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Fungal Community Structure as an Indicator of Soil Agricultural Management Effects in the Cerrado

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ABSTRACT: Forest-to-agriculture conversion and soil management practices for soybean cropping are frequently performed in the Cerrado (Brazilian tropical savanna). However, the effects of these practices on the soil microbial communities are still unknown. We evaluated and compared the fungal community structure in soil from soybean cropland with soil under native Cerrado vegetation at different times of the year in the Tocantins State. Soil samples were collected in two periods after planting (December) and in two periods during the soybean reproductive growth stage (February). Concomitantly, soil samples were collected from an area under native Cerrado vegetation surrounding the agricultural area. The soil DNA was analyzed using a fingerprinting method termed Automated Ribosomal Intergenic Space Analysis (ARISA) to assess the fungal community structure in the soil. Differences in the fungal community structure in the soil were found when comparing soybean cropland with the native vegetation (R = 0.932 for sampling 1 and R = 0.641 for sampling 2). Changes in the fungal community structure after management practices for soybean planting in Cerrado areas were related to changes in soil properties, mainly in copper, calcium, and iron contents, cation exchange capacity, base saturation, and calcium to magnesium ratio. These results show the changes in the fungal community structure in the soil as an effect of agricultural soil management in Cerrado vegetation in the state of Tocantins.

Keywords: change in land use, soil microbiology, molecular ecology, soil fungi.

INTRODUCTION

Agriculture expansion into the Cerrado (Brazilian tropical savanna) environment has led to rapid conversion of this biome into large areas of cattle farming, reforestation, and grain growing, especially soybeans (Bresolin et al., 2010; Lourente et al., 2011). The natural conditions of the Cerrado, such as flat terrains, enable agricultural mechanization and technological development for selection of cultivars highly efficient in nitrogen fixation. These factors have promoted the expansion of soybean cultivation into this biome, making it an economically viable and continuous practice.

Maintaining crop residues on the soil surface is a common practice in a low-management cropping system, and is an alternative for enhancing soil quality through an increase in aggregate stability, porosity, water holding capacity, and reduced water loss through evaporation (Lourente et al., 2011). Despite the adoption of low-management practices in most soybean-growing areas, agricultural expansion for grain cultivation involves the removal of native vegetation and the use of agricultural inputs (Bresolin et al., 2010). When converting native forest areas to grain croplands, some soil chemical and microbiological properties are altered (Costa et al., 2006; Carneiro et al., 2009; Navarrete et al., 2013), which indicates the need to evaluate such properties under the agricultural soil management practices in the Cerrado areas.

One of the strategies used to evaluate changes in the soil environment due to changes in land use and agricultural management practices is to compare the properties of lands under soil management and those of adjacent areas under natural vegetation (Barros and Comerford, 2002). Several studies have highlighted soil management as an important factor influencing soil microbiota structure (Jesus et al., 2009; Taketani and Tsai, 2010; Navarrete et al., 2011). However, the mechanisms involved in changes in soil microbial communities are not yet fully understood (He et al., 2012). These microbial communities can be analyzed by molecular methods, such as Automated Ribosomal Intergenic Spacer Analysis (ARISA). This technique, based on DNA fingerprinting, allows a rapid and reproducible evaluation of the genetic structure of complex communities in different environments by exploring the length polymorphism of the ribosomal DNA region containing two internal transcribed spaces (ITS1 and ITS2) and gene 5, 8S rRNA in the case of fungi (Ranjard et al., 2001; Danovaro et al., 2009). The ARISA technique is based on the use of a fluorescence-labeled primer to amplify the genomic DNA through polymerase chain reaction (PCR), with subsequent discrimination of the size of the amplified fragment in an automated sequencer (Ranjard et al., 2001). This technique has been used for analysis of microbial communities present in environmental samples, including soil samples (Ranjard et al., 2001; Mougel et al., 2006; Blackwood and Buyer, 2007; Navarrete et al., 2010; Jouquet et al., 2013).

The hypothesis of this study is that the conversion of Cerrado lands in the state of Tocantins into soybean croplands may have altered the chemical properties and the fungal community structure in the soil. In this sense, the ARISA technique was used to evaluate the structure of the fungal community (filamentous and yeast) present in soil under agricultural management for soybean cultivation from the Cerrado in Tocantins, and soil from an adjacent area under native vegetation, at different times of the year.

MATERIALS AND METHODS

Sampling areas and collection of soil samples

The study was carried out in Porto Nacional, a municipality located in the state of Tocantins, Brazil. Soil samples were collected in an area under agricultural management (10° 10’ 39.8” South and 48° 40’ 39.1” West) and in an area under native Cerrado vegetation (10° 10’ 31.7” South and 48° 40’ 35.5” West); these samples were denominated only

as “Agricultural” and “Cerrado”, respectively, in this study. At the time of sampling, the agricultural area had been under a no-tillage system (NT) for soybean planting for three consecutive years during the main crop season, and corn and sorghum planting in the off-season. The climate in the region is classified as humid sub-humid, with moderate water deficit (C2wA‘a’ according to the Thornthwaite classification system), with an average annual temperature between 26 and 27 °C, and average annual rainfall between 2,000 and 2,100 mm (Seplan, 2012). The study area is in the Cerrado biome, which has vast areas directed to grain production, and remnant vegetation typical of the Cerrado in *sensu stricto* (the strict sense), which is characterized by the presence of defined arboreal, shrub, and herb strata, with random distribution of trees at different densities.

Soil sampling was performed in the rainy season, December 2012 and February 2013 (sampling 1), and in December 2013 and February 2014 (sampling 2) (Table 1). The month of December corresponds to the post-planting period in the agricultural areas in both samplings, while the month of February corresponds to the soybean reproductive phase, phenological stage R4, in which most pods are in the upper third with a length of 2-4 cm (Ritchie et al., 1982).

A central sampling point was randomly defined in both areas for soil sampling. From the central point, four other points were established, positioned to the south, north, east, and west at 100 m from the central point. Composite soil samples were collected from each of the five sampling points. To do so, five sub-samples were taken at the 0.00-0.10 m depth using the same sample distribution described above, but at 2 m from the central point (Figure 1). Soil samples were transported in polystyrene boxes under ice gel from the field to the laboratory. Part of the sample was sent for chemical analysis, and 0.25 g was used for extracting the genomic DNA from the soil.

Chemical analyses

The following macro- and micronutrients were analyzed: Na, Z, B, Cu, Fe, Mn, Ca and Mg ratio (Ca+Mg), Ca, Mg, Al, K, P, and S. Cation exchange capacity (CEC), base saturation (V), aluminum saturation (m), potential acidity (H+Al), pH, and organic matter percentage (OM) were also analyzed. All analyses were made according to Donagema et al. (2011).

Extraction, amplification, and purification of genomic DNA from the soil

Genomic DNA from the soil was extracted using the Power Lyzer™ Power Soil® DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA), following manufacturer’s guidelines. The concentration and quality of the DNA were determined by NanoDrop spectrophotometer (NanoDrop® ND-1000 UV/vis-spectrophotometer, Peqlab Biotechnologie GmbH, Erlangen, Germany), followed by agarose gel electrophoresis in TBE buffer (200 mmol L^{-1} Tris-HCl (pH 8.4), 500 mmol L^{-1} KCl) subjected to 90 V for 1 h. The DNA extracted was stored at -20 °C until use.

Table 1. Location of the sampling sites and number of soil sampling

Sampling period	Area	Period	Number of samples
1	Agricultural Area	December 2012	5
		February 2013	5
	Cerrado Area	December 2012	5
		February 2013	5
2	Agricultural Area	December 2013	5
		February 2014	5
	Cerrado Area	December 2013	5
		February 2014	5
Total			40

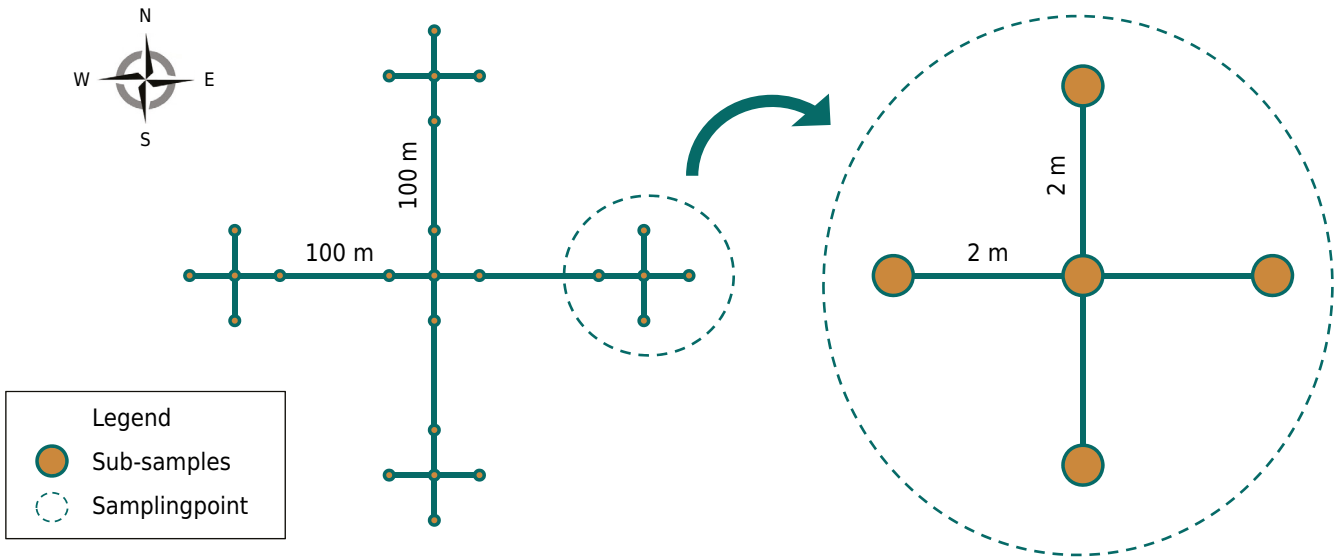

Figure 1. Representative schema of the soil sampling design used in both study areas.

The PCR-ARISA reactions were prepared for a final volume of 25 µL using 1X Taq DNA polymerase buffer (20 mmol L^{-1} Tris-HCl (pH 8.4), 50 mmol L^{-1} KCl) (Invitrogen, Carlsbad, Calif.), 3 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTP's, 5 pmoles of primer 2234Cf-FAM (5'-GTTTCCGTAGGTGAACCTGC-3'), 5 pmoles of primer 3126Tr (5'-ATATGCTTAAGTTCAGCGGGT-3'), both described by Ranjard et al. (2001), 10 ng DNA, 1 U Platinum® Taq DNA Polymerase (Invitrogen, Life Technologies®, Brazil), and 17.3 µL sterile water. Reactions were performed in a thermocycler (GeneAmp PCR System 9700 Applied Biosystems, Foster City, CA) under the following conditions: 94 °C for 3 min, 35 cycles at 94 °C for 30 s, 59 °C for 45 s, 72 °C for 1 min, and 72 °C for 15 min. The amplification product was verified by an electrophoretic run on agarose gel at 90 V for 1 h using TSB buffer (Brody and Kern, 2004). The PCR-ARISA products were purified using the GFX PCR DNA and Gel Band Purification kit (GE Healthcare, GE Brazil), according to manufacturer's instructions.

Automated analysis of ribosomal intergenic spacer - ARISA

Discrimination by automated capillary electrophoresis required by the ARISA technique was performed on an ABI PRISM 3100 Genetic Analyzer automatic sequencer (Applied Biosystems, Foster City, CA). To load the samples into the sequencer, 1 µl of the purified PCR product, 8.8 µL of HiDi formamide, and 0.2 µL of a GeneScanTM 500 ROX standard length (Applied Biosystems, Foster City, CA) were used. The samples were then denatured by heating at 94 °C for 5 min, cooled at 0 °C for 3 min, and loaded into the sequencer.

Data processing and statistical analyses

The electropherograms resulting from capillary electrophoresis in an automated sequencer were analyzed for quality using the Peak Scanner version 1.0 software (Applied Biosystems, Foster City, CA). In summary, in each electropherogram resulting from DNA fingerprinting, the fluorescence units of each peak were converted into data on total fluorescence. To do so, the value attributed to the fluorescence of each peak was divided by the total fluorescence value of the sample (Culman et al., 2008).

Canonical Correspondence Analysis (CCA) was performed with the Canoco for Windows version 4.5 software (Biometris, Wageningen, The Netherlands). Analysis of similarity (ANOSIM) was performed using the Primer version 5.0 software (Plymouth Marine Laboratory, Primer-E, UK). Analysis of similarity is a permutation-based statistical hypothesis test used to test for differences between groups of samples from different

sites or experimental treatments (Mucha et al., 2013). In both the CCA and ANOSIM analyses, the distance between the samples was evaluated using the Bray-Curtis index. The sizes of the intergenic space of 50 to 800 base pairs were considered for the statistical analyses of the data obtained by the ARISA technique. The Tukey test was conducted with the aid of the Past version 2.04 software (Oyvind Hammer, University of Oslo, Norway) to evaluate the differences between the mean values for soil chemical factors.

RESULTS AND DISCUSSION

Changes in soil chemical properties

The Fe and Ca contents, Ca+Mg ratio, base saturation, potential acidity, and pH were significantly different between the soil samples from the soybean cropland and the area under Cerrado vegetation (sampling 1 and 2) (Table 2). In sampling 1, the contents of Mn, Mg, Al, and CTC and m showed significant differences between the two sampling areas. In sampling 2, both in the collections of December of 2013 and February of 2014, Fe content, Ca+Mg ratio, V, and pH showed significant differences between the soybean cropland and the area under Cerrado vegetation. However, the Na, Zn, B, S, and OM contents showed no significant differences between the sampling areas in any of the two samplings.

Agricultural soils had higher pH values, higher OM, and higher macronutrients (P, K, S, Ca, and Mg) contents than the native Cerrado soil. The higher pH in the agricultural area is due to the liming process carried out before soybean planting for pH correction, since the soil is naturally acidic in the area (Bresolin et al., 2010). Liming has a greater effect on soil surface layers, due to its low mobility in the soil profile (Frazão et al.,

Table 2. Chemical properties of the soil under Cerrado vegetation and soybean cropland in two sampling periods

Chemical properties[(1)]	Sampling 1 (December 2012 and February 2013)				Sampling 2 (December 2013 and February 2014)			
	Agri D12[(2)]	Cer D12[(3)]	Agri F13[(4)]	Cer F13[(5)]	Agri D13[(6)]	Cer D13[(7)]	Agri F14[(8)]	Cer F14[(9)]
Na (mg dm^{-3})	2.60a±1.52	2.20a±0.84	14.40a±1.14	14.00a±3.39	14.20a±0.45	13.80a±0.45	14.00a±0.71	13.40a±1.14
Zn (mg dm^{-3})	2.76a±1.19	1.74a±0.61	1.56a±0.77	1.48a±2.75	3.16a±1.45	2.08a±1.28	4.38a±2.71	2.08a±0.95
B (mg dm^{-3})	0.25a±0.04	0.24a±0.02	0.23a±0.06	0.21a±0.05	0.18a±0.05	0.18a±0.06	0.21a±0.05	5.17a±11.09
Cu (mg dm^{-3})	0.38a±0.25	0.26a±0.13	0.60a±0.26	0.40a±0.12	1.80a±0.35	5.48a±8.68	1.90a±0.40	1.24b±0.32
Fe (mg dm^{-3})	47.20b±3.96	98.18a±19.58	43.04b±5.32	71.30a±6.43	60.40b±16.95	121.42a±48.09	67.34b±4.59	145.80a±40.15
Mn (mg dm^{-3})	7.74a±1.99	8.64a±3.20	7.22a±1.40	4.74b±1.92	5.32a±1.45	4.36a±2.22	5.36a±1.29	4.56a±2.26
CEC ($cmol_c$ dm^{-3})	4.75b±0.38	5.99a±0.68	3.78a±0.39	3.85a±0.38	5.71a±0.51	5.40a±1.64	4.84a±0.26	5.83a±1.08
V (%)	61.26a±3.40	26.08b±5.34	55.97a±6.78	20.32b±5.20	68.09a±5.82	37.49b±12.17	64.64a±6.28	38.49b±7.32
m (%)	0.00a±0.00	3.70a±3.46	2.80b±4.37	22.55a±5.31	0.00a±0.00	8.74a±10.57	0.00a±0.00	13.55a±7.72
Ca+Mg	2.76a±0.25	1.50b±0.30	2.06a±0.39	0.72b±0.13	3.78a±0.50	1.86b±0.36	3.06a±0.27	2.18b±0.49
Ca^{2+} ($cmol_c$ dm^{-3})	1.76a±0.25	0.86b±0.15	1.28a±0.24	0.44b±0.05	2.74a±0.30	1.12a±0.22	2.08a±0.28	1.40b±0.33
Mg^{2+} ($cmol_c$ dm^{-3})	1.00a±0.00	0.64a±0.15	0.78a±0.16	0.28b±0.08	1.04a±0.30	0.74a±0.17	0.98a±0.16	0.78a±0.23
Al^{3+}($cmol_c$ dm^{-3})	0.00a±0.00	0.06a±0.05	0.06b±0.09	0.22a±0.04	0.00a±0.00	0.20a±0.28	0.00a±0.00	0.34a±0.21
H+Al ($cmol_c$ dm^{-3})	1.84b±0.23	4.44a±0.71	1.66b±0.26	3.08a±0.50	1.82a±0.38	3.50a±1.63	1.72b±0.38	3.60a±0.91
K (mg dm^{-3})	58.00a±20.33	19.40b±6.07	24.00a±10.20	17.20a±5.40	41.80a±10.50	16.20b±8.41	24.60a±5.32	18.40a±11.57
P (mg dm^{-3})	19.68a±25.29	0.34a±0.09	24.22a±18.44	0.96b±0.39	12.34a±9.23	1.04b±0.73	32.68a±39.17	2.42a±0.86
S (mg dm^{-3})	9.18a±4.08	6.92a±4.18	3.64a±0.95	3.60a±1.40	4.84a±4.12	1.24a±0.50	5.26a±1.53	7.36a±2.04
OM (%)	1.12a±0.13	0.98a±0.37	1.92a±0.70	1.44a±0.40	1.12a±0.37	1.14a±0.57	1.56a±0.77	1.24a±0.57
pH($CaCl_2$)	6.02[(10)]a±0.19[(11)]	4.38b±0.08	5.32a±0.26	4.42b±0.13	5.98[(10)]a±0.37[(10)]	4.44b±0.19	6.28a±0.19	4.52b±0.08

[(1)] Chemical properties analyzed according to Donagema et al. (2011). Agri: agricultural area; Cer: native Cerrado vegetation; D: December; F: February. Tukey test ($p<0.05$) comparing agricultural area and Cerrado [(2) vs (3), (4) vs (5), (6) vs. (7), (8) vs (9)], values followed by the same letter in rows do not differ significantly. [(10)] Average values of the five soil samples from the sample area followed by the standard deviation. [(11)] Standard deviation of the mean for the five soil samples from the sample area.

2008). The structure of the soil microbial communities are strongly influenced by soil pH (Wakelin et al., 2008; Jesus et al., 2009; Val-Moraes et al., 2016).

Acidity and low nutrient levels, such as Ca and Mg, are characteristic of Cerrado soils. When the soil is acid, Al toxicity impairs the growth of plant roots, reducing the ability to take up water and nutrients. Thus, limestone treatment to raise soil pH, neutralize Al, and make Ca and Mg available is necessary to make agricultural production systems possible (Miranda and Miranda, 2007). Soil pH appeared as a factor related to the variability of other soil chemical properties, such as Fe and K, in all the analyses. The diversity of microbial communities is generally correlated with soil acidity, with properties such as pH, H+Al, and V (Jesus et al., 2009; Navarrete et al., 2013). Soil pH is an important feature and is related to changes in other soil properties, such as Al concentration and nutrient availability (McBride, 1994).

Base saturation showed significant values in the two samplings. The values of this property were higher in the agricultural areas. Frazão et al. (2008) evaluated the effect of different land uses and management systems on the chemical properties of a soil in the Cerrado of Mato Grosso. In the study mentioned, the authors also found the lowest V values in the native Cerrado area and observed that V was higher when management systems have been implemented for longer periods (four and five years), which provides better conditions for cultivation. These data corroborate the results of this study.

The OM and microorganism relation has a fundamental role in soil - changes in soil microbial diversity over time have been related to changes in environmental conditions, such as soil moisture and OM (Lombard et al., 2011). Soil moisture affects the physiological state of the microbial community, as well as soil physicochemical properties and plant yield. Increased plant yield may also alter soil microbial communities, as it results in increased soil carbon input (Castro et al., 2010). Microorganisms break down organic materials, which provide nutrients to plants (Schloter et al., 2003; Kujur et al., 2012). This OM present in the soil contributes around 30 to 50 % of total soluble P in most soils (Richardson et al., 2009). In addition, some metabolic reactions occur unevenly in the soil and have large space-time differences along the soil profile. The accumulation of particulate OM, animal waste, and rhizosphere depositions promote the presence of microhabitats with high levels of biological activity and biodiversity (hot spots) (Gonzalez et al., 2012).

Changes in fungal community structure in the soil and their relationship to soil chemical properties

Initially, it is important to note that changes in the fungal community structure in the soil and their relationship to the soil chemical properties reported in this study were observed from soil sampling in the rainy season. The data on fluorescence unit percentage were ordered with soil chemical properties, revealing distinct clusters for the soil samples collected in soybean cropland and in the natural Cerrado vegetation (Figures 2a and 2b). The fungal communities in the areas studied differed in structure, and these differences were related to the soil chemical properties. In sampling 1, the soil chemical properties explained 76.3 % of the variability of the biological data, with a higher influence from Fe, H+Al, V, pH, and the Ca+Mg ratio (Figure 2a). In sampling 2, 93.1 % of the variability of the biological data was explained by the soil chemical properties, with V, pH, Ca+Mg, Ca, Fe, and the H+Al ratio exhibiting a greater relation to the fungal community structure in the soil (Figure 2b).

In general, analysis of similarity revealed significant statistical differences between the areas evaluated, and these differences were related to several soil chemical properties, such as pH and micronutrients contents (Figure 2 and 3). Based on the R-values, the fungal community structure exhibited well separated groups in the ordering spaces. Only the Cerrado vs. Cerrado and Agricultural vs. Agricultural interactions were non-significant, considering the abiotic data (Table 3).

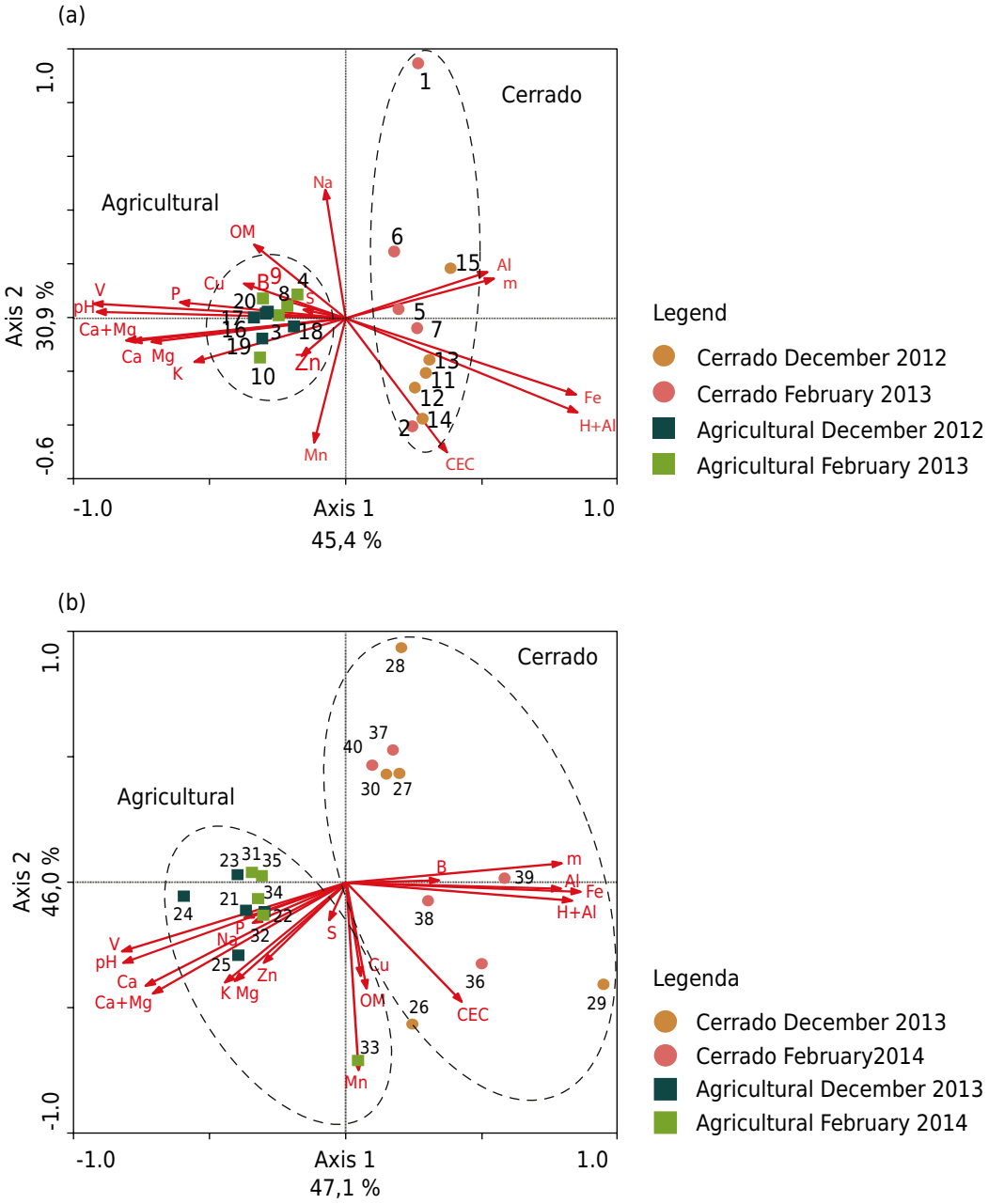

Figure 2. Canonical correspondence analysis based on the fungal community structure and soil chemical properties. (A) sampling 1; (B) sampling 2.

A difference was observed in the fungal community structure (R=0.932) upon comparing the data of the soybean cropland and of the area under Cerrado vegetation in sampling 1 (Table 3). The data from sampling 2 indicated that the differences obtained for this group of samples were lower than those of sampling 1 (R =0.641), and for that reason, the samples were closer in the grouping (Figure 2a). In both campaigns, one grouping was formed from the samples of the soybean cropland and another from the area under Cerrado vegetation, with no overlapping of samples or areas (Figure 2). The agricultural area stands out in samplings 1 and 2 (Figure 3), in which the R-value for the biotic factors was 0.962 (Table 3). This indicates a significant difference between the structures of the fungal communities, which may be associated with the influence of soybean planting in the area. The value of R for abiotic factors was 0.318, and this may be due to the similarity of the chemical treatments performed on the soil before planting in the two sampling campaigns.

The table 4 shows the number of phylotypes present in each study area. In sampling 1, the Cerrado area had a higher total number of phylotypes than that shown for the agricultural

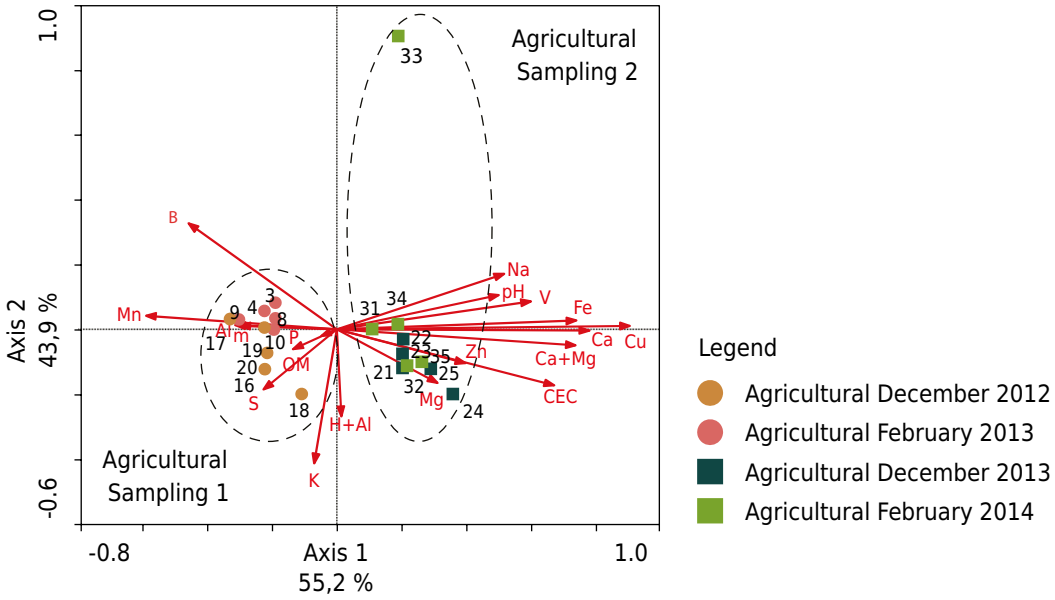

Figure 3. Canonical correspondence analysis based on the fungal community structure and soil chemical properties of the agricultural area (samplings 1 and 2).

Table 3. Similarity analysis considering biotic and abiotic factors of the agricultural and Cerrado area

Interaction[2]	ANOSIM[1]	
	Biotic[3]	Abiotic[4]
Cerrado A1 *vs* Agricultural A1	0.932	0.987
Cerrado A1 *vs* Agricultural A2	0.901	0.948
Cerrado A1 *vs* Cerrado A2	0.711	0.423
Agricultural A1 *vs* Agricultural A2	0.962	0.318
Agricultural A1 *vs* Cerrado A2	0.986	0.944
Agricultural A2 *vs* Cerrado A2	0.641	0.837

[1] Analysis of similarity. All values of "R" are expressed with p<0.001; values >0.75 are statistically different; values >0.5 are overlapping, but are still clearly different; and values <0.5 show no statistical difference. [2] A1: sampling 1; A2: sampling 2. [3] Analysis based on the fungal community structure. [4] Analysis based on soil chemical properties.

Table 4. Phylotypes present in each study area, total number of phylotypes, number of phylotypes with abundance >1 %, number of phylotypes shared between areas

Samp/ Period	SA[1]	Total of phylotypes in each area[2]					TNP[3]	NPA[4] < 1 %	NPA > 1 %	Number of phylotypes with abundance > 1 % shared between:							
		A	B	C	D	E				AS1[5]	AS2[6]	ACS12[7]	AAS12[8]	ACS1[9]	ACS2[10]	AAS1[11]	AAS2[12]
1/D2012	Agri	39	67	86	79	76	191	147	44	3	7	7	8	31	44	25	32
	Cer	93	51	81	113	54	236	164	72								
1/F2013	Agri	72	61	94	61	44	175	115	60								
	Cer	100	39	79	49	59	197	117	80								
2/D2013	Agri	108	104	127	113	116	326	239	87								
	Cer	126	91	120	124	135	343	261	82								
2/F2014	Agri	152	136	109	158	84	338	254	84								
	Cer	139	131	116	148	63	332	246	86								

Samp: Sampling; D: December; F: February; Agri: agricultural area; Cer: native Cerrado vegetation.[1] Study area. [2] A, B, C, D, and E represent the sampling locations in each study area. [3] Total number of phylotypes: the phylotypes that appeared in two or more places in the same area were counted only once, considering only presence or absence. [4] Number of phylotypes with abundance. [5] Areas of Sampling 1. [6] Areas of Sampling 2. [7] Areas of Cerrado (Sampling 1 and 2). [8] Agricultural Areas (Sampling 1 and 2). [9] Areas of Cerrado (Sampling 1). [10] Areas of Cerrado (Sampling 2). [11] Agricultural Areas (Sampling 1). [12] Agricultural Areas (Sampling 2).

area. In sampling 2, the total number of phylotypes in the agricultural area was higher than that in the Cerrado area only in the February period. Upon considering the number of phylotypes with abundance >1 %, in sampling 1, the Cerrado area had a higher number of phylotypes; in sampling 2, the agricultural area had a value higher than that of the Cerrado area in the period of December. Fragments with relative abundance >1 % are considered the dominant organisms in this community (Lehours et al., 2005).

When we evaluated the number of phylotypes with abundance >1 % shared between the areas, higher values were found for sharing when compared to the same sampling, i.e., in a period near collection. When a comparison is made between samplings and between the different areas within the same sampling, the number of phylotypes with abundance >1 % exhibited less sharing. These observations suggest a clear structural difference in the fungal community between the samplings and the study areas (Agricultural and Cerrado). The Cerrado areas of sampling 2 had the highest number of shared phylotypes with abundance >1 %, thus suggesting that these areas are structurally similar.

The fungal community, mainly associated with the degradation of plant residues, apparently undergoes changes due to vegetation type more intensely (Lorenzo et al., 2010), since the physicochemical properties of the soils affect plant physiology and the composition of organic substances exuded by their roots, which in turn influence the composition of the soil microbiota (Beattie and Lindow, 1999). Therefore, the changes that occurred due to agricultural use of the soil may also have resulted in changes essential to soil functioning, which affects the microorganisms present in this environment. These data corroborate descriptions in the literature that show that changes in substrate quality and nutrient availability alter the fungal community and the community roles in a given environment (Allison et al., 2007; Lauber et al., 2008).

Although there was no identification of fungal species in this study, the repetition of time-space molecular and chemical analyses and statistical analyses evidenced that soil management and vegetation are factors that affect the fungal community present in the soil. As the quantity of nutrients required by each fungal species is different (Murray et al., 2010), the differences in soil abiotic factors, as well as heterogeneous availability of nutrients, may explain the differences in fungal abundance in soils with soybean crops and in native Cerrado areas, corroborating the results obtained by Navarrete et al. (2013) and Freitas et al. (2014). In addition, the accumulation of residues on the soil surface, a common practice in NT, may be responsible for the presence of fungal species in the soil that the phylloplane previously inhabited, which also contributed to the differences observed. However, more in-depth studies on the interaction of phylloplane microbiota with the soil microbiota are needed to confirm this inference.

The areas that constitute the Cerrado require studies on the response of the fungal community to the various types of land use. In addition, changes in the fungal community resulting from each land use system can provide important information for soil management and environmental impact assessment (Fracetto et al., 2013). In this context, the fungi present in the soil should be evaluated for their potential to indicate the predominant soil physicochemical changes, i.e., changes in the soil environment.

Thus, the results suggest that soils under agricultural activity have a fungal community distinct from that of soils under native Cerrado vegetation. Recent research involving soil fungal ecology, coupled with PCR techniques from soil genomic DNA, has significantly increased the understanding of fungal ecology in this environment. Up to the time of submission of this manuscript and to the best of our knowledge, this is the first study devoted to evaluating the fungal community structure through independent cultivation techniques in Cerrado areas under agricultural management and comparing it to native vegetation areas in the state of Tocantins.

CONCLUSION

The use of molecular techniques to analyze the fungal community present in the soil, together with determination of soil chemical properties, revealed the effects of conversion of Cerrado areas into soybean cropland on the fungal community structure in the soil. Considering the ability of fungal community structure to respond to changes in soil chemistry from land-use conversion, our results open the possibility of considering such an ecological aspect on the fungal community level as a potential indicator of the effects of soil management on the soil of the Cerrado area in the state of Tocantins.

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