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Impact of Amazon land use on the community of soil fungi

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Introduction

The Amazon is considered a major reserve of biodiversity, mainly of plant species which may contribute to a greater diversity of microorganisms inhabiting the soil (Brodie et al., 2003; Jesus et al., 2009). Constantly, a fraction of the Amazon rainforest is cut down for agricultural activities to be established. The intensification of anthropogenic activities, which culminate with the conversion of forests into extensive pastures, results in loss of biodiversity, possibly influencing the biological processes important for maintaining productivity and sustainability of ecosystems (Islam and Weil, 2000; Moreira et al., 2006). Biodiversity loss also occurs in soil in which there is great diversity of living organisms that depends on the vegetation, as well as on the quantity and quality of organic material produced (Fierer and Jackson, 2006).

Fungi are also affected by human interference in soils, which may result in changes in the ecosystem balance. The roles of fungi in the soil are complex and critical to maintain the functionality of the biome. The fungi can act in nutrient cycling and develop symbiotic or pathogenic associations with plants and animals besides interacting with other microorganisms (Anderson and Cairney, 2004; Bridge and Spooner, 2001; Thorn, 1997). The magnitude of the functions performed by fungi can be better understood by considering the diversity of this group of organisms. Estimates point to 1.5 million species worldwide of which only about 99.000 have been described using classical taxonomic approaches (Hawks-
worth, 1991; 2001; Hawksworth and Rossman, 1997;

ABSTRACT: Considered as one of the most biodiverse biomes, the Amazon has a featured role in the discovery of new species of plants, animals and microorganisms, which may be important for the functionality of different ecosystems. However, studies on the impacts resulted from changes in the Amazon land use on microbial communities and their functions are still limited. In this context, the soil fungal diversity can act as an important indicator of environmental stress caused by land use of the Amazon. This study describes changes in soil fungal communities caused by different systems of land use (primary forest, secondary forest, agroforestry, agriculture and pasture). Communities were observed in each of the areas using denaturing gradient gel electrophoresis (DGGE) of 18S rRNA gene combined with the non-metric multidimensional scaling (NMDS). Unique bands indicated the dominance of particular fungal groups in each of the specific treatments, mainly in areas converted to pasture, which differed greatly from samples of other systems of land use (SLU). The analysis of partial sequence of the 18S rRNA gene of fungi in soils under primary forest, agriculture and pasture showed differences ($p = 0.001$), evidencing the fungal community response to such changes. Most abundant phyla were the Zygomycota in the soil under primary forest and agricultural land, and Basidiomycota in the soil under pasture. The results show that the Amazon soil is an ecosystem susceptible to environmental changes in regarding the fungi community inhabiting this niche.

Keywords: DGGE, 18S rRNA, fungal diversity, soil biology

Schmit and Mueller, 2007). Several fungi do not grow in a culture medium (Van Elsas et al., 2000) and less than 20% of fungal species can be grown in culture media (Bridge and Spooner, 2001). In addition to the limitations of cultivation, there is lack of taxonomic knowledge making the identification of some species in the soil difficult (Kirk et al., 2004).

Although molecular techniques of fingerprinting, cloning and sequencing have been more used for the evaluation of fungal communities, data on fungal communities in soil ecosystems with natural floristic vegetation that have suffered the impact of agricultural practices are scarce (Kasel et al., 2008; Lauber et al., 2008; Lupatini et al., 2012). Comparing fungal community evaluated by T-RFLP in pasture, native and planted forests in Australia, Kasel et al. (2008) found that the soils from the same land use grouped together in NMDS ordination, in spite of geographic separations of 175 km (native eucalyptus forests) and 215 km (*Pinus radiata* plantations). On the other hand, comparing hardwood and pine forests, agriculture and pasture in USA, it was found that specific soil properties may predict changes in microbial community, but the distinct land uses not necessarily have distinct soil fungal or bacterial community in (Lauber et al., 2008). In Pampa Biome, southern Brazil, Lupatini et al. (2012) concluded that both land use shifting and soil type were drivers of archaeal and fungal community structure in soil when comparing native grassland, native forest, Eucalyptus and Acacia plantations, soybean and watermelon field.

Despite the demonstration of fungal community response to different types of land use, few are known

about that issue in Amazon forest. Besides, changes in fungal communities resulting from each land use system can provide important information for soil management and assessment of the environmental impact.

The aim of this study was to determine whether land use affects the structure and diversity of fungal communities using PCR-DGGE and sequencing gene fragments of 18S rRNA from fungi. This gene was used because of the availability of sequences in public databases for the identification of fungi belonging to the five phyla (Ascomycota, Basidiomycota, Glomeromycota, Chytridiomycota and Zygomycota).

Materials and Methods

Location and site description

The studied areas are located in the municipality of Benjamin Constant, Amazonas State, Brazil, between geographic coordinates 4°20' and 4°26' South and 69°36' and 70°2' West along the Solimões River (Figure 1). The climate is classified as Af (Köppen classification) with average annual temperature of 25.7 °C and average rainfall of 2,562 mm. The deforestation rate in this region is low mainly due to the difficult access and low population density. Local communities use the land for agriculture, but some areas of pasture are also present as a result of policies on the Amazon land use implemented by the Federal Government during the 1970s.

Six grids containing between 16-20 sampling points each, were distributed in three areas along the Solimões River in a way that all land use systems - agriculture, pasture, agroforestry, secondary forest and primary forest - were represented (Figure 1). The description of the systems of land use (SLU) was defined by Fidalgo et al. (2005), and the points chosen for the evaluation of fungal communities in the soil are described in Table 1. Two local communities - Nova Aliança and Guanabara II - and a site near the urban area of Benjamin Constant were included. The distance between points in the grids was generally 100 m, except by few points for which the distance was 50 m when more replicates per land use system were necessary. One composited soil sample was taken at each point of the grids. Those samples were formed by 12 subsamples, collected at a depth of 0-20 cm, using a soil auger, in two concentric circles with a 3-6 m radius from the centre, according to the protocol of the CSM-BGBD. The samples were stored in sterile plastic bags Millipore® and kept in a cold environment until the arrival at the laboratory. Finally the samples were stored at -80°C.

DNA extraction

Total DNA was extracted from 500 mg of soil using the Fast DNA kit (Qbiogene, Irvine, CA, USA), according to the manufacturer's instructions. The integrity of DNA was determined after electrophoresis in 0.8 % agarose gel and stained with Vistra Green (GE Healthcare, São Paulo, Brazil). The DNA concentration was determined

by densitometry using the tracer mass DNA Mass Ladder (Invitrogen, São Paulo, Brazil), and the Fragment Analyses program (GE Healthcare, São Paulo, Brazil).

PCR-DGGE analyses

A fragment of 18S rRNA gene of fungi was amplified by nested PCR according to Oros-Sichler et al. (2006). For the initial amplification, the primers used were NS1 (5'-GTA GTC ATA TGC TTG TCT C-3') (White et al., 1990) and EF3 (5'-TCC TCT AAA TGA CCA AGT TTG-3') (Smit et al., 1999) generating a fragment of 1,700 bp. Amplification was performed in a solution containing approximately 2 ng of DNA; 2.5 µL buffer of PCR 10

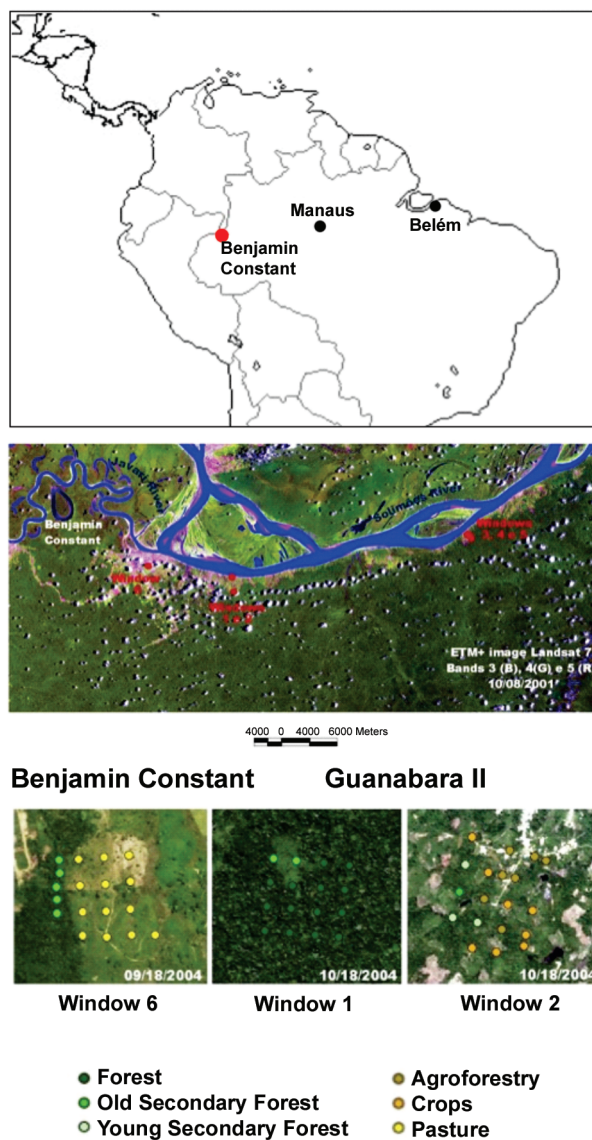


Figure 1 – Location of study areas in the map of South America (red dots), the distribution of “windows” along the Rio Solimões and the distribution of sampling points in “windows” 1, 2 and 6 (Fidalgo et al., 2005).

Table 1 – Characterization of different systems of land use (SLU) and identification of points of the Amazonian soil used for the study of fungal diversity.

SLU	Characterization	Points used
Primary forest	Original forest formation areas without the occurrence of deforestation.	08, 09, 10, 40, 57
Secondary forest	Secondary vegetation under several stages of succession. Systems crops under shifting cultivation or fallow.	29, 63, 75, 79, 80
Agroforestry	Extensive system of production forest, where much of the vegetation is formed by the spontaneous regeneration of secondary forest species. Predominant occurrence of tree species.	20, 22, 24, 24A, 66
Agriculture	Represents areas that are covered by annual or semi-perennial crops such as banana cultivation.	28, 33, 44, 58, 72
Pasture	Includes areas for livestock production, covered by grass.	84, 86, 93, 94, 96

x; 0.2 mM of dNTP; 3.75 mM MgCl₂; 0.2 mM of each primer; one unit of recombinant Taq DNA polymerase (Invitrogen), Milli-Q sterile water to a final volume of 25 µL. DNA amplification was performed under the following conditions: 94 °C for 5 min, 25 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 3 min and a final extension of 72 °C for 10 min.

The first PCR amplicons were diluted at 1:50 (v:v) of sterile water and used for the second amplification with primers NS1 (5'-GTA GTC ATA TGC TTG TCT C-3') and FR1GC (5'-CCC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GCC GAI CCA TTC AAT CCG TAI T-3') (Vainio and Hantula, 2000) under the conditions described for the first amplification, except for the annealing temperature (48 °C), and the number of cycles (used 20 cycles). The amplicons were examined and quantified as described above.

In DGGE, the amplicons (300 ng) were separated in gel containing 6 % acrylamide: bisacrilamida (37,5:1, m:m) and a linear gradient of 25-38 % urea and formamide (100 % denaturant solution containing 40 % of formamide and 7 M urea). Electrophoresis was performed at 55 °C, 200 V constant for 15 hours in a DCode system (BioRad), using a 0.5 x TAE buffer. After electrophoresis, DNA was stained with SYBR-Green I (Molecular Probes) and the gel image captured by scanning in a laser densitometer and the FluorImager Fragment Analysis program (GE Healthcare). The gels were analyzed using the Diversity Database program (BioRad) to determine the richness of amplicons (Sa). Analyses of Non-metric Multidimensional Scaling (NMDS) were conducted on amplicon profiles of fungal communities and analyses of similarity (ANOSIM) were carried out to determine statistical differences among land use systems using the program Primer 5 (Primer-E Ltd.).

Cloning and sequencing of 18S rRNA gene clones

For construction of 18S rRNA gene clone libraries, the total DNA of soil under forest, agriculture and pasture was amplified by nested PCR using primers NS1 (White et al., 1990) and EF3 (Smit et al., 1999) in the first amplification reaction, and NS4 (White et al., 1990) and NS31 (Simon et al., 1992) in the second amplification. We used the same amplification program described for NS1-EF3.

Equal amounts of amplicons of five soil samples under the same system use were mixed to create a composite sample for each of the three types of land use. The amplicons were cloned into vector pGEM-T Easy (Promega, Madison, WI, USA). Competent cells of *Escherichia coli* DH5α were transformed and plated in a Luria Bertani medium (LB-agar) containing ampicillin (50 µg mL⁻¹) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside, 20 µg mL⁻¹). The recombinant plasmids were extracted by alkaline lysis after cultivation of transformed bacteria in a liquid LB medium.

The sequencing was performed using 200-500 ng of plasmid DNA, 10 pmol of primer M13f (5'-GTA AAA CGA CGG CCA G-3'), 2 µL of DYEnamic ET Terminator (GE Healthcare, São Paulo, Brazil), 2 µL of buffer (200 mM Tris-HCl pH 9.0, 5 mM MgCl₂.6H₂O) and ultrapure water to a final volume of 10 µL. DNA amplification was performed as follows: 25 cycles for 20 s at 95 °C, 15 s at 50 °C and 1 min at 60 °C. The sequencing was performed on an ABI 3100 automated capillary sequencer (Applied Biosystems, São Paulo, Brazil), according to the manufacturer's recommendations.

Analysis of 18S rRNA gene sequences

The similarity of DNA sequences available in GenBank database was determined by the Basic Local Alignment Search Tool (BLAST) at NCBI (nr/nt) (Altschul et al., 1990). The existence of chimeras was determined using the Chimera Check version 2.7 of the "Ribosomal Database Project" (RDP) (Maidak et al., 1999). We considered the sequences potentially chimerical if they have a distinct break point of chimera >40 in Chimera Check. To confirm the indication of the Chimera Check program, the sequences were re-analyzed using the BLAST after deletion of previous nucleotides and after the break point of the chimera. If one of the two segments i.e., prior to or subsequent to the break point, was similar to the other organism than the organism more similar to the original sequence complete, then the sequence was considered as a chimera. In addition, very divergent sequences that did not allow the use of ClustalW alignment were removed.

For the grouping of sequences in operational taxonomic units (OTUs), the DOTUR program (Schloss and Handelsman, 2005) was employed. For that purpose, we used an evolutionary distance matrix calculated in

the DNADIST program, with Jukes-Cantor algorithm, after aligning the clone sequences from the libraries of each type of land use. The sequences with a similarity > 99% were considered to belong to the same OTUs. The estimated richness of OTUs, by non-parametric methods ACE-1 (Chao and Lee, 1992) and Chao1 (Chao, 1984), the levels of Shannon diversity, Simpson reciprocal index coverage and sampling were carried out in the SPADE program (Chao and Shen, 2003). Statistical comparison of clone libraries was made in the program webLIBSHUFF (Singleton et al., 2001), using evolutionary distance matrices previously calculated.

An environmental sequence of each OTU of the three land uses and the sequences with the highest scores in the analysis of Megablast were aligned in 565 positions by means of ClustalW in the program MEGA 4.0 (Tamura et al., 2007). The taxonomic relationships among the fungal sequences were inferred through the analysis of neighbor joining (NJ) (Saitou and Nei, 1987), minimum evolution (ME) (Rzhetsky and Nei, 1992) and maximum parsimony (MP) (Eck and Dayhoff, 1966) in the program MEGA 4.0. Sequences of the same region of 18S rRNA gene in plants were used as outgroup. In the NJ and ME analyses, the gaps and "missing data" were completely deleted, and the substitution model was the Jukes-Cantor with uniform rates across sites. Still for the ME analysis, the maximum number of trees retained in the search method was 10, using the algorithm Close Neighbor Interchange (CNI) (Nei and Kumar, 2000) with search level of 2 and the initial tree was obtained by the NJ method. For the MP analysis, the search method was the CNI algorithm with level 2, in which the initial trees were obtained with the addition of random sequences of 10 repetitions. All positions with gaps or "missing data" were eliminated. The robustness of the topology inferred in NJ, MP and ME was tested with 1000 bootstrap replicates.

Results

Analysis of fungal communities by PCR-DGGE

The fungal communities represented by the bands profile after DGGE were more similar between samples of the same system of land use than samples of different

land use systems (Figure 2). Some of the replicates were distinct from the majority of samples in a determined treatment, which can indicate the natural variation in the soil of a land use. However, the grouping of samples by NMDS shows that there is a tendency for samples of soil under pasture to separate from samples of other SLU, which are closer to each other (Figure 3a). Then, we removed the pasture samples from NMDS analysis to better visualize the grouping and, thereby, we can notice that the fungal communities are different among the land uses (Figure 3b).

The levels of stress in this study are 0.13 and 0.15, indicating that the two-dimensional representation is valid, but not entirely clear about the separation of areas. In order to confirm the observations made in the NMDS, the ANOSIM was used, which displayed significant differences (global $R = 0.551$, $p < 0.001$) among all the different SLU (Table 2).

Analysis of fungal communities by 18S rRNA gene clones sequencing

The three libraries used in this study are composed of 88 valid sequences for forest samples, 61 of agriculture and 42 of pasture. Table 3 shows the diversity indices (Shannon and Simpson) and the estimated richness of OTUs (Chao1 and ACE methods) for each library analyzed considering the evolutionary distance of 0.01

Table 2 – Pairwise test results (matching) based on the profiles of 18S rRNA gene amplicons of fungi from the Amazon soils under different land use after denaturing gradient gel electrophoresis (DGGE) ($p < 0.001$).

Systems of land use (SLU)	Pairwise Test
Secondary forest, forest	0.35
Secondary forest, agriculture	0.68
Secondary forest, agroforestry	0.64
Secondary forest, pasture	0.62
Forest, agriculture	0.52
Forest, agroforestry	0.43
Forest, pasture	0.70
Agriculture, agroforestry	0.63
Agriculture, pasture	0.66
Agroforestry, pasture	0.59

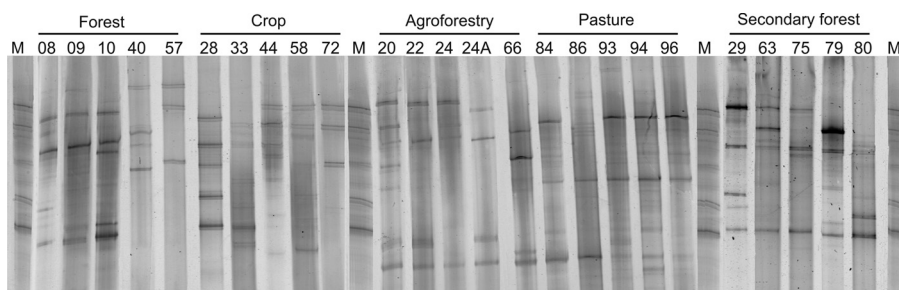


Figure 2 – Denaturing gradient gel electrophoresis (DGGE) gel of 25-38% urea and formamide, generated by separation of 18S rRNA gene fragments amplified with NS1/EF3 + NS1/FR1-GC of soil samples under different natural systems of land use (SLU). M - marker.

Table 3 – Estimates of operational taxonomic units (OTUs), diversity indices and coverage of sampling, calculated from libraries of 18S rRNA of fungi from Amazonian soils under different land use systems.

Community	NS ^a	NU ^b	Estimated richness of OTUs		Diversity indices		ECS ^e
			ACE-1	Chao1	Shannon ^c	1/D ^d	
D = 0.01							
Forest	88	44	541 (204; 1590)	248 (106; 720)	3.24 (3.0; 3.48)	13.87 (27.94; 9.23)	0.602
Agriculture	61	35	222 (88; 687)	166 (73; 483)	3.07 (2.78; 3.36)	10.97 (28.47; 6.79)	0.541
Pasture	42	31	333 (113; 1139)	144 (63; 422)	3.25 (3.04; 3.46)	19.6 (34.79; 13.64)	0.381

^aNumber of sequences. ^bNumber of OTUs determined by DOTUR. ^cEstimator maximum similarity. ^dReciprocal Simpson index (estimator of maximum similarity).

^eEstimated of coverage sampling. Values in parentheses represent the range with 95% confidence.

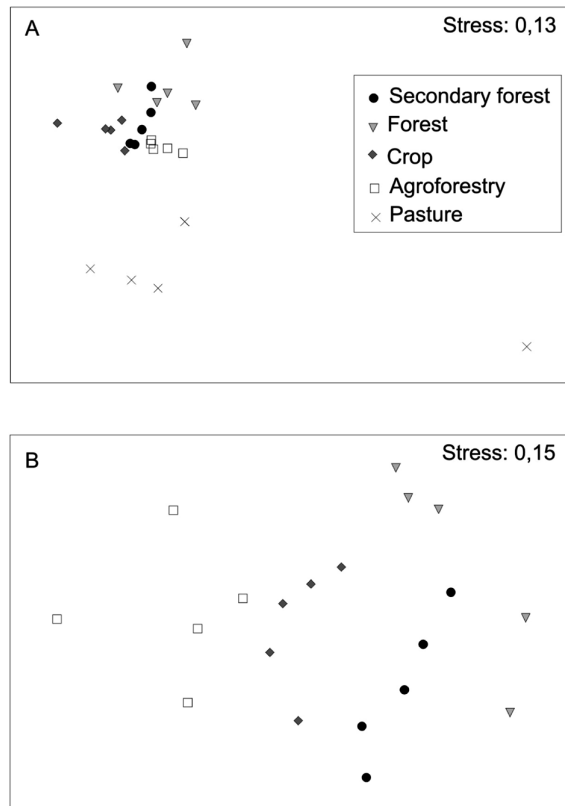


Figure 3 – Analysis of multidimensional scaling (NMDS) profiles of 18S rRNA gene amplicons obtained from denaturing gradient gel electrophoresis (DGGE) of natural soil of the Amazon in different systems of land use: secondary forest, forest, agriculture, agroforestry and pasture (a), and secondary forest, forest, agriculture and agroforestry (b).

for the definition of OTUs. Values for richness ranged between 31 and 44 OTUs, but without differences (95 % confidence) (Table 3). On the other hand, the analysis of homologous and heterologous coverage curves of clone libraries using the program webLIBSHUFF showed that the fungal communities of the three systems are different ($p < 0.05$) (Figure 4).

Considering the phylogeny of the sequences obtained, the NJ topology was built using a representative sequence per OTU and SLU (Figure 5). The first

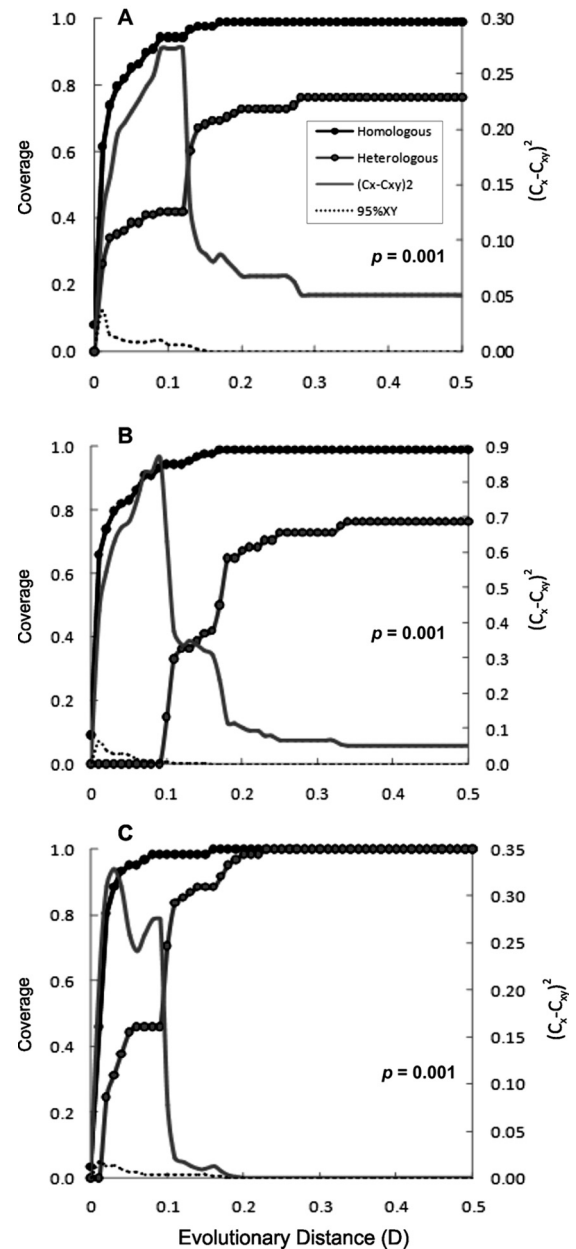


Figure 4 – LIBSHUFF analysis of fungal communities of natural soil of the Amazon. A, Forest (homologous) × Agriculture; B, Forest (homologous) × Pasture; C, Agriculture (homologous) × Pasture. Communities are different at $p = 0.001$.



Figure 5 – Topology of neighbor joining of 18S rDNA sequences obtained from soil under three systems of land use (in bold) and representation rate obtained by the Megablast analysis. Numbers on branches indicate the percentage of 1,000 repetitions of the bootstrap analysis of neighbor joining (NJ), minimum evolution (ME) and maximum parsimony (MP), respectively. Each environmental sequence represents an operational taxonomic units OTU of each system. Values in parentheses are the percentage of sequences of each OTU within each land use system. OTUs with similarity less than 96 % compared to the GenBank sequences: - Forest OTUs, - Agriculture OTUs and - Pasture OTUs. The bar size represents a substitution for 100 nucleotides.

observation showed the existence of sequences that may represent new taxonomic groups due to low similarity to previously described groups. Many of the sequences have less than 96 % of similarity with GenBank sequences (25.3 % of the sequences of forest; 30.3 % of agriculture, and 25 % of pasture).

All sequences from forest and 91 % sequences from agriculture are related to Zygomycota phylum. Taking into account the known groups, 57.5 % of the forest sequences and 78.8 % of agriculture are phylogenetically related to the order Mortierellales (Figure 5). A clade composed entirely of forest sequences (39.1 % of the sequences) has relations to the order Mucorales. Conversely, the pasture had a greater number of sequences related to different fungi phyla, mostly related to the Basidiomycota, Order Tremellales (79.2 % of the sequences), not represented in other libraries. The remaining sequences of the pasture library are related to Ascomycota (12.5 %), to Chytridiomycota (4.2 %) and to order Zoopogales (4.2 %) of the Zygomycota phylum.

Discussion

Changes in land use affect the community structure of soil fungi. Although the number and diversity of OTUs are not different, the composition of each community differs between the SLU as in the LIBSHUFF analysis. The NMDS and ANOSIM made with DGGE data also indicated community separation in different SLU. Possibly the type of vegetation on the soil, i.e., systems based on tree species (forest), grasses (pasture), other herbaceous (agriculture) and the mixture of tree and herbaceous vegetation (agroforestry), plays a key role in this change.

The forest areas sampled have higher plant diversity than the other SLU (agriculture, pasture and agroforestry). A high diversity of plants could promote greater microbial species richness due to the greater number of niches in the rhizosphere or specific interactions between plants and microorganisms. On the other hand, a low diversity of plants may be associated with a reduced microbial diversity (Brodie et al., 2003; Pfenning, 2006). However, we did not find lower fungi diversity in the environment with lower plant diversity as that found in the pasture compared to the forest samples.

Differences in community structure of bacteria in the same soil were also observed and significantly correlated with changes in soil properties, especially those related to acidity and concentration of nutrients in the soil (Jesus et al., 2009). Because the soil type was same type for all locations (Inceptisols are the predominant soil class), the authors assumed that land use was the main factor leading to changes in soil properties. In addition, the conversion of forest to pasture and agriculture did not reduce bacterial diversity (Jesus et al., 2009) and did not reduce the fungi diversity, as observed in this study.

In the sampled areas, the forest and agroforestry systems are composed primarily of tree species while agriculture areas are covered by annual or semi-perennial crops. Therefore, the data do not corroborate the hypothesis of reduced fungal diversity in soil with the reduction of plant diversity, but sustain that the decrease of plant diversity can lead to changes in fungal community of the soil. Fungal communities in pasture samples were the most distinct from the others, which can be attributed to the anthropogenic intervention and the unique occurrence of grasses in the area. A similar effect of vegetation on fungi communities was demonstrated by Brodie et al. (2003) comparing the fungal communities in soils under pastures with a floral transition zone using the PCR-DGGE and T-RFLP. Similarly, however using the RISA technique, changes in fungal communities according to the type of vegetation were also detected by Leckie et al. (2004), when comparing two types of forests found in Canada, with soils presenting different nitrogen availability. Even presenting the same system of land use, when in different locations, soils show similarities in fungal community composition indicating the key role of vegetation in modulating the fungal community structure in the soil (Kasel et al., 2008).

Comparing the three most discrepant SLU (forest, agriculture and pasture), the sequence analysis of 18S rRNA gene clones also revealed differences ($p < 0.05$) among fungal community structures. This fact reinforces the idea that change in land use may not compromise the community diversity, but the functionality of these communities may undergo changes since the relative abundance of species may be changed. It is possible that the observed changes are essential in the functioning of soils under the new use conditions. These data corroborate descriptions in the literature showing that changes in the substrate quality and nutrient availability alter the fungal community and the roles of the community in a given environment (Allison et al., 2007; Lauber et al., 2008). Therefore, in this study, the number of niches does not diminish, but new fungal niches appear when there is a decrease in plant diversity (forest converted to pasture) as a result of anthropogenic influence.

For this study, we used the nested PCR system with primers NS1/EF3 and NS1/FR1-GC 18S rRNA gene, presumably specific for the three major phyla of fungi: Ascomycota, Basidiomycota and Zygomycota (Gomes et al., 2003; Oros-Sichler et al., 2006). However, several sequences obtained showed high similarity to the phylum Cercozoa and were excluded from the analysis. These observations were also made by Malosso et al. (2006), who studied the diversity fungi in soils of Antarctica and found sequences with high similarity to amoeba using primers for 18S rRNA gene. Another limitation of the phylogenetic affiliation of 18S rRNA gene sequence of fungi is the low representation in the database compared with prokaryotes.

The NCBI GenBank contains 98095 of 18S rRNA gene sequences of fungi except for environmental se-

quences (accessed 05/29/2011). The sequences of the same gene with identification of Genbank phylum are mostly Ascomycota (63,885) followed by Basidiomycota (24,169) and Zygomycota (2,326). By comparison, the 16S rRNA bacterial gene have 294,692 sequences (not environmental) deposited in Genbank. This deficiency deposit sequences of fungi can make it difficult to identify environmental sequences. The provision of these sequences will not only allow us to better identify fungi, but it will also lead to a better design of PCR primers for the characterization of fungal communities in natural environments. However, the results show a high proportion of fungi belonging to the phylum Zygomycota in areas of forest and Agriculture. Fungi of the order Mortierellales are found in soils with native vegetation and have been reported in several articles, similarly to results from Pfenning (2006), who studied Amazonian soil fungi, including an area of primary forest and cocoa crops, annual crops and pasture and found zygomycetes of the *Mortierella* genus as the most common in soils of primary forests. Likewise, natural soils in Australia, including soils planted with *Araucaria* sp., showed predominance of this group of fungi (He et al., 2005). A clade composed entirely of forest sequences showed great relation to the order Mucorales, within species of *Umbelopsis*. The frequency of these species is generally high in decomposing needles litter layer (Osono et al., 2006). The data obtained in this work, along with the results reported in the literature, demonstrate the importance of this phylum in the cycling of organic matter in forestry soils.

Excepting pasture, the studied areas did not suffer significant anthropogenic interventions, however, the results indicate that the intensification of land use led to changes in fungal communities. Corrective inputs, fertilizers and products for pests and diseases control as well as irrigation are not used in any of the systems. Following human interventions such as deforestation, burning and planting subsistence crops (rice) in a tropical forest in the Ivory Coast, there was a considerable change in the fungi communities. However, with subsequent abandonment of the area, the rebuilding of these communities occurred rapidly (Persiani et al., 1998).

In conclusion, the community structure of fungi in natural soils of the Amazon forest is different for each system of land use. The conversion of forest to pasture and agriculture does not reduce the fungal diversity, but changes the population levels of species that make up this ecosystem indicating changes in fungal niches in the soil.

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