Anthropic Impacts on Microbiota and Chemical Properties of Cerrado Soil Through Soybean Cultivation

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Abstract

Population growth and improved gross domestic product may increase food consumption. Soybean is the main source of protein, lipids and mineral salts for human and domestic animals' foods. Brazil is responsible of most of the soybeans produced in the world. However, soybean production in Tocantins/Brazil state caused a decrease in the Cerrado's biome. Therefore, the aim of this study was to evaluate the anthropic impact of planting of soybean on microbial and physical-chemical properties of Cerrado's soil. Soil samples were collected in three soybean farms (SF) of the Tocantins/Brazil state. They were collected in the soybean field, in native vegetation field, and in anthropogenic fragmentation area in the dry and wet seasons. The diversity of arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria (NFB) were analyzed by denaturing gradient gel electrophoresis (DGGE). Regardless of the SF, physico-chemical indicators did not present significant differences between the seasons. The DGGE profiles of NFB and AMF genes were different between the soybean field and native vegetation field in both seasons. The viable cells counts and NFBs and AMFs diversity were influenced by the substitution of native vegetation for soybean. The increase of the agricultural production in Cerrado soil is worrisome, due to the endemic microorganisms that was observed in this study. In addition, anthropic action on the microbial community was more effective in the soybean field during the dry season, which showed the importance of maintaining an environmental reserve area within agricultural production units.

Keywords: environmental impacts, mycorrhizal, nitrogen-fixing bacteria, soil resistance, native vegetation

1. Introduction

World population in July 2017 was 7.55 billion inhabitants and in 2050 it will be about 10.00 billions (UFNPA/ONU, 2017). If this population grows in geometric progression, Malthusian theory, it has an annual growth rate of about 0.78. This rate is higher in poor and developing countries than in developed countries. Therefore, the human population in poor and developing countries, between 2017 and 2030, will have an increase of 83 millions (UFNPA/ONU, 2017).

According to the World Bank, the gross domestic product (GDP) in 2018 will have an increase of 3.1% in the world and 4.5% in developing countries (Exame Magazine, 2018). The GDP growth in developing countries may be due to commodities exports. Growths in population and further improvement in GDP may increase food consumption and subsequently increase planting of soybean. This commodity is the main source of protein, lipids and mineral salts of human and domestic animal foods.

Brazil is responsible of most of the soybeans produced in the world. The country has approximately 33.890 million hectares planted with soybean that represent the world largest area planted with this plant (Conab, 2018). The agricultural regions of Maranhão (MA), Tocantins (TO), Piauí (PI) and Bahia (BA) states, which it is known as MATOPIBA, has been responsible for a big part of the country's soybean production. The Tocantins state has about 1.3 million hectares of soybean that is the highest area of soybean planting among high altitude regions (SEAGRO, 2017).

The soybean production in Tocantins state has caused a decrease of Cerrado biome in replacing native vegetation by soybean. This substitution may have changed the physical, chemical and biological properties of the soil, mainly in the agricultural layer. This layer houses microorganisms that degrade organic matter and rocks. Microbial decomposition of the rocks from the mineral soils of the Cerrado. Therefore, identification of these microorganisms is an important parameter to evaluate the changes caused in the soil fauna by the conversion of native vegetation to soybean field.

Thus, the aim of the study was to evaluate the anthropic impact of planting of soybean on microbial and physical-chemical properties of Cerrado soil in regions of Tocantins state in Brazil.

2. Materials and Methods

2.1 Site Location and Characterization

The study was carried out in three regions of Tocantins/Brazil State, the highest soybean (*Glycine max* (L.) *Merril*) producers, including (i) Central region, (ii) Throat region and (iii) Campos Lindos and Petro Afonso region. The cities concerned per region were Porto Nacional, Silvanópolis and Santa Rosa in the Central region and Mateiros and Dianópolis in the Throat region (Table 1). The climate, soil, and vegetation are peculiar to the region (Table 1).

Table 1. Climate, soil declivity, vegetation and soil type of the Tocantins/Brazil state for collection of soil samples

Tocantins/Brazil regions	Cities	Climate	Declivity	Soil	Phytoecology
Central	Porto Nacional Silvanópolis Santa Rosa	C2wA'a'' (Humid and subhumid with moderate water deficiency)	Class A (< 5%)	Latosol Plintosols	Cerrado biome Ecological Pressure (Disjunction Cerrado/ Seasonal Forest
Campos Lindos and Pedro Afonso				Neosol Latosol	
	Mateiros			Neosol	
Throat	Dianópolis	C2w2A'a' (Humid and subhumid with small water deficiency)	Classe C (10-15%)	Cambisol Neosol	

Source: Tocantins atlas (2012).

2.2 Sample Collection Plan

The soil samples were obtained in three soybean farms (SF) of the Tocantins/Brazil. They were collected in three different areas of soybean planting or native vegetation and four sampling points (Table 1).

In the Throat region (SF1), soil samples were collected in (i) the soybean field, (ii) in the native vegetation of Serra Geral field, and (iii) in the native vegetation field adjacent to the soybean field.

In Pedro Afonso/Tocantins/Brazil city (SF2), soil samples were collected in (i) one soybean field with little dead plant cover (soybean field 1), (ii) one native vegetation field of an ephemeral stream, surrounded by soybean field, and (iii) one soybean field with moderate dead plant cover (soybean field 2). The soybean fields 1 and 2 had about three years of cultivation of the crop. The soils were light gray and red-yellow, respectively. In the native vegetation, the relief was uneven and gravelly.

In the Central region (SF3), soil samples were collected in (i) one area of crop-livestock system integrating a beef cattle herd and soybean cultivation (soybean field), (ii) one native vegetation field, and (iii) one anthropogenic fragmentation area. The soil in this area had little dead vegetation cover, red-yellow color and enough gravel on the surface and along the profile. In points, P25-P26 has also invasive plants (Tables 2 to 5). The native vegetation area had foliage on the soil surface, plenty of gravel, adventitious roots and a natural runoff of water. The anthropogenic fragmentation area that is an island in the soybean field has dark red soil, arboreal vegetation of 15 to 20 m tall, abundant foliage on the surface and adventitious roots along the soil profile.

The soil samples were collected in 0 to 10, 10 to 20, 20 to 30 and 90 to 100 cm of depths in the dry period of September 2016 and wet period of January 2017. At each depth, three samples (10 g) were collected using a Dutch auger, according to the methodology of Raij (2001). The depth of 0-30 cm was defined as the effective

depth for soybean roots (~40 cm). The 90-100 cm depth was used to analyze the leaching effect of chemical elements from fertilizers and agrochemicals. In these soil samples were assessed the physical-chemical indicators and microbiota soil (item 2.4). The physico-chemical indicators were determined according to Standard Methods (APHA, 2005). Soil resistance to root penetration in depths of 0 to 60 cm and 2.5 cm interval was measured with the aid of a penetrometer (Falker-PLG 1020).

2.3 Analysis of the Soil Microbiota in the Dry and Wet Seasons

These analyses were performed as described in Carvalho et al. (2018).

2.3.1 Viable Microbial Cells in Soil

Ten grams of soil sample were used to quantify the viable microorganisms. The quantification of bacterial cell was performed in the nutrient agar culture medium containing 0.3 ml of nystatin at pH 7 (Sabino, 2007). The plates were incubated at 25 °C for 3 days.

Martin medium containing rose bengal (0.1% w/v) and 1 ml of streptomycin (0.3 mg/ml) was used to filamentous fungi count (Martin, 1950). The pH of this culture medium was pH 5.8. The plates were incubated at 25 °C for 7 days.

The actinomycete counts in selective medium containing glycerol (Rodrigues, 2007). The plates were incubated at 25 °C for 7 days.

The microbial cells were estimated as logarithm of the colony-forming unit (CFU) per gram of soil.

2.3.2 Microbial Diversity by DGGE Profile

The diversity of arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing bacteria (NFB) were analyzed by denaturing gradient gel electrophoresis (DGGE). Fungal and bacterial DNA were extracted from the soil samples using a soil DNA Mega Prep Kit (Kit-MO BIO, Ultraclean TM, Quiagen, USA). This extraction was done with 0.5 g of soil in plastic tubes (Eppendorf type) containing polypropylene beads. The *nifH* and *18S rDNA* genes amplification were performed by polymerase chain reaction (PCR) from the total DNA for analysis of NFB and AMF, respectively.

The PCR and Nested-PCR of the *nifH* gene were done with the 19F and 407R primers (Ueda et al., 1995) and 19F-GC (with GC clamp) and 278R primers (Direito & Teixeira, 2002), respectively. The *18S rDNA* gene amplification was performed with AM1, NS31, NS31-GC (with GC clamp) and Glo1 primers (Simon et al., 1992; Helgason et al., 1998; Kowalchuk et al., 2002; Cornejo et al., 2004).

DGGE analysis of the Nested-PCR fragments was performed (Model DCodeTM Systems—BIO-RAD, California, USA). Twenty µL of these fragments (150 to 200 ng of DNA) were loaded onto polyacrylamide gel (8%, w/v) in 1xTAE buffer.

2.4 Statistical Analysis

The estimates of the physical-chemical and biological parameters were performed at 95% confidence level (p < 0.05) based on the coefficient of variation (CV). Pimentel-Gomes (2000) classifies the experimental variations in low variation (CV < 10%), medium (10-20%), high (20-30%), and very high (CV > 30%).

The DGGE profiles were analyzed in the Bionumerics software Version 5.1 (Applied Maths, Belgium). The software has generated dendrograms of the unweighted pair group method with arithmetic mean (UPGMA) using the Jaccard similarity index. The similarity of bands was determined at 0.5% probability by the post-hoc Bonferroni test.

Excel, Surfer, SigmaPLOT12.0 and Minitab 17 software were also used in these analyses.

3. Results and Discussions

3.1 Physical-Chemical Indicators Determination of Soil Quality

We did not observe any difference in the physico-chemical indicators between seasons in the soybean farms (Figure 1).

In SF1, differences were observed in the Fe, K and P contents, base saturation, aluminum saturation and sand between sample collection points and soil depths (Figures 1A and 1B). These physico-chemical indicators and Mn content and clay had also no difference among the soil depths, soil moisture and samples collection points of SF2 (Figures 1C and 1D). In SF3, only Fe and K contents, organic matter, base saturation were different between soil depths and between sample collection points. Thus, although SF2 is closer to SF3 than to SF1, its

physico-chemical indicators of this farm were similar to those of SF1 (Figure 1). Furthermore, we did not observe any difference in the physico-chemical indicators between seasons in the soybean farms (Figure 1).

In SF3, it was observed the anthropic influence on the physico-chemical indicators (Figure 2C). In addition, between the soybean field and the native vegetation field differed for Fe and K contents (Figure 2). These results showed the importance of maintaining an area with native vegetation cover within soybean farms.
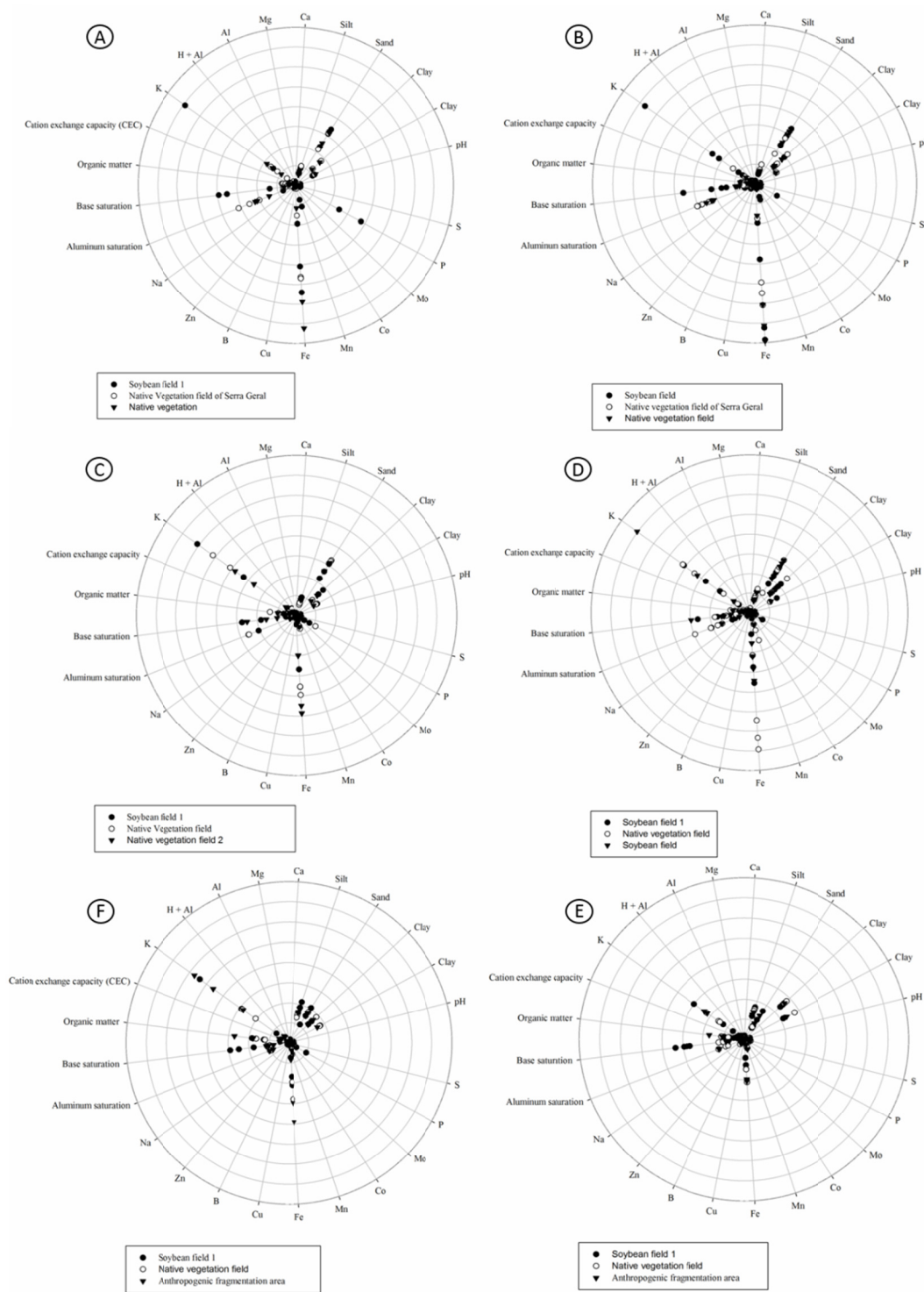

Figure 1. Physico-chemical indicators of soil in the soybean farms, SF1 (A and B), SF2 (C and D) and SF3 (E and F), in dry (A, C and E) and wet (B, D and F) seasons and soil depths of 0-10, 20-30, 30-40 plus 90-100 cm.

The depth of 90-100 was performed only for the wet season, due to high resistance to
soil penetration in dry season

3.2 Resistance to Soybean Roots Penetration

The resistance to root penetration (RP) in the three farms was measured under two soil moisture conditions (Figure 2). Plant growth has a negative correlation with soil compaction. The resistance to root penetration is the most used soil physical indicator to evaluate this condition (Beulter & Centurion, 2004).
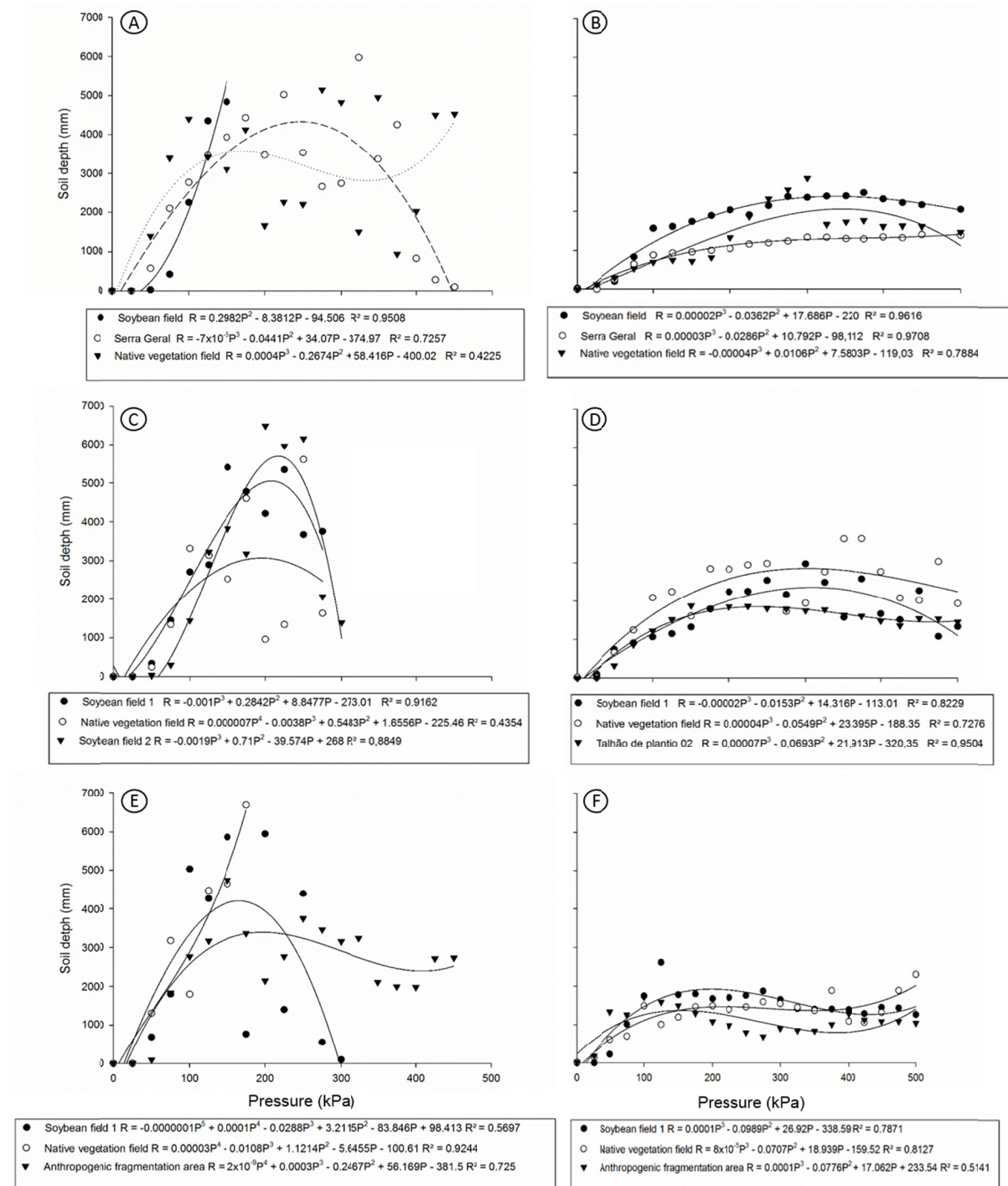

Figure 2. Soil resistance to root penetration in soybean farms, SF1 (A and B), SF2 (C and D) and SF3 (E and F), during the dry (A, C and E) and wet (B, D and F) seasons

The RP represent the critical limit of soil compaction (Figure 2). In soils of native vegetation has a roots depth threefold greater than in soil of soybean field, because of the reduction in RP and the increase of soil moisture (Genro Junior et al., 2004).

The soil of SF1 had RP of 2 MPa in the dry season (Figure 2A). According to Tormena et al. (1998), this value is used as a critical limit to the soybean roots growth. The estimation of roots' depth in this area was higher than 50 mm in the dry season (Figures 2A and 2B).

In soybean field, the roots depth was higher than in other areas of SF1, because of the plowing and the harrowing that contributed to the reduction of RP by the breakdown of micro particles and increase of soil porosity. However, the removal of native vegetation and soil homogenization for soybean planting, might have contributed to increased soil moisture loss and RP if plowing and harrowing were not done.

3.5 Biological Indicators of Soil Quality

The biological indicators' estimation in soil samples did not concern samples collected from 90-100 cm, because of small amount of microbial cell present at that depth. Carvalho et al. (2018) did not observed bands in the DGGE profile of NFB and AMF genes at this soil depth. According to the authors, there was no viable fungal cell in the soil at a depth beyond 30 cm.

Viable bacterial cells counts were higher than those of actinomycete and fungi regardless of the soybean farm, sample collection points, soil depth, and season (Tables 3 to 5). These results are similar to those obtained by Silva et al. (2018) in soil samples from Cerrado. In addition, a high amount of actinomycete cells was observed in the farms. These microorganisms are mainly responsible of nitrogen fixation and its presence in the soybean field can reduce the requirement of nitrogen fertilization (Faleiro, 2011; Moreira & Siqueira, 2006). Da Silva (2012) identified a predominance of the *nifH* gene in actinomycete when compared to other bacterial groups in Cerrado soil.

In both seasons, the DGGE profiles of NFB and AMF genes were different in soybean farms (Figures 3 to 5). The diversity of *nifH* gene in Cerrado soil was also changed by environmental conditions (Da Silva, 2012). The *18S rDNA* gene amplified using NS1 and FR1-GC primers presented alterations in the DGGE profile for different times of incubation (Gomes et al., 2003). NS1 primer was also used in this study to evaluate the AMFs diversity in soybean farm of the Tocantins state (Figures 3 to 5). Thus, the soybean farm had the different microbial diversity that shows the need for the preservation of Cerrado biome.

In these farms, bacterial diversity was greater than that of fungi, which confirms the results of viable cell counts (Tables 2 to 4) and Silva et al. (2018). This author analyzed the microbial communities in the soil with native vegetation cover. Furthermore, the soil microbial cell count may vary depending on the technique, the depth and the culture medium (Silva et al., 2018; Faleiro, 2011).

In SF1, the viable cell counts were lower in soybean field compared to native vegetation field (Table 2). This result showed the depressive effect of agricultural activity on the number of microbial cells that maight have affected their diversity in soybean fields. It was also observed that the microbial diversity of Serra Geral was intermediate between those of soybean field and native vegetation field. In the Serra Geral, human activities were greater than those observed in native vegetation field, which may have influenced in viable cell counts. The relative abundance of microorganisms in Cerrado soil after removal of native forest by anthropic activities has not been intensively studied (Monteiro et al., 2004). According to the authors, there are changes in the bacterial population after replacement the of native vegetation cover by planting of Eucalyptus and/or pinus.

The depth and moisture of soil had an unlike distinct influence on viable cell counts (Table 2). The counts were inversely proportional to soil depth. Monteiro et al. (2004) also observed this negative correlation between the microbial cell and soil depth. The fungal cell was not observed in the soil depth of 20-30 cm (Table 2). The cell count was higher in the wet season than the dry season that shows the influence of water availability on microbial growth. This increase of cell count in wet season may be due to spore germination or growth in size and number of cells favored by soil humidity. In fact, water is one of the parameters that most influence on microbial metabolism (Madigan et al., 2010 Tortora et al., 2014). Furthermore, in native vegetation field in the wet season, fungal cells were observed along the entire soil profile (0-30 cm). These fungal cells may be due to spore germination or cells percolation under moist condition. According to Gomes et al. (2003), fungi are in greatest quantity in the rhizosphere.

In SF1, the dry season's *16S-nifH* profile differed from the wet season's one (Figure 3). In the dry season, bands from the same soil depth (SD1, SD2 and SD3) were grouped in the same cluster showing that bacterial distribution was a function of soil depth.

Table 2. Viable microbial cells count in dry and wet seasons, of the soil samples of soybean farm 1 (SF1).

Sampling area	Sample code	Dry season			Wet season		
		Actinomycetes	Bacteria	Fungi	Actinomycetes	Bacteria	Fungi
		------------------------------------- Log (Colony forming unit g^{-1}) -------------------------------------					
Soybean field	P1SD1	7.73±0.22	12.7±0.89	6.27±0.25	9.52±0.49	13.29±0.40	7.88±0.71
	P1SD2	6.22±0.10	9.90± 0.98	5.08±0.20	7.62±0.50	10.85±0.38	6.89±0.72
	P1SD3	5.31±0.11	7.80± 0.90		6.47±0.51	9.19±0.27	
	P2SD1	9.39±0.11	12.8 ±0.61	7.76±0.24	11.36±0.45	13.10±0.47	8.69±0.73
	P2SD2	8.31±0.11	9.21± 0.61	5.62±0.20	9.20±0.58	10.20±0.41	6.26±0.73
	P2SD3	6.11±0.11	7.21± 0.70		7.30±0.59	9.56±0.30	
	P3SD1	10.48±0.10	13.1± 0.65	8.41±0.24	12.44±0.54	13.13±0.46	8.82±0.74
	P3SD2	7.54±0.10	10.2± 0.23	6.54±0.26	8.90±0.59	10.82±0.37	4.96±0.75
	P3SD3	5.76±0.10	8.71± 0.27		6.75±0.55	9.83±0.28	
	P4SD1	10.82±0.10	12.32±0.36	8.35±0.22	12.62±0.44	13.89±0.39	9.28±0.75
	P4SD2	9.51±0.11	10.63±0.39	7.36±0.22	10.03±0.10	10.92±0.33	6.79±0.76
	P4SD3	7.89±0.11	8.92±0.70		9.09±0.29	9.87±0.27	
Native vegetation field of Serra Geral	P5SD1	10.38±0.10	12.50±0.66	8.71±0.24	11.90±0.30	13.06±0.44	9.06±0.77
	P5SD2	8.64±0.11	10.10±0.28	7.68±0.22	9.84 ±0.30	10.25±0.34	7.58±0.77
	P5SD3	5.91±0.11	7.20±0.74		6.69±0.31	9.06±0.28	
	P6SD1	11.98±0.10	13.0±0.55	9.05±0.22	12.49±0.32	13.13±0.38	9.81±0.78
	P6SD2	9.68±0.11	10.20±0.49	7.94±0.25	9.84±0.32	10.24±0.36	8.64±0.79
	P6SD3	7.25±0.10	8.70±0.32		8.07±0.47	9.42±0.28	
	P7SD1	11.61±0.11	13.20±0.98	8.50±0.22	11.85±0.45	13.16±0.41	9.26±0.79
	P7SD2	8.99±0.11	10.10±0.70	6.43±0.24	9.90±0.46	10.90±0.34	8.17±0.80
	P7SD3	6.43±0.11	7.61±0.66		7.92±0.47	9.48±0.29	
	P8SD1	11.79±0.11	13.41±0.43	8.22±0.20	12.44±0.49	13.64±0.42	9.62±0.73
	P8SD2	10.57±0.11	10.81±0.44	5.95±0.24	10.87±0.50	11.72±0.34	8.69±0.74
	P8SD3	7.88±0.10	8.32±0.37		9.54±0.46	9.94±0.27	
Native vegetation field	P9SD1	11.83±0.11	13.72±0.95	8.38±0.25	14.22±0.49	14.67±0.42	9.84±0.75
	P9SD2	10.49±0.10	11.12±0.47	5.96±0.22	11.53±0.45	11.89±0.37	6.78±0.75
	P9SD3	8.24±0.11	8.43±0.96		8.79±0.29	8.97±0.29	2.54±0.44
	P10SD1	12.03±0.11	13.51±0.63	8.66±0.24	13.20±0.30	14.2± 0.40	9.51±0.76
	P10SD2	10.26±0.11	10.93±0.56	7.35±0.22	11.04±0.31	11.32±0.30	8.75±0.76
	P10SD3	5.55±0.11	8.20 ±0.47		6.98±0.31	9.22±0.47	2.68±0.34
	P11SD1	12.49±0.11	13.92±0.70	8.58±0.25	14.48±0.54	14.51±0.70	10.77±0.25
	P11SD2	9.20±0.11	10.93±0.70	8.14±0.22	10.91±0.55	11.16±0.70	9.72±0.22
	P11SD3	6.46±0.11	8.51±0.66		7.41±0.56	9.45±0.66	2.57±0.31
	P12SD1	12.45±0.11	13.40±0.28	8.76±0.24	14.21±0.11	14.40±0.28	10.92±0.24
	P12SD2	9.62±0.11	10.40±0.74	8.70±0.22	10.01±0.49	11.44±0.74	9.37±0.22
	P12SD3	6.81±0.10	8.72±0.55		7.90±0.32	9.30±0.55	2.64±0.32

Note. P: Sample collection point, SD: Soil depth (SD1 = 0-10, SD2 = 10-20, SD3 = 20-30 cm).
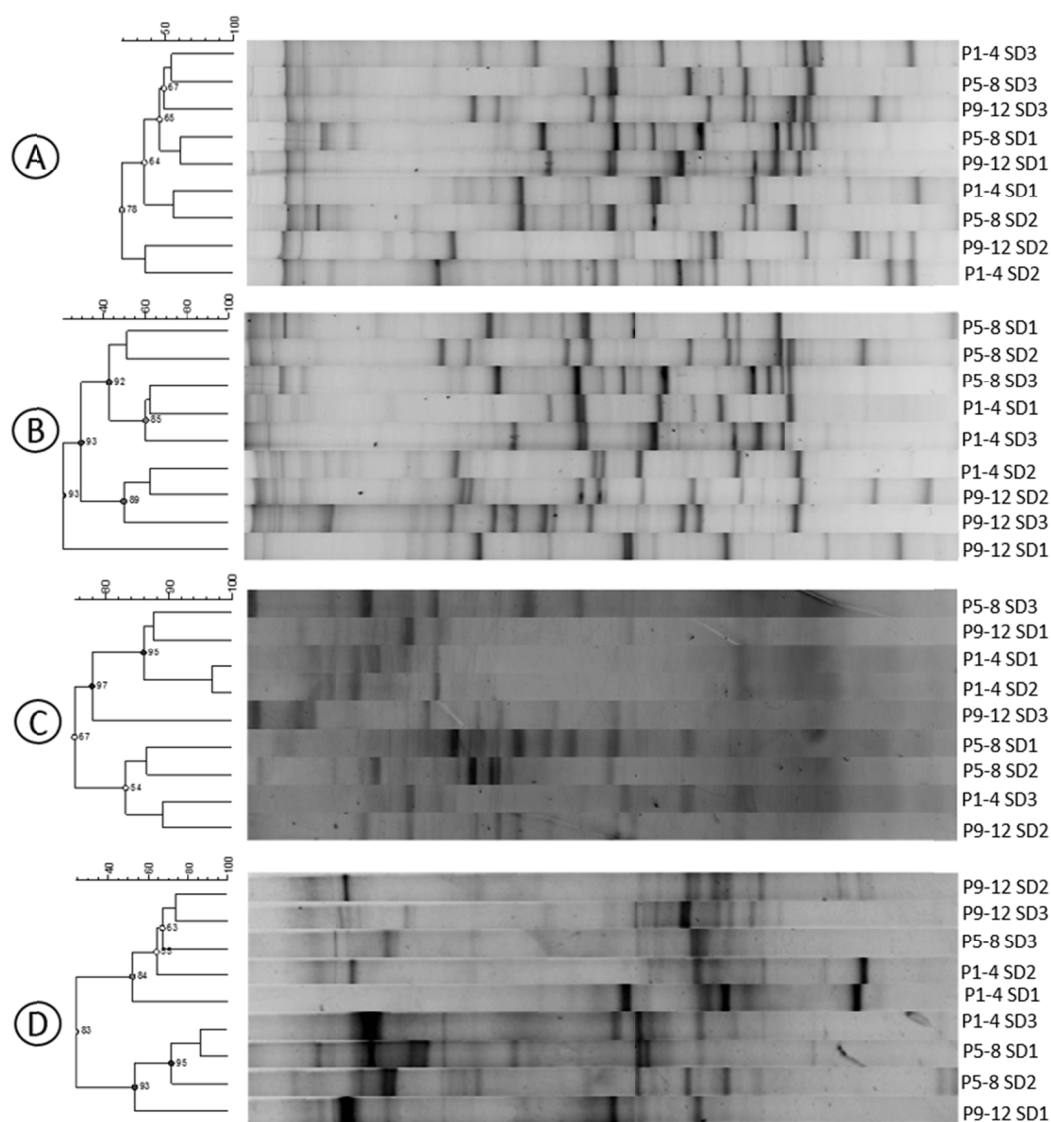
Figure 3. The DGGE profile of the *16S rDNA*, *nifH* (A and B) and *18S rDNA* and *arbuscular mycorrhizal* genes (C and D) of soils from the soybean farm 1 in dry (A and C) and wet (B and D) seasons
(Cf Table 2 for samples' codes)

On soil depth of 0-10 cm soil depth (SD1), there was 65% of similarity of bacterial communities between soybean field (P1-4), native vegetation field of Serra Geral (P5-8) and native vegetation field (E9-12). At 10-20 cm (SD2) and 20-30 cm (SD3), the similarities were 64% and 67%, respectively. Therefore, the bacterial community of the native vegetation field was also found in the other areas that showed that the anthropic changes have not yet affected these bacterial groups.

In the wet season, bands clustered on the basis of the soil sampling points (Figure 3B). The areas of native vegetation had 89 and 85% of similarity for *16S-nifH* bands in the three depth. These results show the influence of water infiltration on the bacterial community. This is to say that floods and infiltration had evenly distributed the bacteria communities along the soil profile.

The fungal community was similar in all areas points of samples collection of SF1, regardless of anthropic action and soil depth (Figures 3C and 3D). In the wet season, an increase in the intensity of the DGGE bands was observed (Figure 3D). This increase was proportional to the viable fungal cells amount. Barbosa (2014) also observed an increase in the number of DGGE bands and the fungal cells amount in the wet season.

In SF2, the viable microbial cell count was lower in the soybean field than in native vegetation field that showed once again the preservative action of native vegetation cover on the soil microbiota (Table 3).

The effect of the moisture and depth of soil in SF2 on the viable cell count was similar to the results of SF1 (Table 3). In fact, the lowest count was at 20-30 cm depth and in the dry season. The fungal growth was limited in anoxic sites (Madigan et al., 2010, Tortora et al., 2014).

Table 3. Viable microbial cells count in dry and wet seasons, of the soil samples of soybean farm 2 (SF2)

Sampling area	Sample code	Dry season			Wet season		
		Actinomycete	Bacteria	Fungi	Actinomycete	Bacteria	Fungi
		---------------------------------- Log (Colony forming unit g^{-1}) ----------------------------------					
Soybean field 1	P13SD1	7.47±0.45	10.00±0.29	6.18±0.32	9.12±0.89	12.20±0.42	7.55±0.39
	P13SD2	5.73±0.47	8.20±0.31	5.18±0.29	6.95±0.95	9.90±0.36	6.29±0.33
	P13SD3	4.01±0.48	6.10±0.31		6.04±0.48	7.30±0.52	
	P14SD1	9.02±0.50	10.90±0.32	6.90±0.46	10.82±0.61	13.10±0.21	8.28±0.31
	P14SD2	8.54±0.45	9.30±0.32	6.29±0.49	10.18±0.61	11.10±0.45	7.51±0.37
	P14SD3	4.98±0.46	7.50±0.32		7.09±0.70	8.90±0.41	
	P15SD1	10.10±0.46	11.40±0.29	6.83±0.30	11.90±0.65	13.40±0.58	8.04±0.31
	P15SD2	7.32±0.48	9.20±0.30	5.38±0.31	8.57±0.23	10.80±0.34	6.30±0.33
	P15SD3	4.79±0.49	7.40±0.31		6.74±0.27	8.70±0.31	
	P16SD1	8.18±0.45	9.90±0.31	6.74±0.57	11.78±0.36	11.50±0.53	7.79±0.11
	P16SD2	6.96±0.47	8.60±0.32	5.98±0.59	10.31±0.39	9.90±0.59	6.88±0.12
	P16SD3	4.89±0.49	7.70±0.32		9.02±0.70	8.80±0.27	2.77±0.80
Native vegetation field of ephemeral stream	P17SD1	11.36±0.47	12.60±0.30	6.69±0.56	13.63±0.47	14.10±0.58	8.03±0.06
	P17SD2	9.80±0.48	10.30±0.31	6.23±0.56	11.69±0.96	12.20±0.45	7.42±0.60
	P17SD3	5.51±0.49	8.20±0.31		10.08±0.62	9.70±0.50	
	P18SD1	11.48±0.46	11.70±0.32	6.99±0.30	13.52±0.56	13.80±0.48	8.24±0.26
	P18SD2	7.55±0.46	8.60±0.32	5.83±0.48	11.19±0.47	10.10±0.47	6.82±0.30
	P18SD3	5.60±0.49	8.00±0.30		10.01±0.70	9.30±0.52	
	P19SD1	10.99±0.50	11.70±0.31	7.10±0.49	13.88±0.63	13.60±0.47	8.22±0.17
	P19SD2	10.31±0.50	10.50±0.49	6.46±0.32	11.80±0.39	12.00±1.67	7.43±0.09
	P19SD3	5.00±0.45	9.50±0.51		6.90±0.47	10.90±0.94	
	P20SD1	11.91±0.46	12.30±0.46	7.34±0.31	13.50±0.74	14.00±0.40	8.34±0.23
	P20SD2	7.70±0.47	9.00±0.48	6.99±0.46	10.90±0.59	10.10±0.47	7.90±0.19
	P20SD3	5.61±0.48	8.30±0.32		9.60±0.81	9.30±0.70	
Soybean field 2	P21SD1	9.72±0.50	11.80±0.29	7.11±0.48	11.05±0.66	13.50±0.35	8.09±0.18
	P21SD2	7.94±0.45	9.00±0.31	6.19±0.49	8.97±0.74	10.10±0.40	7.00±0.15
	P21SD3	4.69±0.46	7.80±0.31		6.40±0.55	8.80±0.45	
	P22SD1	9.27±0.48	10.80±0.32	7.56±0.58	12.59±0.49	12.10±0.72	8.44±0.15
	P22SD2	9.35±0.49	10.50±0.29	6.85±0.56	10.38±0.32	11.70±0.66	7.61±0.95
	P22SD3	4.50±0.46	8.20±0.30		9.40±0.98	9.00±0.90	
	P23SD1	10.93±0.49	12.00±0.31	7.23±0.60	12.01±0.70	13.20±0.63	7.95±0.17
	P23SD2	8.63±0.50	10.50±0.31	5.60±0.54	9.42±0.66	11.50±0.39	6.12±0.09
	P23SD3	4.77±0.51	8.20± 0.32		7.36±0.43	8.90±0.47	
	P24SD1	10.77±0.45	10.80±0.32	6.13±0.54	13.16±0.44	13.20±0.74	7.50±0.23
	P24SD2	8.13±0.46	9.30±0.29	4.68±0.57	12.30±0.37	11.30±0.59	5.68±0.19
	P24SD3	5.92±0.46	7.20±0.29		10.76±0.95	8.70±0.81	

Note. P: Sample collection point, SD: Soil depth (SD1 = 0-10, SD2 = 10-20, SD3 = 20-30 cm).

The viable microbial cell counts in soybean field 2 were similar to the cell counts from native vegetation field of the ephemeral stream (Table 3). This result may be due to the time of planting soybean and soil type.

In SF2, we also observed that the NFBs and AMFs profiles depended on the season (Figure 4). Similar to the result of SF1, in the dry season, samples formed clusters for *16S-nifH* gene based on soil depth (Figure 4A). Therefore, in same soil depth, there was no difference in microbial diversity among soybean field 1 (P13-16), native vegetation field (P17-20) and soybean field 2 (E21-24). This result may be due to the few microbial groups that can grow in low water availability. Silva et al. (2018) also obtained clusters of *nifH* and *18S rDNA* genes by soil depth in the Cerrado.

In the wet season, there was an increase in the intensity of *16S-nifH* bands (Figure 4B). These bands were more evident in the upper part of the gel. Therefore, there was not an increase in bacterial diversity. Furthermore, only on the soil surface, there was a cluster between sample collection points, because of the influence of water on the microbial community.

In SF2, the bacterial community of native vegetation field was similar to the soybean field (Figures 4A and 4B). The soil resilience and the short time anthropic activity had not considerably affected bacteria in the soybean field.

The fungal community was present in all sample collection points, but with higher intensity in the wet season (Figures 4C and 4D). In dry season, there was no difference between bands of AMFs profile from 20-30 cm soil depth (Figure 4C). At this depth, the lack of oxygen and other nutrients led to low survival of the aerobic microorganisms. In addition, in the wet season, there was an increase in the intensity of the AMF bands (Figure 4D). Therefore, the highest fungal cell amount in the wet season was due to the water availability that contributes to spore germination.
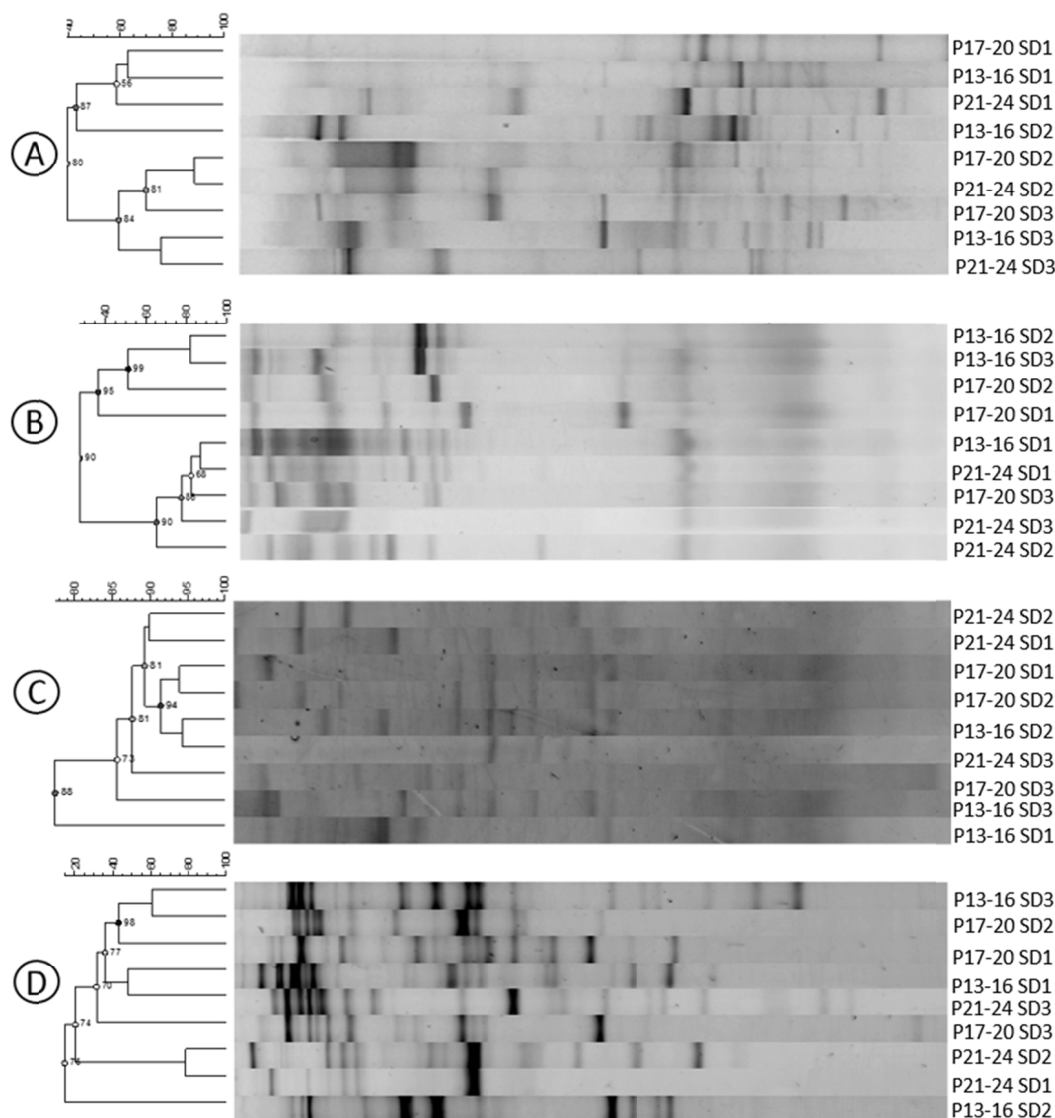
Figure 4. DGGE profiles of the *16S rDNA*, *nifH* (A and B) and *18S rDNA* and *arbuscular mycorrhizal* genes (C and D) of soil from the soybean farm 2 in dry (A and C) and wet (B and D) seasons
(Cf table 4 for samples' codes)

In SF3, viable microbial cell counts were higher in native vegetation field than in soybean field and anthropogenic fragmentation (Table 4). In the anthropogenic fragmentation area, the cell count was the lowest due to soil compaction and disposal of food residues and others human utensils. In fact, the anthropogenic fragmentation area was the place of food residues disposal, of rest and sanitation of the rural workers. Food residues may have influenced the growth of fungi in 20-30 cm depth layer. These results show the importance of delimiting environmental preservation areas within of the soybean field.

The effect of depth and moisture soil on the quantity of microbial cells in SF3 was similar to the one observed in the others soybean farms (SF1 and SF2) (Tables 2 to 4). SF3 had the highest endemism rate showing profile of NFBs and AMFs different from those obtained from the other soybean farms (Figures 3 to 5). Furthermore, some bands of *16S-nifH* gene profile of soybean field were not found in native vegetation field and anthropic fragmentation DGGE profiles (Figure 5A). These results reinforce the need for an environmental preservation policy in this soybean farm.

In SF3, an increase in the number of bands of *16S-nifH* gene was also observed in the wet season (Figure 5B). In the dry season, the *16S-nifH* gene clustering by the soil depth (and sample collection points. The clustering for *16S-nifH* gene was only a function the sample collection points in the wet season (Figures 5A and 5B).

Table 4. Count of viable microbial cells in dry and wet seasons, of the soil samples of soybean farm 3 (SF3)

Sampling area	Sample code	Dry season			Wet season		
		Actinomycetes	Bacteria	Fungi	Actinomycetes	Bacteria	Fungi
		------------------------------------- Log (Colony forming unit g^{-1}) -------------------------------------					
Soybean field 01	P25SD1	6.59±0.29	10.80±0.42	5.45±0.17	8.06±0.23	13.17±0.45	6.66±0.85
	P25SD2	4.90±0.28	8.50±0.40	4.43±0.17	5.95±0.08	10.31±0.36	5.38±0.69
	P25SD3	3.95±0.26	5.80±0.37		4.76±0.27	6.96±0.78	
	P26SD1	8.01±0.29	11.70±0.42	6.13±0.17	9.61±0.56	13.99±0.51	7.35±0.95
	P26SD2	7.30±0.28	9.50±0.40	5.38±0.17	8.70±0.73	11.34±0.45	6.42±0.83
	P26SD3	4.71±0.26	7.00±0.37		5.58±0.31	8.30±0.69	
	P27SD1	8.92±0.29	11.90±0.42	6.0±0.17	10.50±0.85	13.99±0.51	7.10±0.93
	P27SD2	6.26±0.28	9.30±0.40	4.6±0.17	7.33±0.43	10.84±0.39	5.39±0.71
	P27SD3	4.56±0.26	6.80±0.37		5.31±0.30	7.94±0.54	
	P28SD1	9.04±0.29	10.20±0.42	5.98±0.17	10.46±0.49	11.82±0.52	6.92±0.93
	P28SD2	7.66±0.28	8.50±0.40	5.11±0.17	8.82±0.44	9.78±0.44	5.88±0.79
	P28SD3	6.22±0.26	6.90±0.37	1.57±0.15	7.11±0.84	7.92±0.14	1.79±0.24
Native vegetation field	P29SD1	9.55±0.32	13.20±0.46	6.99±0.19	10.86±0.84	15.05±0.61	7.95±0.88
	P29SD2	7.58±0.31	9.70±0.45	5.92±0.19	8.57±0.81	10.95±0.52	6.69±0.92
	P29SD3	5.05±0.29	7.80±0.42	1.58±0.17	5.68±0.31	8.76±0.14	1.78±0.25
	P30SD1	11.25±0.33	12.10±0.47	7.55±0.20	12.58±0.92	13.51±0.68	8.4±0.80
	P30SD2	7.99±0.28	10.00±0.40	5.85±0.17	8.88±0.89	11.12±0.53	6.51±0.91
	P30SD3	6.70±0.26	7.10±0.37	1.82±0.15	7.41±0.97	7.86±0.16	2.01±0.28
	P31SD1	10.74±0.32	13.00±0.46	7.11±0.19	11.81±0.81	14.28±0.65	7.81±1.10
	P31SD2	7.55±0.29	10.10±0.41	4.90±0.17	8.25±0.74	11.02±0.45	5.36±0.76
	P31SD3	5.34±0.26	7.00±0.37	1.73±0.15	5.80±0.54	7.65±0.16	1.88±0.27
	P32SD1	10.53±0.32	12.90±0.46	6.00±0.19	12.87±0.88	15.74±0.49	7.33±0.93
	P32SD2	8.66±0.28	9.7±0.40	4.00±0.17	10.52±0.66	11.73±0.33	4.86±0.62
	P32SD3	7.03±0.26	6.80± 0.37	1.87±0.15	5.48±0.66	8.24± 0.15	2.26±0.29
Anthropogenic fragmentation area	P33SD1	4.35±0.13	5.40± 0.18	2.56±0.07	5.22±0.48	6.48± 0.21	3.07±0.40
	P33SD2	2.50±0.08	3.10± 0.12	1.59±0.05	2.98±0.27	3.73± 0.13	1.90±0.25
	P33SD3	6.70±0.26	7.70± 0.37	1.95±0.15	7.94±0.86	9.09± 0.16	2.32±0.30
	P34SD1	4.46±0.13	5.40± 0.18	2.72±0.08	5.25±0.53	6.32± 0.23	3.20±0.42
	P34SD2	2.23±0.08	2.40± 0.11	1.36±0.05	2.61±0.32	2.76± 0.12	1.59±0.21
	P34SD3	6.78±0.26	7.30± 0.37		7.89±0.95	8.55± 0.14	
	P35SD1	4.39±0.12	5.00± 0.17	2.60±0.07	5.08±0.58	5.75± 0.22	3.01±0.40
	P35SD2	2.50±0.08	2.90± 0.12	1.58±0.05	2.90±0.31	3.39± 0.14	1.82±0.24
	P35SD3	3.20±0.17	5.80± 0.25		3.70±0.04	6.59± 0.16	
	P36SD1	3.70±0.10	4.40± 0.15	2.28±0.06	4.20±0.46	4.95± 0.20	2.60±0.35
	P36SD2	2.00±0.07	2.10± 0.10	1.47±0.04	2.30±0.30	2.41± 0.13	1.66±0.23
	P36SD3	5.80±0.22	6.30± 0.32		6.60±0.82	7.11±0.55	

Note. P: Samples collection point, SD: Soil depth (SD1 = 0-10, SD2 = 10-20, SD3 = 20-30 cm).

SF3 bands' profiles of *18S-AMFs* gene were also different from the profiles of the other soybean farms (Figures 3 to 5). In the dry season, the band's profiles of anthropogenic fragmentation were more closer to the profile of the soybean field than to that of the native vegetation field (Figure 5C). However, in the wet season, an inversion occurs of this result (Figure 5D). This demonstrate that the anthropogenic action is most evident in the period of water scarcity.
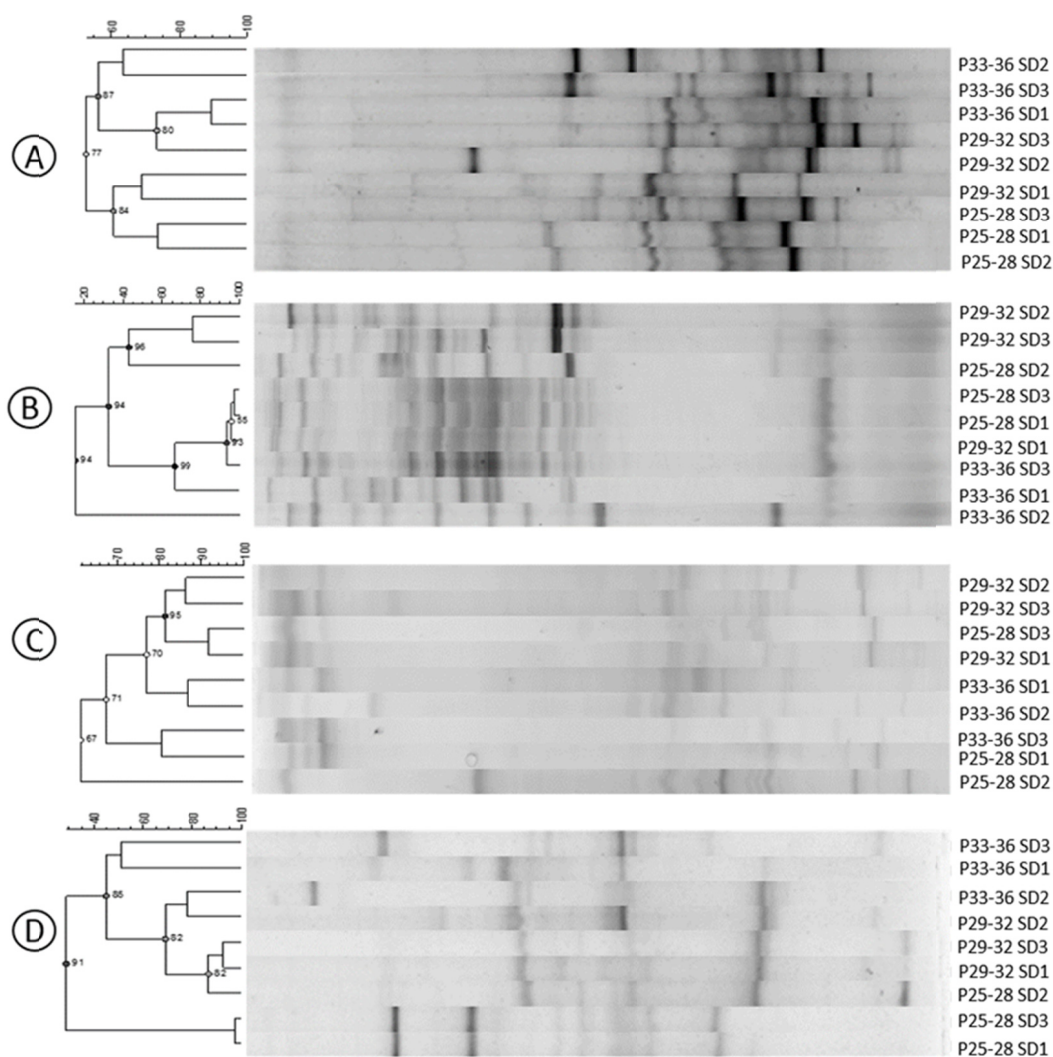
Figure 5. The DGGE profiles of the *16S rDNA*, *nifH* (A and B) and *18S rDNA* and *arbuscular mycorrhizal* genes (C and D) of soils from the soybean farm 2 in dry (A and C) and wet (B and D) seasons (Cf Table 4 for samples' codes)

Thus, the DGGE profile of *18S-AMFs* was adequate to analyze differences in the AMF community before and after environmental changes. Vainio and Hantula (2000) also using the amplification of *18S rDNA* genes and NS1 primers for the study of the fungal community by DGGE showed that such amplification, have high-resolution bands. They concluded that DGGE is suitable for the detection of fungal groups under different crop and sampling conditions.

The increase of the agricultural production area in the Cerrado, especially in Porto Nacional/TO city is worrisome due to the endemic microorganisms rate that was observed in this study. Thus, the maintenance of areas with native vegetation cover is very important for the sustainability of agribusiness.

4. Conclusion

The average monthly temperature of the regions is not a limiting factor for soybean production; however, rainfall limits its cultivation from November to April. In these Cerrado regions, soils with native vegetation cover do not differ in dry and wet seasons, how much to the root growth. However, the removal of natural vegetation for soybean cultivation influences communities as well as NFBs and AMFs diversities. In addition, anthropic action on the microbial community is more effective in the period of water scarcity, which shows the importance of maintaining an environmental reserve area within agricultural production units.

Acknowledgements

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