (2004).

Denaturing gradient gel electrophoresis of the amplified gene sequences was performed using a DCode System (universal mutation detection system; Bio-Rad). The GC-clamped amplicons were applied to a 6% polyacrylamide gel with a gradient of 45–70% denaturant (urea and formamide). All gels were loaded with DNA markers in the first and last lanes, surrounding the lanes with samples, to allow gel standardisation according to the manufacturer's instructions. The gels were run in 1× Tris–acetate–EDTA buffer at 60°C at a constant voltage of 70 V for 17 h. The DGGE gels were stained with Sybr Green and visualised using a Storm 860 Imaging System (GE Healthcare). The DGGE numeric matrices were generated by Bionumerics software (Applied Maths, Ghent, Belgium) using bands with quantitative value.

Statistical analyses

All univariate data (chemical and biochemical variables) were analysed by one-way analysis of variance. Statistical differences between means were assessed by Tukey's test at 0.05 significance. These analyses were performed in S-Plus® 8.0 (Insightful, Seattle, WA, USA).

Each DGGE-derived community dataset was ordinated using non-metric multidimensional scaling (NMS) (Kruskal 1964; Mather 1976) based on Sørensen distances (Bray and Curtis 1957). Before ordinations, all DGGE matrices were normalised by row

totals to eliminate differences in PCR yield among samples. All resulting bi-dimensional ordinations were graphically displayed after rotation to maximise the clustering of NC samples on the left side of axis 1. A secondary matrix was used to overlay the major gradients of chemical and biochemical variables, thus allowing a direct assessment of the relationship between each of these environmental variables and variations in the microbial profiles. Similar a NMS ordination analysis was performed using a matrix of chemical and biochemical soil properties to compare the soil quality profiles of the studied areas.

Statistical differences in the microbial community compositions were analysed using a multi-response permutation procedure (MRPP) based on the Sørensen distance matrix. MRPP is a non-parametric method for testing group differences in multidimensional space (Biondini et al. 1985). A Mantel test (Douglas and Endler 1982), which evaluates the null hypothesis of no relationship between two distance matrices, was used to evaluate the association between the three DGGE fingerprints and between these fingerprints and a matrix of chemical and biochemical properties. All Mantel analyses were run using Mantel's asymptotic approximation method and the Sørensen distance. All multivariate analyses were performed using the PC-ORD statistical package V5 (MjM Software, Gleneden Beach, OR, USA).

Shannon's diversity index was calculated based on the DGGE results using PC-ORD. The Shannon index (H') (Shannon and Weaver 1949) was calculated according to the following equation: $H' = -\sum \pi \log \pi$, where π is the proportion represented by each DGGE band relative to the total number of bands. The indices obtained were statistically analysed as described for other univariate data.

Results

Soil chemical and biochemical characterisation

Chemical and biochemical properties of soils from the NC and cultivated areas under NT and CT are shown in Table 1. There were no significant differences in the amounts of Al, H + Al, SOM, and MBC among the three studied areas. Soil pH and nutrients were generally higher in the cultivated areas

Table 1 Soil chemical and biochemical properties of areas cultivated for 11 years under no-tillage (NT) and conventional tillage (CT), and of a native Cerrado (NC) area

	pH	Al (cmol$_c$ dm^{-3})	H + Al (cmol$_c$ dm^{-3})	Ca (cmol$_c$ dm^{-3})	Ca + Mg (cmol$_c$ dm^{-3})	P (mg dm^{-3})	K (mg dm^{-3})	SOM (%)	MBC (mg C kg^{-1})	β-gluc. (μg p-nitrophenol h^{-1} g^{-1})	Phos. (μg p-nitrophenol h^{-1} g^{-1})
NT/0–5 cm	5.1 A	0.04 A	3.9 A	4.1 A	5.5 A	7.2 A	73.0 A	3.7 A	277 A	230 A	1009 A
NT/5–20 cm	5.2 A	0.19 A	4.0 A	1.4 C	2.2 B	10.0 A	70.0 A	2.8 A	159 A	70 B	497 B
CT/0–5 cm	5.3 A	0.07 A	3.7 A	2.1 B	3.0 B	4.6 A	53.0 AB	2.7 A	152 A	71 B	488 B
CT/5–20 cm	5.2 A	0.10 A	3.9 A	2.1 B	2.7 B	4.3 A	59.7 AB	2.6 A	218 A	77 B	541 B
NC/0–5 cm	4.4 B	0.81 A	5.3 A	0.0 D	0.3 C	0.2 B	25.0 B	2.4 A	451 A	81 B	1284 A
NC/5–20 cm	4.4 B	0.96 A	6.4 A	0.0 D	0.2 C	0.2 B	25.3 B	2.7 A	369 A	30 B	980 A

Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey's test ($N = 3$)

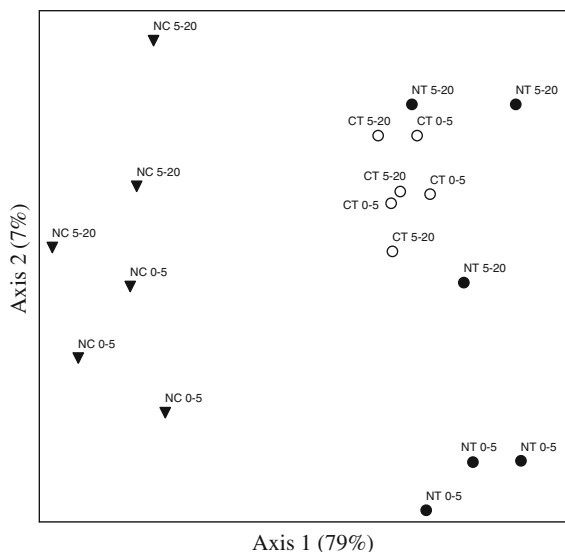SOM soil organic matter; MBC microbial biomass carbon; β-gluc. β-glucosidase activity; Phos. acid phosphatase activity

Fig. 1 Non-metric multidimensional scaling ordination of chemical and biochemical soil characteristics obtained from soil samples collected from CT, NT, and NC areas. The depths of sampling were 0–5 cm and 5–20 cm. The fraction of the total variance accounted for in each axis is indicated in *parentheses*

compared to the NC area. The soil pH ranged from 5.1 to 5.3 in the NT and CT areas and was 4.4 in the NC area. The lowest levels of available Ca, Ca + Mg, P and K were found in the NC area. There were no significant differences in soil properties between the 0–5 cm and 5–20 cm soil layers of the CT and NC areas. However, the highest values of Ca, Ca + Mg, and of β-glucosidase were found in the top 0–5 cm layer from the NT area (Table 1), while the highest values of acid phosphatase activities were observed in the top 0–5 cm layer from NC areas.

Figure 1 shows the NMS plot representing the dissimilarities among the study areas regarding all chemical and microbiological (MBC and soil enzymes) soil properties evaluated. It was possible to note a clear separation between NC and the cropped areas under NT and CT managements. Moreover, samples from 0–5 to 5–20 cm depths were separated in both the NC and NT areas, whereas no differences were found between the CT samples from the two soil depths. NT samples taken from 5 to 20 cm presented close similarity to CT samples from both soil layers, but these were significantly different from 0 to 5 cm NT samples (Fig. 1).

DGGE analysis of soils under tillage and NT managements

Non-metric multidimensional scaling ordinations of DGGE fingerprints using total bacterial *rpoB* and 16S rRNA genes based primers and the specific *Pseudomonas* primers showed significant differences between the BCSs from areas under different soil use or management (Table 2; Fig. 2a–c). The bacterial *rpo*B ordination separated soil samples mainly along axis 1, with NC samples grouped on the left side, NT samples in an intermediate position and CT samples grouped mostly on the right side of this axis (Fig. 2a). These differences in BCS were statistically significant, according to pairwise comparisons using MRPP analysis (Table 2). NC samples were also grouped on the left side of axis 1 in both the 16S rRNA gene and *Pseudomonas* ordinations, but separation of NC and CT samples along this axis was not as clear as in the *rpo*B ordination (Fig. 2a, b). Despite this fact, MRPP pairwise analyses showed significant differences in the BCSs of all three studied areas in both the 16S rRNA gene and *Pseudomonas* fingerprints (Table 2).

Spatial variability in the BCS within each area, estimated by the three sampling replicates, was higher under CT than under NT or in NC, according to the *rpoB* and bacterial 16S rRNA gene ordinations (Fig. 2a, b). In contrast, this variability seemed to be homogeneous among the three areas in the *Pseudomonas* ordination, considering that variation in NT samples was mostly associated with differences between BCSs from 0–5 to 5–20 cm soil layers (Fig. 2c). MRPP analysis showed significant

Table 2 *P*-value for MRPP comparisons of microbial community profiles obtained by DGGE fingerprints of bacterial 16S rRNA, *rpoB*, and *Pseudomonas* gene fragments amplified from 0–5 to 5–20 cm soil samples collected from a native Cerrado (NC), and areas cultivated for 11 years under conventional tillage (CT), and no-tillage (NT) management systems

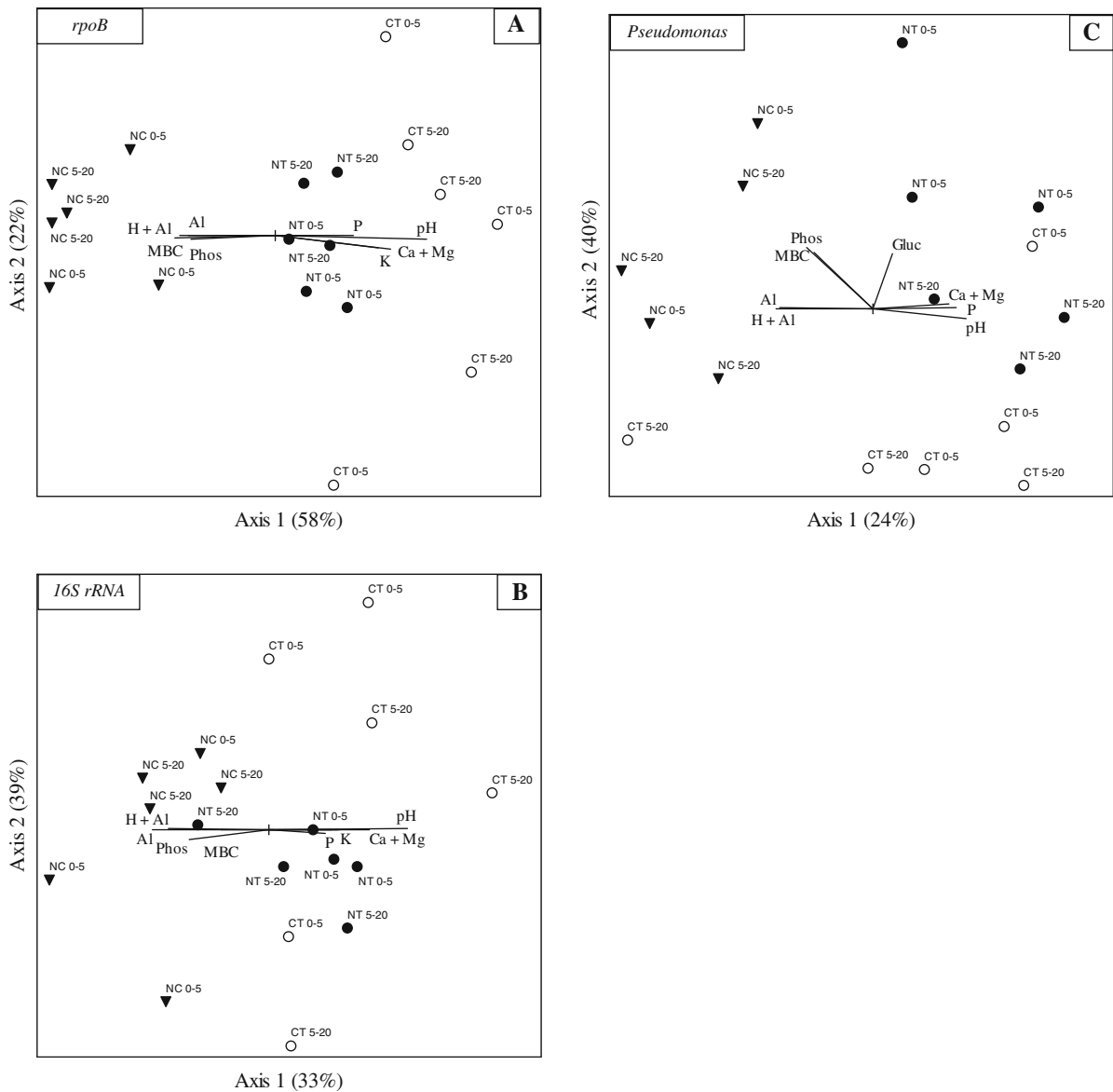| Comparison	*rpoB*	16S rRNA	*Pseudomonas*
NC vs. NT	0.0005	0.010	0.003
NC vs. CT	0.0006	0.002	0.009
CT vs. NT	0.006	0.011	0.043
NT 0–5 vs. NT 5–20	0.029	0.033	0.032
CT 0–5 vs. CT 5–20	n.s.	n.s.	n.s.
NC 0–5 vs. NC 5–20	0.023	0.022	n.s.

Fig. 2 Non-metric multidimensional scaling ordination of DGGE fingerprints of *rpoB* (**a**), 16S rRNA (**b**) and *Pseudomonas* (**c**) gene fragments amplified from soil samples collected from different managements represented by CT, NT, and NC. The sampling depths were 0–5 and 5–20 cm. The fraction of the total variance accounted for in each axis is indicated in *parentheses*. The angles and lengths of the *radiating lines* indicate the direction and strength of relationships of soil chemical and biochemical variables with the ordination scores

differences between the BCSs from 0–5 cm and 5–20 cm soil layers of NT areas, based on all gene fingerprints, and of NC, based on the bacterial 16S rRNA and *rpoB* genes fingerprints (Table 2). No differences were observed in microbial fingerprints obtained from 0–5 cm and 5–20 cm samples from the CT area.

Joint plots shown in Fig. 2 allowed verification of the direction and strength of relationships of the soil chemical and biochemical variables (Table 1) with the ordination scores. Accordingly, the BCSs of NC samples were related in all ordinations to high levels of soil acidity, expressed by low pH and high Al and H + Al contents, and increased amounts of MBC and

Table 3 Standardized Mantel statistic (r) of the relationship between matrices of DGGE fingerprints and a matrix including the chemical and biochemical properties showed in Fig. 1

Matrix	Mantel statistic (r)			
	16S rRNA	rpoB	Pseudomonas	Chemical/ biochemical profile
16S rRNA	1	0.44***	0.21*	0.16[+]
rpoB		1	0.18*	0.47***
Pseudomonas			1	0.36***

*** $P < 0.001$; * $P < 0.05$; [+] $P < 0.10$

phosphatase activity (Fig. 2a–c). In contrast, the BCSs from cultivated areas were related to high levels of Ca + Mg, P and K in the 16S rRNA and *rpoB* genes ordinations and to Ca + Mg and P concentrations in the *Pseudomonas* ordination. The separation of *Pseudomonas* communities from different soil layers in the NT area was mostly associated with higher levels of MBC and activities of β-glucosidase and phosphatase in the 0–5 cm layer of this soil (Fig. 2c).

There was a significant correlation between the DGGE matrices of *rpoB*, 16S rRNA genes, and *Pseudomonas* group, as indicated by the Mantel test (Table 3). The Mantel test also revealed a significant correlation of DGGE matrices with the matrix of chemical and biochemical properties for all three studied areas (Table 3). This correlation, though, was stronger with *rpoB* gene ($r = 0.47$; $P < 0.001$) and *Pseudomonas* ($r = 0.36$; $P < 0.001$) than with the 16S rRNA gene matrix ($r = 0.16$; $P < 0.10$).

Table 4 Shannon's diversity index calculated from DGGE gene fragments of bacterial 16S rRNA gene fragments amplified from 0–5 to 5–20 cm soil samples collected from native Cerrado (NC), and areas cultivated for 11 years under conventional tillage (CT), and no-tillage (NT)

Area/soil depth	16S rRNA	rpoB	Pseudomonas
NT/0–5 cm	3.49 A	2.58 A	1.84 A
NT/5–20 cm	3.09 AB	2.86 A	2.19 A
CT/0–5 cm	2.87 B	2.13 B	1.93 A
CT/5–20 cm	3.02 B	2.26 B	2.33 A
NC/0–5 cm	2.75 B	2.64 A	2.02 A
NC/5–20 cm	2.89 B	2.68 A	2.64 A

Values in columns sharing the same letter do not differ significantly ($P < 0.05$) as determined by the Tukey's test ($N = 3$)

Calculation of Shannon's diversity index based on DGGE gene fragments suggested a high diversity of bacteria, based on 16S rRNA gene, in the 0–5 cm soil layer of the NT area ($P < 0.05$) (Table 4). The NT and NC areas also presented higher Shannon's indices than CT in both soil layers. There were no differences in this index among the three areas for the DGGE gene fragments of *Pseudomonas*.

Discussion

In this study, we evaluated changes in the BCS of Cerrado soils cultivated for 11 years under either CT or NT managements and from a soil collected from an adjacent are of NC. In addition, several chemical and biochemical properties were analysed in these soils, which revealed important differences between the NC area and both the NT and CT areas. For instance, the cultivated areas had higher pH and nutrient levels compared to the NC areas (Table 1), which may be attributed to the application of lime and fertiliser in the former. NC soils are characterized by low pH and low nutrient contents therefore soil liming and the addition of NPK fertilizers are necessary in order to provide the adequate conditions to achieve sound crop yields (Sousa and Lobato 2004). In contrast, even after 11 years of soil cultivation, no differences were found in the SOM and MBC between NC and cultivated areas. This result diverges from other studies that showed decreases in SOM and MBC in cultivated areas of Cerrado (Mendes et al. 2003) or in other soils (Paustian et al. 1997; Sharma et al. 1998; Sá et al. 2001; Valpassos et al. 2001) compared to areas with undisturbed native vegetation.

Despite the unchanged SOM and MBC levels, phosphatase activity was lower in CT areas compared to NC areas (Table 1). This lower activity may be the result of the application of phosphate fertilisers in the CT area. Acid phosphatase is an important enzyme involved in organic phosphorus mineralisation in areas under native vegetation (Matsuoka et al. 2003). High levels of inorganic P in the soil, as a consequence of the application of phosphate fertilisers, may reduce the activity of phosphatases because orthophosphate is a competitive inhibitor of these enzymes (Tabatabai 1994).

The activity of acid phosphatase under NT management (at 0–5 cm) was also significantly superior

to that found in CT. A similar difference has been reported in Cerrado soils (Mendes et al. 2003), where higher acid phosphatase activity was found in the NT system relative to an area under CT. According to these authors, this difference could be related to the reduced soil mixing in the NT system, which favoured a localised concentration of phosphate at the soil surface, mainly restricted to the line of fertiliser application. Therefore, phosphatase inhibition by inorganic P may be reduced under NT management compared to CT, where the fertiliser is incorporated during tilling. Conte et al. (2002) also verified that the increase in soil P availability in NT areas did not result in a reduction of acid phosphatase activity. They attributed this fact to the high affinity of P for soil organo-mineral colloids, which may increase the adsorption of phosphate ions, reducing their inhibition of phosphatases.

β-Glucosidase activity was more than three times higher in soil samples collected in the 0–5 cm layer of NT areas compared to the 5–20 cm samples of this same soil and to samples from the other two areas (Table 1). Mendes et al. (2003) also reported higher activity of β-glucosidase in micro- and macro-aggregates from 0 to 5 cm soil layer of a Cerrado area cultivated for 21 years under NT, compared to areas under CT or native vegetation. The β-glucosidase enzyme acts in the final stage of cellulose degradation and is responsible for the hydrolysis of cellobiose residues into D-glucose (Tabatabai 1994; Paul and Clark 1996). The activity of this enzyme in soils can be strictly related to the inputs of cellulosic material into the soil and to soil physico-chemical conditions that regulate the activity of decomposing microorganisms. Therefore, the higher level of β-glucosidase activity in NT areas compared to CT areas could be explained by the high concentration of plant residues at the soil surface, as previously suggested (Bandick and Dick 1999). The difference relative to the NC area, however, could be related to higher acidity of the native soil relative to that of NT areas. The acidity of Cerrado soils can interfere negatively with the activity of β-glucosidase, which is positively correlated with soil pH (Ekenler and Tabatabai 2003). This effect may be related to a stimulation of microbial growth and activity caused by liming, which in turn may increase the activity of soil enzymes. According to Mendes and Reis Junior (2004), the higher β-glucosidase activity in soils under NT management

compared to that in NC soils may be related to a more labile organic material presented in the crop litter compared to that found in NC vegetation. The higher litter complexity in native vegetation may implicate in lower β-glucosidase activity because other enzymes such as cellulases and ligninases would have a greater participation in the decomposition process.

The Mantel test showed a significant correlation between all analysed BCS (total bacteria, assessed by the use of two molecular markers, the 16S rRNA and *rpoB* genes, and the *Pseudomonas* group) (Table 3), indicating that these targeted communities are related to a certain degree and show similar patterns of differences among the three studied areas. In fact, NMS ordinations obtained by all three gene markers showed a relative degree of similarity, like the evident difference in the BCS of NC relative to the two cultivated areas, or between NT areas and NC (Table 2; Fig. 2a–c). Peixoto et al. (2005) also demonstrated that soil BCS, based on PCR/DGGE using both the *rrs* and *rpoB* biomarkers, differed in response to tillage and soil depth in a different Cerrado area and speculated that this behaviour might also be observed in specific microbial groups. We demonstrated that land use also affected the profile of *Pseudomonas* populations, indicating that agricultural systems can also be responsible for changes within specific microbial niches. Sharma et al. (1998), compared functional microbial diversity in CT and NT microcosms and also demonstrated clear shifts in many microbial parameters, as CLPP (community level physiological parameters), in soils amended with litter when compared to soils without litter.

Pseudomonas sp. encompasses many known PGPR species and is an important taxonomic group of soil microbial communities. The genomic complexity and plasticity of fluorescent *Pseudomonas* spp. (Moore et al. 1996) generates an intra-specific diversity that gives this group a great ability to persist in changing environments (Costa et al. 2006). Costa et al. (2006) described that the age and cultivar of the plant with which *Pseudomonas* is associated are responsible for shifts in the structure of *Pseudomonas* communities in rhizosphere samples, indicating the sensitivity of this bacterial group to environmental changes.

Although all analysed markers provided similar results, it was possible to detect different degrees of responses within each targeted population. For

instance, we observed that the spatial variability in BCS within each area was higher in soils under CT management compared to NT and NC areas, according to *rpoB* and 16S rRNA genes ordinations. In contrast, in CT soil samples, there was less variability in the *Pseudomonas* group than in NT or NC samples (Fig. 2c). MRPP analysis, based on all generated profiles, also indicated differences between soil sampling layers of NT areas. Profiles generated by 16S rRNA and *rpoB* genes from NC also differed between the 0–5 cm and 5–20 cm layers. The lack of difference in the BCSs of different CT soil layers may be associated with the mixing of soil caused by successive soil tillage operations. It could also be expected that soil tillage would also lead to a more homogeneous BCS in CT areas, but as discussed above, the BCS of CT areas presented the highest variability among the three studied areas (Fig. 2a, b). Contrariwise, in the NC and NT areas, the absence of tillage practices may allow the stratification of microbial and chemical properties in the soil profile, which is corroborated by our results. Maybe this could also explain why Shannon's diversity index of 16S rRNA gene DGGE profiles was higher in NT and NC than in CT.

Microorganisms are the key drivers of soil biogeochemical processes (Allison and Martiny 2008; Ndaw et al. 2009). Thus, it is important to evaluate how land use and soil management affect soil physico-chemical properties, and how these changes could be related to different profiles of soil BCS. The results shown here confirmed the positive connection of those factors, revealing a significant relationship between the BCS and the chemical and biochemical status of the soil. For instance, it was noted that the BCSs based on all gene markers obtained for the NC area were correlated with low pH, high Al, and high H + Al, as well as high values of MBC and phosphatase activity (Fig. 2a–c). In the other hand high levels of P, K, and Ca + Mg were related to the BCSs of cultivated areas, indicating that, in the studied area, land use concomitantly affects soil chemical properties and microbial diversity, biomass and activity. This result is not surprising because all these soil variables are in some way connected and dependent on each other. For instance, the use of lime and fertilisers regulates soil pH and nutrient availability, which in turn may select specific bacterial populations that are better able to grow in the new soil chemical conditions. Fierer et al. (2009) postulated that soil microbial communities are related to soil pH, C and N ratios. The newly selected population may also provide a different functional profile to the soil, which may affect several soil biochemical processes (Chaer et al. 2009).

Microbiologists have been studying the impacts of abiotic variables on microbial diversity since the 1960s, and there is growing interest in the relationships between microbial diversity, ecological functions and soil resilience (Garbeva et al. 2004; Allison and Martiny 2008; Chaer et al. 2009). Resistance, resilience and redundancy in microbial communities are important issues, and microbial community composition is often ignored in ecosystem modelling, as recently reviewed by Allison and Martiny (2008). The authors noted that, generally, most microbial communities sensitive to soil disturbances or stresses are not immediately resilient, regardless of their high flexibility and physiological tolerance to environmental changes. For example, Chaer et al. (2009) showed that the resilience of specific microbial functions in a tropical soil can be affected by land use and soil agricultural management.

In this study, we demonstrated that microbial profiles of total bacteria and of the *Pseudomonas* group from Cerrado soils were significantly different in CT, NT and NC areas and that the observed changes were significantly related to chemical and biochemical variations in the soils. These results provide a small but important piece of information necessary for a better understanding of soil microbial diversity in the Cerrado, which is fundamental to helping us conduct sustainable practices in this biome and predict the responses of this biodiversity hotspot to changes in land use or soil management.

References

Adámoli J, Macêdo J, Azevedo LG, Madeira Netto JM (1987) Caracterização da região dos Cerrados. In: Goedert WJ (ed) Solos dos Cerrados—tecnologias e estratégias de manejo. Nobel e Embrapa CPAC, São Paulo e Brasília, pp 33–74

Allison SD, Martiny JBH (2008) Resistance, resilience and redundancy in microbial communities. PNAS 105: 11512–11519

Bandick AK, Dick RP (1999) Field management effects on soil enzyme activities. Soil Biol Biochem 31:1471–1479

Bardas GA, Lagopodi AL, Kadoglidou K, Tzavella-Klonari K (2009) Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365. Biol Control 49:139–145

Biondini ME, Bonham CD, Redente EF (1985) Secondary successional patterns in a sagebrush (*Artemisia tridentata*) community as they relate to soil disturbance and soil biological activity. Vegetatio 60:25–36

Bray JR, Curtis JT (1957) An ordination of the upland forest communities in southern Wisconsin. Ecol Monit 27: 325–349

Castro AP, Quirino BF, Pappas GJ, Kurokawa AS, Neto EL, Krüger RH (2008) Diversity of soil fungal communities of Cerrado and its closely surrounding agriculture fields. Arch Microbiol 190(2):129–139

Chaer G, Fernandes M, Myrold D, Bottomley P (2009) Comparative resistance and resilience of soil microbial communities and enzyme activities in adjacent native forest and agricultural soils. Microb Ecol 8(2):414–424

Conte E, Anghinoni E, Rheinheimer DS (2002) Frações de fósforo acumuladas em Latossolo argiloso pela aplicação de fosfato no sistema plantio direto. Rev Bras Cienc Solo 26:925–930

Cook RJ (2006) Toward cropping systems that enhance productivity and sustainability. PNAS 103(49):18389–18394

Costa R, Götz M, Mrotzek N, Berg G, Lottmann J, Smalla K (2006) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of different microbial guilds. FEMS Microbiol Ecol 56:236–249

Douglas ME, Endler JA (1982) Quantitative matrix comparisons in ecological and evolutionary investigations. J Theor Biol 99:777–795

Ekenler M, Tabatabai MA (2003) Effects of liming and tillage systems on microbial biomass and glycosidases in soils. Biol Fertil Soils 39:51–61

EMBRAPA—Empresa Brasileira de Pesquisa Agropecuária—Centro Nacional de Pesquisa de Solos (1997) Manual de métodos de análises de solo, 2nd ed. Rio de Janeiro, 212 pp

EMBRAPA—Empresa Brasileira de Pesquisa Agropecuária (2009) www.embrapa.br/imprensa/noticias/2009/junho/4a-semana

Evans FF, Seldin L, Sebastian GV, Kjelleberg S, Holmström C, Rosado AS (2004) Influence of petroleum contamination and biostimulation treatment on the diversity of *Pseudomonas* spp. in soil microcosms as evaluated by 16S rRNA based-PCR and DGGE. Lett Appl Microbiol 38:93–98

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC (2009) Global patterns in belowground communities. Ecol Lett 12:1238–1249

Garbeva P, van Venn JA, van Elsas JD (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopatol 42:243–270

Heuer H, Smalla K (1997) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis for studying soil microbial communities. In: van Elsas JD, Trevors J, Wellington EMH (eds) Modern soil microbiology. Marcel Dekker Inc., New York, pp 353–373

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil—V: a method for measuring soil biomass. Soil Biol Biochem 8:209–213

Kruskal JB (1964) Nonmetric multidimensional scaling: a numerical method. Psychometrika 29:115–129

Kumar S, Pandey P, Maheshwari DK (2009) Reduction in dose of chemical fertilizers and growth enhancement of sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. Eur J Soil Biol 45:334–340

Machado PLOA, Silva CA (2001) Soil management under no-tillage systems in the tropics with special reference to Brazil. Nutr Cycl Agroecosyst 61:119–130

Marris E (2005) The forgotten ecosystem. Nature 437:944–945

Mather PM (1976) Computational methods of multivariate analysis in physical geography. Wiley, London

Matsuoka M, Mendes IC, Loureiro MF (2003) Biomassa Microbiana e Atividade Enzimática em Solos sob Vegetação Nativa e Sistemas Agrícolas Anuais e Perenes na Região de Primavera do Leste (Mt). Rev Bras Cienc Solo 27:425–433

Mendes IC, Reis Junior FB (2004) Uso de parâmetros microbiológicos como indicadores para avaliar a qualidade do solo e a sustentabilidade dos agroecossistemas. Embrapa Cerrados, Planaltina, DF, 34 pp

Mendes IC, Souza LV, Resck DVS, Gomes AC (2003) Propriedades biológicas em agregados de um LE sob plantio convencional e direto no Cerrado. Rev Bras Cienc Solo 27:435–443

Moore ERB, Mau M, Arnscheidt A, Böttger EC, Hutson RA, Collins MD, van de Peer Y, de Wachter R, Timmis KN (1996) The determination and comparison of the 16S rRNA gene sequences of the genus *Pseudomonas* (sensu stricto) and estimation of the natural intrageneric relationships. Syst Appl Microbiol 19:478–492

Myers N, Mittermeier R, Mittermeier C, da Fonseca G, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772):853–858

Ndaw SM, Gama-Rodrigues AC, Gama-Rodrigues EF, Sales KR, Rosado AS (2009) Relationships between bacterial diversity, microbial biomass, and litter quality in soils under different plant covers in northern Rio de Janeiro State, Brazil. Can J Microbiol 55(9):1089–1095

Oliveira JRA, Mendes IC, Vivaldi L (2001) Biomassa microbiana de carbono em solos de cerrado sob vegetação nativa e sob cultivo: avaliação dos métodos fumigação-incubação e fumigação-extração. Rev Bras Cienc Solo 25:863–871

Paul EA, Clark FE (1996) Soil microbiology and biochemistry. Academic Press, San Diego, 340 pp

Paustian K, Collins HP, Paul EA (1997) Management controls on soil carbon. In: Paul GEA, Paustian K, Elliott ET, Vole CV (eds) Organic matter in temperate agroecosystems. CRC Press, Boca Raton, FL, pp 15–49

Peixoto RS, da Costa Coutinho HL, Rumjanek NG, Macrae A, Rosado AS (2002) Use of *rpoB* and 16S rRNA genes to analyse bacterial diversity of a tropical soil using PCR and DGGE. Lett Appl Microbiol 35(4):316–320

Peixoto RS, Coutinho HLC, Madari B, Machado PLOA, Rumjanek NG, van Elsas JDL, Seldin L, Rosado AS (2005) Soil aggregation and bacterial community structure as affected by tillage and cover cropping in the Brazilian Cerrados. Soil Tillage Res 90:16–28

Ratter JA, Ribeiro JF, Bridgewater S (1997) The Brazilian Cerrado vegetation and threats to its biodiversity. Ann Bot 80:223–230

Recep K, Fikrettin S, Erkol D, Cafer E (2009) Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. Biol Control 50:194–198

Rosas SB, Avanzini G, Carlier E, Pasluosta C, Pastor N, Rovera M (2009) Root colonization and growth promotion of wheat and maize by *Pseudomonas aurantiaca* SR1. Soil Biol Biochem 41:1802–1806

Sá JCM, Cerri CC, Dick WA, Lal R, Venzke Filho SP, Piccolo MC, Feigl BE (2001) Organic matter dynamic and carbon sequestration rates for a tillage chronosequence in a Brazilian Oxisol. Soil Sci Soc Am J 65:1486–1499

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Dlinois Press, Champaign, IL

Sharma S, Rangger A, Insam H (1998) Effects of decomposing maize litter on community level physiological profiles of soil bacteria. Microb Ecol 35:301–310

Soil Survey Staff (1999) Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. USDA Natural Resource Conservation Service Agriculture Handbook, 436, U.S. Government Printing Office, Washington DC

Sousa DMG, Lobato E (2004) Adubação com nitrogênio. In: Sousa DMG, Lobato E (eds) Cerrado: correção do solo e adubação, 2nd edn. Planaltina, Embrapa Cerrados, pp 129–144

Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) Methods of soil analysis, part 2: microbiological and biochemical properties. Soil Science Society of America, Madison, pp 778–833

Timms-Wilson TM, Kilshaw K, Bailey MJ (2004) Risk assessment for engineered bacteria used in biocontrol of fungal disease in agricultural crops. Plant Soil 266:57–67

Valpassos MAR, Cavalcante EGS, Cassiolato AMR, Alves MC (2001) Effects of soil management systems on soil microbial activity, bulk density and chemical properties. Pesq Agropec Bras 36:1539–1545

Widmer F, Sedler RJ, Gillevet PM, Watrud LS, Di Giovanni GD (1998) A high selective PCR protocol for detecting 16S rRNA genes of the genus *Pseudomonas* (*sensu stricto*) in environment samples. Appl Environ Microbiol 64:2545–2553