Diversity of filamentous fungi in different systems of land use

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Abstract Microbial population and activity can be influenced by changes in the physical and chemical conditions of the soil. The objective of this study was to compare fungal diversity under different agricultural management systems and associated differences in soil properties. This research was carried out in three areas, representing the Atlantic Forest, AFS and cassava (Manihot esculenta Crantz) monoculture system. Five composite samples were collected during the rainy and dry seasons from each area. Using the composite soil samples, fungal isolation was carried out using a serial dilution technique. Physical, chemical and DGGE analyses of the filamentous fungi community were performed. The fungal isolation data were used to calculate ecological indices of diversity, species richness, equitability, dominance, similarity and density. In general, Atlantic Forest soil presented the highest ecological indices followed by the AFS. The DGGE technique revealed that the structure of the soil mycobiota of the Atlantic Forest and AFS are more than 50% similar. The data indicate that the similarity of the structure and composition of soil mycobiota between AFS and Atlantic Forest is mainly due to the conservation of above-ground plant diversity, and the

conservation of soil characteristics can be attributed to the absence of pesticides and fertilizers.

Keywords Species richness - Mycobiota - Agroforestry System - Atlantic Rain Forest -Manihot esculenta

Introduction

In natural environments, soil microbial communities exhibit complex interactions. The dynamic state of soil microorganisms and the diversity of these communities depend on plant diversity (Naeem et al. [2000\)](#page-8-0). However, anthropogenic activities can affect diversity, resulting in an ecological imbalance with unpredictable consequences that sometimes leads to the extinction of key species essential to ecosystem maintenance (Reber [1992](#page-8-0); Tótola and Chaer [2002\)](#page-8-0).

Soils are degraded in several regions of Brazil as a result of inadequate land use mainly due to the dependence that monocultures have on external input, such as chemical fertilizers and plowing (Neves et al. [2004\)](#page-8-0). In tropical soils, the clearing of native vegetation for the introduction of monocultures changes plant species composition, organic matter, nutrient levels and soil microbial community structure (Tótola and Chaer [2002\)](#page-8-0).

As an alternative to the conventional model of agriculture, Agroforestry Systems (AFS) have been

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suggested. These systems include combining trees, shrubs, agriculture and/or animals in the same area, simultaneously or sequentially, in order to optimize the aggregation of socio-economic-cultural and environmental values. AFS take advantage of biodiversity increments as a dynamic balancing factor as seen in natural ecosystems (MacDicken and Vergara [1990](#page-8-0); Nair [1993](#page-8-0)).

Crops with higher plant diversity have shown that biodiversity can be an important tool in balancing the interaction between plants, insects and microorganisms (Altieri [2002](#page-7-0)). Among the microorganisms found in soil, the fungi are exceptional for their heterotrophic activity on organic matter and potential as agents of biological control of other fungi, nematodes and arthropods besides being associated with most plant in symbiotic (mycorrhiza) or parasitic (diseases) relationships (Alexopoulos et al. [1996](#page-7-0)).

Biodiversity, particularly fungal diversity, can be reduced in soils submitted to conventional agricultural practices compared to natural environments. Although the changes introduced by agricultural practices can benefit microorganisms more adapted to the new conditions, especially bacteria that easily increase populations' densities, these effects can cause an imbalance in the soil environment (Valpassos et al. [2001\)](#page-8-0). However, agricultural activities aimed at the conservation of environmental resources can support populations of soil-dwelling organisms, resulting in an increase of beneficial organisms and/or enabling the establishment of new species in the (eco)system (Altieri [2002](#page-7-0)). Therefore, the aim of this paper was to study the community diversity of filamentous fungi in different systems of land use.

Materials and methods

Study areas

This research was carried out in three areas with different land uses: the AFS, conventional cassava (Manihot esculenta Crantz) monoculture and the native Atlantic Forest, located in the municipality of Abreu e Lima in the metropolitan region of Recife, Pernambuco, Brazil. Annual average rainfall in the region is approximately 1,700 mm with two welldefined seasons: rainy from April to September and dry from October to March (Lamepe [2010\)](#page-8-0). Chemical

properties and soil texture for the three studied areas are presented in Table [1](#page-2-0).

Native Atlantic Forest soil was collected from a conservation area $(7^{\circ}52'22''S; 34^{\circ}55'54''W)$ of approximately 17 ha. Vegetation physiognomy in this area corresponds to Dense Ombrophilous Atlantic Forest, characterized by evergreen forest with a crown of up to 15 m with emergent trees reaching 40 m in height beside dense shrub vegetation composed of arborescent ferns, bromeliads and palms. Climbing plants, epiphytes (bromeliad and orchids) and ferns are also abundant.

Soil samples from the AFS were collected in the São João smallholding, (7°53'13"S; 34°53'43"W). This property has more than 75 plant species, including fruit and wood trees in an area of 1 ha. Management of the area includes pruning and incorporation of the residues to the soil in addition to the local decomposition of the leftovers of short cycle plants for fertilization. Pesticides and chemical fertilizers are banned, and nitrogen is supplied by fertilizing plants.

Soil samples from a cassava monoculture were collected in the Dona Luciana smallholding (7°52'32"S; 34°57'58"W). The sampled area corresponds to 1 ha. of cassava plantation inside the property. The cassava monoculture was established in this area 12 years ago and, in some years, after the cassava was harvested yam or corn was planted until the time for the next cassava crop. This conventional culture system (monoculture) has only one type of plant at a time arranged in simple lines with 1-m spaces between plants.

Soil samplings

Two soil samplings were carried out in each of the three areas. The first was in July/2009 and the second in February/2010, corresponding to the rainy and dry seasons, respectively. The AFS and the monoculture areas were 1 ha each and, therefore, a 1 ha plot was randomly set inside the 17 ha native Atlantic Forest to standardize the size of the sampling area.

In each area, a 5 m^2 $(2.5 \times 2.0 \text{ m})$ plot was centrally set, and 4 equidistant 5 m^2 plots were marked in the North, South, East and West directions from this point, totaling 5 sampling plots/area. In the plots, 4 soil subsamples (0–15 cm deep) were taken to make one composite sample. From each composite soil sample, aliquots were taken for isolation of fungal

Table 1 Chemical properties and texture of soil under native Atlantic Forest, cassava monoculture and AFS of land use

System of land use	Chemical properties of soil											Textural			
	mg/dm ³				pH	cmol _c /dm ^{3 a} $\%$							class		
	Fe	Cu	Zn	Mn	\mathbf{P}		K^b	Na ^c	Al	Ca	Mg H		CEC	OM	
Native Forest	189.54	0.10	1.20	0.58		3.20 4.52	0.06	0.04	0.76 0.92			0.48 6.74 9.00		3.80	Sandy clay loam
Agroforestry	116.02	0.38	4.64	6.00	108.00		5.62 0.12 0.03		0.04	3.18		0.61 2.62 6.60		2.72	Sandy loam
Monoculture	242.64	0.08	1.04	1.88	4.80	5.40	0.05	0.02	0.08	2.06	0.29	3.22 5.73		1.47	Sandy clay loam

OM organic matter

e.mg/100cm³ = cmol_c/dm³

ppm K = cmol_c/dm³ \times 390

 $^{\circ}$ ppm Na = cmol_c/dm³ \times 230

colonies and analysis of filamentous fungi community structure using denaturing gradient gel electrophoresis (DGGE). Soil samples for DGGE analysis were kept at -20 ^oC and at 4^oC for colony isolation.

Ecological indices analyses for filamentous soil fungi

Initially, filamentous fungi isolation from soil samples was carried out using a serial dilution technique. One milliliter of the 10^{-3} dilution was spread onto Sabouraud Agar (AS) (g/l of distilled water: 40 dextrose, 10 peptone, 15 agar, pH 5.5) with chloramphenicol (170 mg/ml) and Bengal rose (0.05 g/l). The Petri dishes were incubated at $27 \pm 2^{\circ}$ C for 10 days. After incubation, the colonies were sub-cultured to SA plus chloramphenicol (170 mg/ml) until single colonies were obtained, and the fungi were identified with the help of personnel from the URM Culture Collection, Mycology Department, Universidade Federal de Pernambuco, Brazil.

After the identification of the filamentous fungal species from the different soils, the ecological indices were applied:

Shannon–Wiener's Diversity (H') : $H' = -\sum pi (ln \theta)$ pi) where $pi = nilN$; $N = total$ number of fungi sampled; $ni =$ number of sampled fungi in a particular taxonomic group; $ln =$ natural logarithm.

Pielou's Equitability $(J') : J = H'/H'_{\text{max}}$ where H'_{max} is the maximum possible diversity to be observed if all species present have equal abundance. $H'_{\text{max}} =$ $\log S$ where $S =$ total number of sampled species.

Berger–Parker's Dominance (d): $d = N_{\text{max}}/N_T$ where N_{max} is the number of individuals of the most abundant species, and N_T is the total number of individuals in the sample.

Species Richness: consists of the total number of species (S) in a sample unit.

Shannon-Wiener's Diversity (H') , Pielou's Equitability (J') and Berger-Parker's Dominance (d) were calculated using the PAST 1.7 software (Hammer et al. [2001](#page-8-0)).

Fungal community structure analyses using denaturant gradient gel electrophoresis (DGGE)

Total DNA from soil samples taken from different study areas was extracted according to Griffiths et al. [\(2000](#page-8-0)). Five composite soil samples from each study area were employed. Briefly, 0.5 g of soil was placed into microcentrifuge tubes to which 500 μ l of 5% CTAB (hexadecyltrimethylammonium bromide) buffer, 500 µl of phenol–chloroform–isoamyl alcohol $(25:24:1 \text{ v/v/v}, \text{pH 8})$ and 0.5 g of acid washed glass beads were added. The tubes were placed in a FastPrep[®] homogenizer and were shaken to break the cells. Extracted DNA was precipitated with sodium acetate 3 M (0.1 v) and isopropanol (0.6 v) on ice for 2 h. DNA was subsequently washed with 70% ethanol, dried and dissolved in 30 µl sterile ultrapure water and stored at -20° C until used. Total soil DNA extracted was checked on 1% agarose gel electrophoresis.

Soil fungal DNA was PCR amplified using the primer pair FR1(GC) (5'-ccc ccg ccg cgc gcg gcg ggc

ggg gcg ggg gca cgg gcc gAI CCA TTC AAT CGG TAI T-3') and FF390 (5'-CGA TAA CGA ACG AGA CCT-3'), giving approximately 400 bp fragments of the 18S rDNA (Vainio and Hantula [2000\)](#page-8-0).

The reaction mix was prepared with $1 \times PCR$ buffer (Tris–HCl 20 mM pH 8.4; NH₄), 2.0 mM MgCl₂, 0.20 mM dNTP mix, $0.40 \mu M$ of each primer, 0.04 U/µl Taq DNA polymerase (Fermentas Life Sciences), 25 ng DNA and sterile ultra-pure water up to 30 µl. Amplification was carried out in a thermocycler programmed for 8 min at 95° C initial denaturation followed by 35 cycles of 30 s at 95° C, 45 s at 47 \degree C and 3 min at 72 \degree C, and 10 min final extension at 72° C.

DGGE was carried out according to Malosso et al. [\(2006](#page-8-0)). Fungal PCR products were separated in 6% acrylamide gel with a denaturing gradient varying from 30 to 50%. The electrophoresis was conducted at 200 V , 60° C for approximately 4 h. Following electrophoresis, the gel was stained with SYBR Green I (Invitrogen) and visualized under UV light. The gel images were analyzed using Quantity One 4.4.0 (The Discovery Series, BioRad). A binary matrix was produced and used to calculate the similarity matrix (DICE coefficient, Sorensen) and to plot an UPGMA dendrogram using NTSYSpc 2.10.

Results and discussion

Ecological data of filamentous soil fungi

In this work, 84 fungal species have been identified. In the first sampling, 63 species were detected; 56 species were identified in the second sampling with 34 common species between the two sampling events. A detailed taxonomic characterization of the species is beyond the scope of this paper; however, it is worth mentioning that the largest numbers of filamentous fungi species were found in the Atlantic Forest followed by the AFS. Taking all soil samples into account, 1329 CFU were isolated, including species of Basidiomycota, Ascomycota and Zygomycota.

According to Domsch et al. [\(2007](#page-7-0)), the identified fungi are considered common soil inhabitants that can occur in soils of forests and fields, sandy soils and cultivated areas. However, the distribution of the soil fungal community is related to the climate, vegetation and the quality of the soil organic matter. Most of the identified species can be considered saprotrophic. Among the saprotrophic fungi, it was also possible to isolate those that are antagonists, such as Trichoderma and Penicillium. Aspergillus was the most frequent genus in the cassava monoculture soil in both sampling periods. Due to the production of different structures, such as conidia, chlamidospores and/or sclerotia, the isolation of Trichoderma, Penicillium and Aspergillus is common. The main fungal genera isolated in this work were similar to the general isolation pattern found in soil ecosystems (Azaz [2003;](#page-7-0) Cavalcanti et al. [2006;](#page-7-0) Prade et al. [2007](#page-8-0); Tangjang et al. [2009\)](#page-8-0).

A higher diversity and species richness was observed in the Atlantic Forest soil in both sampling periods followed by the AFS soil (Table 2). Fungal diversity found in the Atlantic Forest soil reflects the equilibrium between production and decomposition inside this system, as forest soils present a higher abundance of plant species and a leaf litter layer over the soil, thereby leading to increased richness and abundance of fungi. However, the use of soil for agriculture affects microorganisms, including fungi, and their vital processes due to changes in plant composition. Plant species substitution can change the quantity and quality of organic matter under decomposition, as the plant residues will be different. The fungal communities are selected by different plants,

Table 2 Species richness, Shannon-Wiener's Diversity (H') , Pielou's Equitability (J') and Berger-Parker's Dominance (d) in soils from different systems of land use, sampled during wet (C) (July/2009) and dry (E) (February/2010) periods

System of land use	Richness		Diversity		Equitability		Dominance		
	Sampling		Sampling		Sampling		Sampling		
		Е	C	Е	C	E		E	
Native forest	38	27	3.29	2.90	0.90	0.88	0.10	0.15	
Agroforestry	37	30	3.04	2.91	0.84	0.86	0.16	0.15	
Monoculture	16	25	2.43	2.06	0.88	0.64	0.25	0.41	

due to the chemical composition of dead organic matter. The permanence of a population in the ecosystem is conditioned to its ability to adapt and respond to these environmental changes (Persiani et al. [1998\)](#page-8-0).

In the AFS soil, it was observed that fungal species diversity and richness are similar to that of the natural Atlantic Forest environment, indicating that the AFS management does not disturb the soil. Fungal diversity found in soils of these systems is directly associated with plant species diversity that is responsible for providing decomposable residues of particular chemical composition to the soil surface. Calegari ([1998\)](#page-7-0) affirms that available organic matter in soil favors the development of microorganisms, including fungi that utilize this material as one of their main sources of energy. Therefore, higher species diversity contributes to more efficient use of available resources (Tótola and Chaer [2002](#page-8-0)).

Naeem et al. [\(2000](#page-8-0)) showed that diversity of microorganisms can depend on the diversity of producers. In terrestrial environments, producers are predominantly plants, while the decomposers are constituted by macro and microarthropods, protozoa, nematodes, and, principally, bacteria and fungi. As they carry out complementary functions, microorganisms and producers interact. In this interaction, producers provide organic materials and exudates to the soil that represent a nutrient source to the fungi and bacteria. In turn, fungi and bacteria hydrolyze complex organic molecules into simple inorganic molecules that are nutrients to the producers. Therefore, according to Naeem et al. ([2000\)](#page-8-0), the increment in the diversity of producers or microorganisms increases the efficiency of biological processes, such as nutrient cycling.

The management of AFS by incorporation of crop and pruning residues into the soil increases the availability of soil organic matter, favoring the development of microorganisms. Bardgett and Shine [\(1999](#page-7-0)) suggested that the increasing diversity of microorganisms is due to a greater variety of plant residues with different chemical characteristics in the soil. As microorganism groups vary in their biochemical capacities of exploiting different resources, a larger variety of detritus allows for a better use of different resources by a broader range of microorganisms, thereby speeding up the process of nutrient release to the soil.

A lower fungal species diversity and richness was observed in cassava monoculture soil in both sampling periods (Table [2](#page-3-0)). Altieri [\(2002\)](#page-7-0) considers that the biological diversity of agroecosystems can be disturbed, due to inadequate management, which results in a population imbalance for the different organisms. Thus, anthropogenic activities can affect ecosystem functioning and decrease its diversity, resulting in ecological perturbation and disappearances of species essential to ecosystem maintenance. Reber ([1992\)](#page-8-0) verified that there was lower nutrient cycling and lower plant growth when there was a decrease in soil microbial diversity.

Species distribution of isolates was more uniform in the native forest followed by the AFS with an equitability index varying from 0.90 to 0.84. However, in the first sampling, the soil of cassava monoculture presented an equitability that was 0.04 higher than that found for the AFS (Table [2](#page-3-0)). This result is attributed to the low fungal density (32 isolates), which was distributed among 16 species.

An elevated dominance and low fungal diversity in the cassava monoculture soil has been found (Table [2](#page-3-0)). This result is related to disturbances referent to conventional soil management on the soil fungal community diversity. According to Gliessman [\(2005](#page-7-0)), the dominance of different species is related to soil and culture management that can influence soil organisms' population dynamics. According to Lodge and Cantrell ([1995\)](#page-8-0) and Valpassos et al. ([2001](#page-8-0)), changes in soil organism's diversity when a type of agricultural management is set can lead to an ecological imbalance due to an increase in population density of the most adapted species to the new environment. Thus, disturbed environments favor diversity and dominance of determined fungal groups, which is reflected in the niche amplitude inside the agrosystem, presenting generalist or specialist species.

Fungal community structure analysis using denaturant gradient gel electrophoresis

Metagenomic DNA was successfully extracted from soil samples from the native Atlantic Forest and the AFS. Monoculture soil did not yield amplifiable DNA, possibly due to a lower fungal biomass. In support of this, several studies have reported limitations related to total DNA extraction from soils, mainly the coextraction of contaminant compounds, such as humic

acids, polysaccharides and tannin that can be coprecipitated with DNA and RNA and which impair extracted nucleic acid amplification (Miller et al. [1999;](#page-8-0) Anderson and Cairney [2004;](#page-7-0) Mitchell and Zuccaro [2006](#page-8-0)). Thus, variations can occur in the applied techniques for different types of soil (Ogram [2000\)](#page-8-0). Miller et al. [\(1999](#page-8-0)) observed that soil complexity and multiples factors can affect the performance of a method during DNA extraction, thereby resulting in different yields from the same technique.

Traditional fungal isolation methods showed variations in fungal populations. Cassava monoculture soil presented a smaller number of fungal species compared to the other land-use systems. According to Mitchell and Zuccaro [\(2006](#page-8-0)), the efficiency of nucleic acid extraction depends on the species present, on the environmental sample substrate and on the method used. The quantity of total soil DNA in the Atlantic Forest and AFS samples varied from 60 to 120 ng/g of soil.

The amplification of the 18S rDNA with primers FR390/FR1GC produced bands of the expected 400-bp size. This result is in accordance with the protocol described by Vainio and Hantula [\(2000](#page-8-0)) that indicates a 390-bp product was amplified with the same primers. FR390 and $FR1_{GC}$ are described as specific to amplify the 18S rDNA region of the three major Phyla: Ascomycota, Basidiomycota and Zygomycota of the Kingdom Fungi (Vainio and Hantula [2000;](#page-8-0) Malosso et al. [2006\)](#page-8-0). Studies have shown that amplicons generated from this primer pair can be successfully used for generating DGGE profiles of soil fungal communities (Costa et al. [2006;](#page-7-0) Malosso et al. [2006\)](#page-8-0).

The dendrograms constructed using the band profiles of soil fungal communities from native forest and AFS showed the influence of the type of vegetation cover. Therefore, the DGGE pattern of these soil fungal communities showed that the comparative evaluation of the band profiles highlights characteristic bands to each community. According to Garbeva et al. ([2004\)](#page-7-0), the type of plants present is a factor determining the soil microbial community structure, as plants are the greatest providers of specific types of carbon and energy sources.

The total number of bands detected in the first period of soil sampling (July/2009) was 49 in the AFS and 45 bands in the Atlantic Forest. The number of different band types detected in the AFS and Atlantic

Forest soil samples were 17 and 13, respectively. In the second sampling period (February) there was a decrease in the number of bands in each studied area with 47 bands in the AFS and 32 bands in the Atlantic Forest. The number of band types detected in the AFS and Atlantic Forest soil samples was also lower (14 and 12, respectively). Lambais et al. ([2005\)](#page-8-0) affirmed that samples that present different band patterns indicated that there are differences in the microbial communities. However, if the band patterns do not differ, differences between communities may or may not be present. In this case, it is necessary to employ other techniques to detect community differences.

Traditional isolation methods are considered limited, due to the difficulty implied in detecting a large portion of microbial populations present in environmental samples (Paul and Clark [1989](#page-8-0)), the so-called viable but noncultivable (VBNC, Oliver [2005](#page-8-0)) or nonculturable fungi (Bleve et al. [2003;](#page-7-0) Gams [2003](#page-7-0)). However, the number of amplicons obtained by the DGGE technique was lower than the quantity of species isolated by traditional methods in both sampling periods. According to Smit et al. [\(1999](#page-8-0)) and Mitchell and Zuccaro [\(2006](#page-8-0)), some representative communities may not be detected by the chosen primer pair; therefore, the observed bands may represent the most abundant species in the samples. There is also the possibility that the region amplified by the chosen primers is too conserved between the fungal species present in these soil samples. Malosso et al. [\(2006](#page-8-0)) was able to show larger fungal diversity compared to isolation techniques using these primers. However, their study area (Antarctic soils) is known to be less diverse than tropical areas, allowing the assumption that each band type represents a different taxon, and the culture conditions for psychrophilic organisms are not easily reached. Soil samples from the Atlantic Forest or the AFS are richer in species, and there is a high probability that each band type is represented by more than one DNA sequence of same GC content.

Taking into account the limitations of the DGGE method, as DNA fragments can present similar mobility in the gel, and a band type can represent more than one species, the decreasing of band types followed the same trend of decreasing species richness detected by traditional isolation methods between sampling periods (Table [2](#page-3-0)). The variation of species richness between sampling periods shown by both methods of accessing the fungal community can be related to the decreasing rainfall between sampling periods. The water stress favored the more generalist and resistant species, which explains the decrease in species richness between sampling periods.

Seasonal variation is considered to be one of the main factors determining the microbial community in soils. Silva [\(2004](#page-8-0)), using the DGGE technique with soils from native areas of Cerrado, observed a difference in the microbial community when comparing sampling periods, as this biome presents two distinct climate seasons during the year, one dry and one rainy.

The hierarchical grouping analysis based on the presence and absence of bands detected in all soil samples of the different land uses collected in both periods is depicted in the dendrogram of Fig. 1. In both sampling periods, the samples tended to group in relation to the land use. The dendrogram indicates two large clades, one represented by AFS samples and the other by Atlantic Forest samples.

The similarity between the areas was 47% in the first sampling event and 61% in the second sampling (Fig. 1). The results showed that the AFS caused changes in the fungal community; however, these changes do not affect the fungal richness and diversity found in this agriculture system. Thus, it is possible to affirm that this type of management conserves fungal populations in soil and, consequently, positively influences soil quality.

The average similarity of the mycobiota found in the different periods between the studied forest and the AFS is above 50%. Therefore, it can be considered that soil mycobiota in the AFS is more similar to those of natural environments than those of traditional monoculture systems, remembering that it was not possible to amplify the DNA extracted from the conventional cassava monoculture soil in this study. The fact that the general fungal biomass in the monoculture soil is lower than in the other systems to the point of preventing DNA band detection in a common agarose gel is evidence that the structure of the community is different in this system. Using DGGE, Kowalchuk et al. ([2002\)](#page-8-0) verified that the structure and composition of a microbial community is mainly influenced by plant types present when comparing areas with similar edaphic and climatic conditions.

These results suggest that fungal communities can change, mainly according to the type of vegetation. Forest and agroforest systems are composed of diverse plant species, mainly tree species, while the conventional management of soils keep them covered by a single annual or semi-perennial culture (monoculture). According to Naeem et al. [\(2000](#page-8-0)) and Pfenning and Abreu [\(2006](#page-8-0)), plant diversity is related to microorganism diversity due to the formation of intimate relationships established between specific plant species and microorganisms. Therefore, in environments with high plant diversity, a greater microorganism community richness is found, and environments with low plant diversity can be associated with reduced microbial diversity.

In Brazil, Bresolin et al. ([2010\)](#page-7-0) compared the structure and composition of fungal and bacterial communities in the soils of a native Cerrado and an area of soybean monoculture along the culture cycle using DGGE. These researchers indicated that the soil microbial community structure was affected by changes in the soil cover and by the development of the soybean culture. Similar results were obtained by Castro et al. ([2008\)](#page-7-0), who analyzed fungal communities in soils of native Cerrado, a riparian forest, an area

Fig. 1 Hierarchical grouping of fungal 18S rDNA amplicons from soils under different systems of land use detected by DGGE: a rainy period (July/2009). b Dry period (February/2010). Agro Agroforestry System; Mata Atlantic Rain Forest

converted into soybean plantation and a pasture area using the analysis of the ribosomal intergenic spacer (RISA). These authors detected a reduction in fungal diversity in the soils of the soybean plantation and the pasture area when compared to the native Cerrado soil.

The AFS are presented as production systems that preserve natural resources, mainly the soil and the inhabitant microorganisms, as there is no need to use fertilizers and pesticides. The uncontrolled use of agrotoxics (insecticides and fungicides) and unbalanced fertilization causes diseases in plants and reduces soil biodiversity. This occurs due to the fact that upon agrotoxic application, 50–80% does not reach the plant and falls directly into the soil (Chaim et al. 1999). Sigler and Turco ([2002\)](#page-8-0) showed changes in bacterial and fungal (Zygomycetes and Ascomycetes) communities after application of Chlorothalonil using DGGE. The authors concluded that an increase or inhibition of the dominant communities can occur in soils impacted by this fungicide.

Soil and vegetation cover management is reflected in a soil's physical, chemical and biological characteristics. Thus, understanding the impacts of different agricultural systems in the dynamics of fungal communities is essential for the development of a sustainable agriculture, which may enable the use of more rational methods of soil management.

Conclusion

This research showed that the type of land use that soils are submitted to influences filamentous fungi communities. The conventional cassava monoculture reduces filamentous fungal diversity in soil and favors generalist species that are dominant in this soil system. Conversely, the AFS presents fungal diversity similar to that found in soils of the native Atlantic Forest.

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