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**MUDANÇA DO USO DA TERRA E TIPO DE SOLO
SÃO FATORES DETERMINANTES DAS
COMUNIDADES DE FUNGOS E ARQUEAS NO
BIOMA PAMPA**

DISSERTAÇÃO DE MESTRADO

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**Santa Maria, RS, Brasil
2012**

**MUDANÇA DO USO DA TERRA E TIPO DE SOLO SÃO
FATORES DETERMINANTES DAS COMUNIDADES DE
FUNGOS E ARQUEAS NO BIOMA PAMPA**

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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciência do Solo, Área de Concentração em Biodinâmica e Manejo do Solo, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Mestre em Ciência do Solo.

Orientador: Prof. Rodrigo J. S. Jacques

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DETERMINANTES DAS COMUNIDADES DE FUNGOS E ARQUEAS
NO BIOMA PAMPA**

elaborada por
Manoeli Lupatini

como requisito parcial para obtenção do grau de
Mestre em Ciência do Solo

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**“...Porque eu não quero deixar pro meu filho
A Pampa pobre que herdei de meu pai...”
(Vainê Darde/Gaúcho da Fronteira)**

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciência do Solo
Universidade Federal de Santa Maria

MUDANÇA DO USO DA TERRA E TIPO DE SOLO SÃO FATORES DETERMINANTES DAS COMUNIDADES DE FUNGOS E ARQUEAS NO BIOMA PAMPA

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Data e Local da Defesa: Santa Maria, 29 de fevereiro de 2012

A mudança do uso da terra e o tipo de solo podem exercer impactos significantes sobre a comunidade microbiana do solo. O bioma Pampa Brasileiro, nas últimas décadas, tem sofrido severas mudanças na paisagem devido à mudança no uso da terra, principalmente pela introdução de plantações de árvores exóticas e pelos cultivos agrícolas. Diferentes usos do solo no bioma Pampa foram avaliados para determinar o efeito sobre a estrutura das comunidades microbianas do solo. Além disso, devido à presença de vários tipos de solo presentes neste bioma, foi investigado se diferentes tipos de solos abrigam diferentes comunidades microbianas. Amostras de solo foram coletadas em duas áreas com diferentes usos do solo (pastagem nativa, mata nativa, plantações de árvores exóticas e cultivo agrícola) e em uma topossequência típica no bioma Pampa formado por Argissolo, Planossolo e solos aluviais. A estrutura das comunidades microbianas do solo (arqueas e fungos) foi avaliada por RISA e capacidades funcionais do solo foram mensuradas através de carbono da biomassa microbiana e quociente metabólico. Diferentes padrões foram detectados nas comunidades de fungos e arqueas influenciados pela mudança no uso da terra e pelo tipo de solo, mostrando que ambos são importantes fatores da estrutura e atividade da comunidade microbiana. Florestamentos de acácia e eucalipto apresentaram as comunidades mais diferentes quando comparados com a vegetação natural. Embora diferenças nas comunidades foram detectadas, os diferentes usos e tipos de solos avaliados compartilham grande parte das unidades taxonômicas e mostram que apenas uma parte da comunidade sofre alterações causadas pela interferência humana.

Palavras-chave: *Core* microbiano do solo. Análise do Espaço Intergênico. Biomassa microbiana. Quociente metabólico.

ABSTRACT

Master Dissertation
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LAND-USE CHANGE AND SOIL TYPE ARE DETERMINANTS OF FUNGAL AND ARCHAEAL COMMUNITIES IN PAMPA BIOME

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Date and Local of the Defense: Santa Maria, February 29th 2012.

Land-use change and soil type can have significant impact on microbial communities of soil. The Pampa biome in recent decades has undergone severe changes in landscape due to land-use change, mainly for the introduction of exotic tree plantation and croplands. Different land-use in Pampa biome were evaluated to determine the effect on the structure of soil microbial communities. Furthermore, due to the presence of various soil types present in this biome, we investigated whether different soil type harbor different microbial communities. Soil samples were collected at two sites with different land-uses (native grassland, native forest, exotic tree plantation and cropland) and in a typical toposequence in Pampa biome formed by Paleudult, Albaqualf and alluvial soils. The structure of soil microbial community (archaeal and fungal) was evaluated by RISA and soil functional capabilities were measured by microbial biomass carbon and metabolic quotient. We detected different patterns in fungal and archaeal community driven by land-use change and soil type showing that both factors are significant drivers of microbial community structure and activity. *Acacia* and *Eucalyptus* afforestation presented the most dissimilar communities when compared with natural vegetation. Although differences in the communities were detected, the soils tested shared most of the taxonomic unities and only a proportion of the community suffers changes caused by human interference.

Keywords: Soil core microbiome. Intergenic Spacer Analysis. Microbial biomass. Metabolic quotient.

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INTRODUÇÃO

O bioma Pampa, também chamado de Campos Sulinos, abrange uma área compartilhada pelo Brasil, Argentina e Uruguai, ocupando cerca de 64% do território do Rio Grande do Sul, destacando-se de outros biomas por ser o único cuja ocorrência é restrita a somente um Estado do Brasil e por ser o bioma mais desconhecido de todos os biomas brasileiros, sendo reconhecido oficialmente pelo Instituto Brasileiro de Geografia e Estatística apenas em 2004 (IBGE, 2004). Este ecossistema possui características únicas de vegetação, clima e tipos de solo, sendo considerado uma das áreas prioritárias para a conservação da biodiversidade da fauna e da flora (Ministério do Meio Ambiente, 2002, 2007).

Este bioma abriga uma grande quantidade de espécies de plantas. Estima-se que há em torno de 3.000 espécies que pertencem principalmente às famílias botânicas Poaceae, Asteraceae, Cyperaceae, Fabaceae, Apiaceae, Oxalidaceae, Verbanaceae e Iridaceae (OVERBECK et al., 2006; OVERBECK et al., 2007). Apesar dos Campos Sulinos serem classificados em duas grandes regiões fitoecológicas, as estepes e as savanas (IBGE, 2004), estes termos são considerados inapropriados para descrever a vegetação natural desta região. Overbeck et al. (2007) sugerem que as formações campestres do sul do Brasil devem ser referidos apenas como "Campos", sem no entanto apresentar uma justificativa classificatória.

Apesar da grande riqueza de espécies encontradas, o bioma Pampa não é adequadamente protegido por políticas conservacionistas, sendo considerado um ambiente frágil, principalmente devido à presença de solos arenosos muito suscetíveis à erosão (OVERBECK et al., 2007; ROESCH et al., 2009). Além disto, nas últimas décadas, este bioma tem sofrido severas modificações em sua paisagem devido ao intenso processo de mudança do uso da terra através da conversão da vegetação natural em cultivos agrícolas e em grandes áreas com plantações de árvores exóticas (*Acacia* spp., *Eucalyptus* spp. and *Pinus* spp.). Estima-se que metade da vegetação original deste bioma foi removida e convertida em outros tipos de vegetação (PILLAR, 2009).

Além de alterar a vegetação natural, a mudança do uso da terra no bioma Pampa pode exercer impactos significantes na comunidade de microrganismos do solo. Muitos estudos têm mostrado que a mudança do uso do solo podem provocar inúmeras alterações em suas propriedades, incluindo pH, conteúdo de C e N, matéria orgânica, concentração de P e exsudatos liberados pelas plantas, exercendo modificações nas comunidades microbianas do

solo (LAUBER et al., 2008; BERG;SMALLA, 2009; JESUS et al., 2009; SINGH et al., 2009; CARSON et al., 2010; OSBORNE et al., 2011). Além disso, devido à sua grande extensão, o bioma Pampa possui uma grande variedade de tipos de solo, os quais podem influenciar na composição da microbiota do solo, sendo considerado um dos fatores mais importantes na estruturação das comunidades microbianas (SINGH et al., 2007; CHRIST et al., 2011).

Apesar da enorme importância da conservação da biodiversidade deste bioma, não há estudos no bioma Pampa Brasileiro relacionando o grau de alteração que a mudança do uso da terra pode provocar nas comunidades de microrganismos do solo e nem se distintos tipos de solo abrigam diferentes comunidades microbianas. Devido a esses fatos, neste estudo tentou-se responder uma simples pergunta: Qual fator, tipo de uso da terra ou tipo de solo, é responsável pelas maiores mudanças na comunidade de fungos e arqueas no bioma Pampa brasileiro? Por isso, neste estudo, formulou-se a hipótese de que ambos, mudança no uso do solo e o tipo de solo são fatores responsáveis pelas alterações na estrutura das comunidades de fungos e arqueas do solo. Os objetivos do estudo foram avaliar a alteração das comunidades de fungos e arqueas pela modificação do uso do solo através da implantação de lavouras e florestamento de árvores exóticas em áreas originalmente constituídas por vegetação nativa; comparar a (di)similaridade entre as comunidades microbianas presentes nos diferentes usos do solo; e verificar a influência de diferentes solos, sob mesma vegetação, nas comunidades de fungos e arqueas do bioma Pampa.

Para avaliar o efeito da mudança do uso da terra, dois locais foram escolhidos com o mesmo tipo de solo (Argissolo) e com diferentes usos característicos deste bioma (pastagem natural, floresta natural, cultivos agrícolas e florestas exóticas). Para verificar os efeitos de diferentes tipos de solo nas comunidades microbianas foi escolhida uma sequência típica de solos (topossequência) deste bioma, sob mesma vegetação (pastagem natural). A estrutura da comunidade microbiana do solo foi caracterizada usando RISA e a condição geral desta comunidade foi avaliada através da medição da biomassa microbiana e atividade metabólica.

**Land-use change and soil type are determinants of fungal and archaeal communities in
Pampa biome**

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Abstract

Land-use change and soil type can have significant impact on microbial communities of soil. The Pampa biome in recent decades has undergone severe changes in landscape due to land-use change, mainly for the introduction of exotic tree plantation and croplands. Different land-use in Pampa biome were evaluated to determine the effect on the structure of soil microbial communities. Furthermore, due to the presence of various soil types present in this biome, we investigated whether different soil type harbor different microbial communities. Soil samples were collected at two sites with different land-uses (native grassland, native forest, exotic tree plantation and cropland) and in a typical toposequence in Pampa biome formed by Paleudult, Albaqualf and alluvial soils. The structure of soil microbial community (archaeal and fungal) was evaluated by RISA and soil functional capabilities were measured by microbial biomass carbon and metabolic quotient. We detected different patterns in fungal and archaeal community driven by land-use change and soil type showing that both factors are significant

drivers of microbial community structure and activity. Acacia and eucalyptus afforestation presented the most dissimilar communities when compared with natural vegetation. Although differences in the communities were detected, the soils tested shared most of the taxonomic unities and only a proportion of the community suffers changes caused by human interference.

Keywords: Soil core microbiome. Intergenic Spacer Analysis. Microbial biomass. Metabolic quotient.

1. Introduction

The Brazilian Pampa biome, located in southern of the country, has been subject of attention by researches due to its intense processes of land-use change in recent years. This biome was officially recognized by the Brazilian Institute of Geography and Statistics alone in 2004 (IBGE, 2004) and is considered the most unknown of the Brazilian's biomes. It presents distinctive characteristics of vegetation, climate and types of soils, making it a unique ecosystem on the planet, capable of holding a high biodiversity. Due to this, it is considered one of the priority areas for conservation of flora and fauna biodiversity in Brazil (Ministry of Environmental - MMA, 2002, 2007).

The vegetation of this biome is frequently interpreted as a grassland environment, formed principally by savanna, but according to most classifications, savanna is an inappropriate term to describe the grasslands of southern Brazil because savannas generally are defined as vegetation types possessing a mixture of woody and herbaceous life-forms in distinct strata that occur in tropical regions with strongly seasonal precipitation (Walker, 2001). On the other hand, the typical vegetation in the Pampa is native grassland, with sparse shrub and tree formations. Overbeck et al., (2007) suggest that the southern Brazilian grasslands should be referred simply as "Campos" although without classificatory justification. This biome is home of a huge amount of plant species. It is estimated that there

are around 3.000 grassland species, which belong mainly to the Poaceae, Asteraceae, Cyperaceae, Fabaceae, Apiacea, Oxalidaceae, Verbanaceae, and Iridaceae (Overbeck et al., 2006; Overbeck et al., 2007).

Despite its high species richness and their importance to environmental balance, the Pampa biome is not adequately protected under current policies conservation, being considered a fragile environment mainly due to the presence of sandy soils very susceptible to water and wind erosion (for more information see Overbeck et al., 2007; Roesch et al., 2009). Parallel to this, in recent years, the Pampa biome has suffered severe modifications through land-use change by the conversion of natural vegetation to agricultural land (rice and soybean) and to large areas with exotic tree plantation (*Acacia* spp., *Eucalyptus* spp. and *Pinus* spp). It is estimated that half of its original vegetation was removed and transformed in other types of vegetation (Pillar, 2009).

The land-use change can exert significant impacts on soil microbial communities, which may react differently to biotic and abiotic conditions imposed by these changes (Singh et al., 2009). Several studies have shown that changes in microbial community structure may be associated with numerous alterations in soil properties caused by the land-use change, including pH, C and N content, organic matter, P concentration and exudates released by plants (Berg and Smalla, 2009; Lauber et al., 2008; Jesus et al., 2009; Carson et al., 2010; Osborne et al., 2011). Moreover, the wide variety of soils that occur in the Pampa biome can exert high influence on soil microorganisms. Even with the studies performed until now, it is not yet clear how the environmental changes affect these microbes and their function (Bisset et al., 2011).

Many studies have shown the impact of land-use in the bacteria community of soil (Jesus et al., 2009; Osborne et al., 2011; Wallenius et al., 2011), but little has been studied about the impact on the soil fungal (Lumini et al., 2010) and archaeal communities (Taketani

and Tsai, 2010). Moreover, there are no studies in the Brazilian Pampa biome about the impact caused by land-use change in soil microbial community. Up to now, the degree of alteration caused by the land-use change on the fungal and archaeal communities was not clarified. Also, there is no information about the differences between the microbial communities in different soils of this biome. Due to these factors we attempted here to answer a simple question: Which factor, land-use or soil type, is responsible for the major changes in the fungal and archaeal community in the Brazilian Pampa biome? In this study we hypothesized that both, land-use change (e.g. introduction of exotic tree plantations and agricultural cropland) and different soil types are the major responsible for shifts in the structure of soil fungal and archaeal communities. To study the effect of land-use change under the microbial communities, we sampled two sites with the same soil type (Paleudult) and with different uses (natural grassland, natural forest, agricultural cropland and exotic plantation trees). To verify the effect of different soil types on microbial communities we sampled a typical sequence of soils (toposequence) of this biome. Soil microbial community structure was characterized using RISA and the overall status of the microbial community was evaluated by measuring microbial biomass and metabolic activity.

2. Material and methods

2.1. Study sites and soil sampling

To analyze the impact caused by land-use change in the fungal and archaeal communities, soil samples were collected at two sites in the state of Rio Grande do Sul, Brazil: site A, located in Rosário do Sul municipality, and site B, located in São Gabriel municipality, approximately 23 km far from site A. To minimize the effect of climate and soil type on the microbial communities at each site, samples were collected in the same day (December 17th 2010), in adjacent areas and under the same soil (Paleudult, U.S. Soil

Taxonomy). To analyze the effect of different soil types of the Pampa biome on microbial communities, soil samples were collected in a very common sequence of soils in the landscape of this region (toposequence) with uniform vegetation, formed by native grassland, currently used for grazing of cattle (site C, located in São Gabriel municipality, Rio Grande do Sul, Brazil, approximately 4.5 km far from site B).

At site A, soil samples were collected in areas with four different land-uses: NGA - native grassland currently used for grazing of cattle (30° 00' 38.2" S and 54° 50' 17.4" W, altitude 121 m); NFA - native forest used only for preservation of wildlife (30° 00' 39.7" S and 54° 50' 05.6" W, altitude 150 m); SFA - soybean field cultivated under no-tillage system on oat straw, with plants in early stage of growth (30° 00' 40.3" S and 54° 50' 13.2" W, altitude 137 m); and APA - 9 years old acacia trees plantation (*Acacia mearnsii* Willd.) (30° 00' 27.5" S and 54° 50' 10.2" W, altitude 141 m).

At site B, soil samples were also collected in areas with four different land-uses: NGB - native grassland currently used for grazing of cattle (30° 11' 03.6" S and 54° 42' 48.3" W, altitude 172 m); NFB - native forest used only for preservation of wildlife (30° 10' 57.5" S and 54° 42' 54.8" W, altitude 173 m); WFB - watermelon field cultivated in conventional tillage, with plants in early stage of growth (30° 10' 57.5" S and 54° 42' 54.8" W, altitude 173 m); and EPB - 5 years old *Eucalyptus* trees plantation (*Eucalyptus urophylla*) (30° 10' 57.3" S and 54° 42' 48.5" W, altitude 168 m).

At site C, four soil samples were collected in a line about 120 m from the highest (top of a hill) to the lowest point (near small stream), with distance between sampling points of approximately 30 m: T1C - point with highest altitude, top of a hill, Paleudult soil (30° 13' 34.3" S and 54° 42' 24.6" W, altitude 178 m); T2C - point with upper middle altitude, Paleudult soil (30° 13' 34.1" S and 54° 42' 25.9" W, altitude 175 m); T3C - point with lower middle altitude, Albaqualf soil (30° 13' 33.9" S and 54° 42' 27.4" W, altitude 172 m); and

T4C - point with the lower altitude (30° 13 '34.1" S and 54° 42' 29.8" W, altitude 167 m), alluvial soil constituted by fluvial sediments deposited by stream, because soils in the region are sandy and very susceptible to erosion.

For microbial community analysis, a total of 60 soil samples were collected (20 for each site): five for each land-use in sites A and B and five for each point in the landscape in site C. Each set of five samples was collected after drawing of a square (1m²) and taking samples in every corner and in the center of the square. The soil was collected with a sterile spatula to a depth of 5 cm and stored in sterile bottles in ice, until transport to the laboratory in the same day. Each soil sample was used to extract the total DNA separately, resulting in 120 profiles of microbial community.

For physical, chemical and microbial metabolic activity, soil samples were collected (in the center of the square), in each land-use or point in the landscape, resulting in 12 samples. The samples were collected in the same day that samples for microbial community analysis, with a sterile spatula to a depth of 5 cm and stored in sterile bottles in ice, until transport to the laboratory.

2.2. Physical, chemical and microbial metabolic activity

The physical and chemical analyses were performed according to the recommendations of the Brazilian Society of Soil Science (Silva, 2009). Total Carbon and Nitrogen were determined by combustion using an automated C/N analyzer (Flash EA 1112). Soil moisture content was determined gravimetrically by oven drying soils at 105 °C for 24 h. Clay was determined by densimeter method, soil pH was measured in a 1:1 soil/distilled water suspension. Potassium and phosphorus were extracted by Mehlich-1. Aluminum, Calcium and Magnesium were extracted by KCl 1 mol L⁻¹. Copper and Zinc were extracted by HCl 0.1 mol L⁻¹, S was extracted with Ca(H₂PO₄)₂ and B was extracted with hot water.

Physical and chemical data from the sites studied are shown in Table 1. The estimation of microbial biomass carbon (MBC) was conducted by fumigation-extraction method (Vance et al., 1987) and metabolic quotient (qCO_2) was calculated by ratio between basal respiration and microbial biomass.

2.3. Soil DNA extraction and RISA

Soil DNA was extracted with Kit PowerSoil[®] DNA (MoBio Laboratories, Inc) according to the manufacturer's instruction with the exception that 1 g rather than 0,25 g of soil was used and the final DNA extracts were eluted into 50 μ L of ultrapure H₂O rather than solution C6. DNA concentrations were determined using NanoVue[™] (GE Healthcare) and all DNA samples were stored at -20 °C until needed.

The genetic structure of the dominant portion of the soil fungal and archaeal communities was determined by RISA. The fungal internal transcribed spacer (ITS) was amplified using the primers 2234C (5'-GTTTCCGTAGGTGAACCTGC-3') and 3126T (5'-ATATGCTTAAGTTCAGCGGGT-3') (Sequerra et al., 1997). The amplification reactions were carried out in a 50 μ L containing approximately 100 ng of soil DNA, 0.2 mM of each dNTP, 1.5 mM of MgCl₂, 0.5 μ M of each primer, 5 units of *Taq* DNA Polymerase and 1X PCR buffer (Invitrogen Life Technologies). The following thermocycling pattern was used: 94 °C for 3 min; 94 °C for 45 s; 53 °C for 1 min and 72 °C for 2 min (30 cycles); and 72 °C for 7 min (1 cycle). The archaeal intergenic spacer region was amplified using the primers 21Fa (5'-TTCCGGTTGATCCYGCCGGA-3') and 64R (5'-GCCNRGGCTTATCGCAGCTT-3') (Summit and Baross, 2001) with the same PCR mix mentioned above. The thermocycling pattern used was: 94 °C for 3 min; 94 °C for 45 s; 55 °C for 1 min and 72 °C for 2 min (30 cycles); and 72 °C for 7 min (1 cycle).

An aliquot of 20 μ L PCR products of fungal and archaeal was loaded into 8% nondenaturing polyacrilamide gel (Sambrook and Russell, 2001) in TBE buffer and resolved by electrophoresis for 14 h at 60 V and 5 mA. The gels were stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Molecular Probes) following the manufacturer's instructions.

2.4. Data analysis

The RISA profiles were used to generate a binary matrix (presence/absence) with Gel-Pro Analyzer program (Media Cybernetics, USA) with 5% threshold. The fungal and archaeal data were analyzed using multivariate techniques. To compare the genetic structure between samples, a matrix of similarity (Jaccard similarity coefficient) was calculated from the binary matrix with PRIMER 6 (Clarke and Gorley, 2006). Changes in relative similarities of each site were measured using ranks of similarity represented by non-metric multidimensional scaling (NMDS) based on the Sorensen similarity index with PRIMER 6 (Clarke and Gorley, 2006), where ordination distance ranking was showed using Kruscal's stress value, which should be less than 0.2 to give a good and accurate representation of the similarities between the samples (Clarke et al., 1993). Associated with NMDS, one-way analysis of similarity (ANOSIM) tests were used to assess significant differences in microbial composition of land-uses and soil types groups, which use a similarity matrix (the same of NMDS) to calculate an R test statistic. R values can range from 0 to 1 (Clarke and Gorley, 2006). R values smaller than 0.5 indicate that the microbial composition do not differ statistically between samples and R values greater or equal to 0.5 indicate that the microbial composition differ significantly between samples (Wertz et al., 2007).

To display and analyze how the Operational Taxonomic Unities (OTUs) were partitioned between samples we applied a Network-based analysis. The network was

calculated with the same matrix of presence/absence from previous analysis (NMDS and ANOSIM), generated by using QIIME (Caporaso et al., 2010) and visualized with Cytoscape (Shannon, 2003). In this type of analysis there are two types of nodes representing the treatments (in this case, the land-uses or soil types) and the OTUs found in all treatments. OTUs found in only one node are connected by only one line (named edge) and OTUs found in more than one node are connected by more than one line (Caporaso et al., 2010).

To compare the microbial biomass carbon and the metabolic quotient among different land-uses and soil types we used Tukey's test at $p < 0.05$. Correlations were assessed by Pearson's coefficient.

3. Results

The fungal and archaeal community structure was assessed by RISA fingerprint, which is considered a highly reproducible technique that allows an easy comparison between samples (Elsas and Boersma, 2011). The overall microbial activity was assessed by measurements of microbial biomass and metabolic quotient.

RISA profiles were assumed to be indicative of fungal and archaeal community structure and differences were assumed to reflect variation in communities' composition. The average number of OTUs obtained by RISA from fungal and archaeal communities are presented in Table 2. The number of OTUs from fungal communities differed between land uses in both sites A and B, while the number of OTUs from archaeal in site A were similar among treatments and did not present statistical differences in site B. Contrasting soil types (site C) also reflect differences in the number of OTUs for both fungal and archaeal communities. In both sites A and B, the smallest number of bands from fungal community was found in the cultivated forests followed by the agricultural sites.

To better judge the (di)similarities between microbial communities from different

land-uses and soil type, the RISA profiles were used to calculate nonmetric multidimensional scaling (NMDS) plots associated with analysis of similarities (ANOSIM) of fungal and archaeal communities. These analyses provide a way to test statistically whether there is a significant difference between two or more environments. According to the tests the fungal and archaeal communities were ordered according to the land-uses and soil types (Fig. 1).

Changes in land use affected both fungal and archaeal communities at the two sites evaluated (Table 3). The global R, which is the average pairwise R for each group and indicates the general similarity of the microbial community analyzed, were 0.83 for the fungal community and 0.51 for the archaeal community at site A. At site B, the global R was 0.48 for fungal and 0.80 for archaeal communities. Furthermore the NMDS and ANOSIM tests indicated that the native vegetation (grassland and forest) formed clusters apart from tree plantation and croplands (Fig. 1). Those clusters were more evident at site A than site B.

The highest fungal communities similarities at site A were found between the native vegetation (pairwise R = 0.33) and the highest dissimilarities were found between Acacia plantation and two native vegetation (Pairwise R = 1.0) (Table 3). The highest archaeal communities' similarity at site A was found between the native grassland and the soybean field (Pairwise R = 1.0) (Table 3). At site B, the archaeal communities presented high dissimilarity between all the different land uses with the exception of *Eucalyptus* plantation and native grassland that presented an R-value equal to 0.34. Those soil samples were collected from fields that were about 20 m closer to each other and far from the other sampling points.

In addition to the differences in fungal and archaeal communities from distinctive land uses, we also found that soil types under the same plant cover (site C) were able to support different fungal and archaeal communities. The archaeal communities were most influenced by the soil type than the fungal communities (archaeal global R equal to 0.81 and fungal

global R equal to 0.48) (Table 3). The archaeal communities were grouped according to the soil type while individual clusters of fungal communities from all different soil types were not clearly observed (Fig. 1). The ANOSIM test (Table 3) also demonstrated the high divergence between archaeal communities from different soil types. The highest dissimilarities were observed between the most distant soil samples with contrasting soil features (T1C x T4C) while the highest similarities were observed between closer soil samples (T1C x T2C and T3C x T4C).

Although the NMDS and the ANOSIM testes showed that introduction of exotic tree plantations and agricultural cropland causes a significant change on the structure of soil archaeal and fungal communities and different soil types found in Pampa biome harbor different structures of microorganisms communities, the OTU network showed that a large percentage of OTUs were shared among the four land uses and soil types (Fig. 2). Overall, the network analysis suggests the existence of fungal and archaeal taxa broadly distributed among all soil samples but also reveals the presence of locally abundant taxa restricted to a particular environment. The native grassland presented the highest number of unique OTUs.

The microbial biomass carbon content (MBC) differed significantly ($p < 0.05$) among the land-uses in sites A and B, and among the soil types (site C) (Table 4). At site A, the native forest presented the highest amount of MBC, while at site B, the highest MBC was found at the soil under *Eucalyptus* plantation. The areas under annual cultivation presented the smallest amounts of MBC when compared to the grassland and natural forest. This trend was also observed with the qCO_2 values (Table 4). At site C, the smallest microbial biomass was found at the highest point of the toposequence (T1) and when the samples were collected in sites with smaller elevation a progressive increase in the MBC was observed although this increment was not statistically different at 5% probability. At the toposequence, where native grassland is the predominant vegetation, the soil moisture would be the environmental factor

that exerts the greatest influence. A positive relationship can be found between soil moisture and MBC (Table 1 and Table 4).

4. Discussion

Changes in land-use are common in many landscapes in the world and are among the factors that affect the soil microbial community structure and function (Kasel et al., 2008; Carson et al., 2010). As the microorganisms play key roles in nutrient cycling and other important functions in soils, the shifts in microbial communities caused by land-use change can directly affect the functioning of ecosystems (Berthrong et al., 2009). Another important factor that influence the microbial community is the soil type. Some studies have demonstrated the strong impact that different soil types can exert under the microbiota (Singh et al., 2007).

We hypothesized that land-use change and soil types in Pampa biome can significantly alter soil microbial community. To confirm our hypothesis we used two approaches to analyze the alteration caused by land-uses and soil type in the soil fungal and archaeal communities. One approach was based on the microbial community fingerprint and the other on the microbial metabolic activity.

Our results clearly revealed that land-use change exerted a strong influence on the community structure of soil fungal and archaeal communities in the sites analyzed. Since the selected sites were characterized by the same soil type (Paleudult) and same weather conditions, we consider that the land-use change may be the main factor responsible for alteration in the structure of soil microbial communities. Despite the shifts in fungal and archaeal communities observed in our work, we also found that different land-use and soil types shared several taxonomic unities. Those results indicated the presence of a core community shared among all soil samples. This core community did not suffer any disturbance after the alteration of land-use. Furthermore, supporting the initial hypothesis, our

results demonstrated that different land-uses and soil types also exerted influence on the microbial biomass carbon and metabolic quotient.

In our study, we found that acacia and *Eucalyptus* afforestation presented the greatest impact on the soil fungal community. Previous studies have shown that introduction of exotic tree species in areas under natural vegetation can modify the soil microbial communities (Carson et al., 2010). The fungal communities presented a higher response due to the vegetation modification than other soil microorganisms since the characteristics of the litter can act as selective pressure towards fungal species capable of degrading specific substratum (Lauber et al., 2008; Macdonald et al., 2009). The conversion of grassland to *Eucalyptus* plantations decreased the richness and altered the composition of fungal community (Kasel et al., 2008; Carson et al., 2010). Also, the conversion of grassland to *Acacia* plantations decreased the soil fungal richness and diversity suggesting that this modification may be mainly caused by changes in decomposer communities (Lorenzo et al., 2010).

The effect of land-use change observed in our work was consistent with findings from other studies that have shown that alteration of land-use cause a significant effect on soil fungal and archaeal community. Hossain and Sugiyama (2011) studying 32 sites under different land-uses found that microbial community structure was more similar between forest and grassland than agricultural soil. They suggested that soils that are exposed to frequent human disturbances might present modification or reduction of the soil microbial community. In site A, we also observed that fungal community showed more similarity between native vegetation than introduced vegetation and that archaeal community structure presented major dissimilarity between native grassland and the soybean field.

We found that archaeal was strongly affected by land-use in site B. Taketani and Tsai (2010) studying the influence of land-use change on the structure of archaeal community reported that land-use caused a strong effect on archaeal structure and attributed this

modification to the plant community. We attribute these land-use effects to the actual dominant vegetation found at each soil. The vegetation could affect the soil microbial community due to the release of different carbon compounds (Berthrong et al., 2009), quantity and quality of litter inputs (Kasel et al., 2008) and allelopathic effects on microbial communities (Lorenzo et al., 2010). The plant diversity in native environments release large amounts of metabolites through root exudates that might positively affect the soil microbial population (Zhang et al., 2011). In addition to direct effects of litter on microbial resources, land-use change can indirectly influence soil microbial composition through plant-mediated changes in soil properties like aggregation and soil density, infiltration and evaporation of water and bioavailability of nutrients (Singh et al., 2009; Wakelin et al., 2008). Agricultural practices, like tillage, application of pesticides and nutrients, traffic of machinery used for the establishment and management of exotic forests and croplands can modify the physical and the chemical properties of soil and consequently alter the soil microbial communities and ecological functions (Bisset et al., 2011, Lumini et al., 2010).

The effect of land-use and soil type under fungal and archaeal communities, varied according to the location analyzed. This result showed that the edaphic factors could also influence the fungal and archaeal communities. Kasel et al. (2008) studying the effect of land-use and location on fungal communities in different land uses find that location influenced the community structure of soil fungal. Soil microbial communities patterns had not been extensively studied yet but some authors suggest the complex plant-soil-microbe-environment interactions as the main origin of geographical patterns of soil microbes (Hossain and Sugiyama, 2011).

Although soil type is considered to be a primary factor determining microbial composition (Girvan et al., 2003) we found that fungal community structure were less affected by soil type than archaeal community. Wakelin et al. (2008) also found that the soil

fungus community structure was less related to soil type than the community of soil bacteria and suggests that soil physicochemical properties might be less important as a primary driver of fungus community structure than other factors like soil management. On the other hand, the major driver of the differentiation in archaeal community might be the soil attributes (Taketani and Tsai, 2010). According to Grossman et al. (2010), the archaeal community can be grouped according to the different soil types, however they also suggest that the soil microbial community composition is more controlled by historical soil management than by soil type. Apparently, the fungus communities, mainly associated with the degradation of plant residues, suffer more intensely the effects of replacement of natural vegetation, while archaeal communities are better indicators of differences in the natural soil properties.

Although specific impacts on fungus and archaeal communities' structure were found, different land-use and soil types shared a large number of OTUs. Microbial communities might present some degree of resilience maintaining its structure unchanged after disturbance (Allison and Martiny, 2008). Microbial communities can also be driven by historical events (e.g. prevalence of any type of vegetation, weather conditions) that might influence present-day community structure (Martiny et al., 2006). Our results suggest the prevalence of a resilient core soil fungus and archaeal community that not suffer any change related to land-use, soil type or edaphic conditions. Even detecting the presence of high number of shared OTUs, we additionally found unique OTUs mainly associated with the native vegetation suggesting that introduced plant species might cause the loss of some microbial populations.

Changes in microbial composition might not affect the ecosystems process because the new community may contain taxonomic groups that are functionally redundant. The old community or the new community can carry out the same processes even having a different structure (Allison and Martiny, 2008). To investigate the effect of land-use and soil types on the overall microbial activity we measured the microbial biomass and metabolic quotient at

each sampling site. The results demonstrated that different land-uses and soil types also exerted influence on the microbial biomass carbon and the metabolic quotient. The microbial biomass control many important functions in soil and can be used as an indicator of environmental disturbance cause by land-use changes. Significant differences ($p < 0.05$) among land-uses were detected in both sites A and B indicating that the microbial biomass was affected by the land-use and the vegetal species. Areas under natural condition (grassland and natural forest) presented the greatest amount of MBC than croplands in both sites. This effect was already detected in grassland and native forests (Bissett et al., 2011; DuPont et al., 2010; Hossain and Sugiyama, 2011) might be attributed to the high plant diversity and consequent high release metabolites through root exudates that might positively affect the soil microbial community (Zhang et al., 2011). Many factors are associated with the decreased microbial biomass in cropland. Soil tillage, chemical inputs (Treseder, 2008), herbicide application (Singh and Ghoshal, 2010) reduced inputs from plants through root exudation and rhizodeposition (Waldrop et al., 2006) are among the major factors responsible for the reduction of MBC.

We found a positive correlation between soil moisture and BMC in site C. At this site, since the vegetation was uniform, the main factor contributing to the increment of microbial biomass was the soil moisture. The results are in agreement with Bissett et al. (2011), who suggested that the moisture is probably the most important factor in dry land soils to determine the microbial biomass. The water content is essential for all soil biogeochemical processes and an important factor that determines microbial activity (Brockett et al., 2012).

The metabolic quotient (qCO_2) is an index that expresses the soil quality, representing the efficiency in which organisms use the ecosystem resources (Zhang et al., 2011). High values indicate low C efficiency because there is a great loss of CO_2 and consequently lower amount of C incorporated into microbial biomass. In our study, the metabolic quotient

differed significantly between land-uses only at site A, where soybean cropland showed the highest qCO_2 value, indicating that the soil microorganisms were living under environmental stress in this land-use. The lower metabolic quotient under natural grassland indicates the better soil quality.

5. Conclusion

With this study we attempted to answer a simple question: Which factor, land-use or soil type, is responsible for the major changes in the fungal and archaeal community in the Brazilian Pampa biome? The sampling strategy and the molecular and biochemical approaches applied here allowed us to detect different patterns in fungal and archaeal community driven by land-use change and soil type showing that both factors are significant drivers of microbial community structure and activity. *Acacia* and *Eucalyptus* afforestation presented the most dissimilar communities when compared with natural vegetation. Nevertheless, irrespective of the land-use or soil type, the soils tested shared most of the taxonomic unities and only a proportion of the community suffers changes caused by human interference.

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References

- Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci.* 105, 11512-11519.
- Berg, G., Smalla, K., 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microb. Ecol.* 68, 1-13.
- Berthrong, S.T., Christopher, W.S., Piñeiro, G., Jackson, R.B., 2009. Afforestation alters the composition of functional genes in soil and biogeochemical processes in South American Grasslands. *Appl. Environ. Microbiol.* 75, 6240-6248.
- Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil microbial community structure and function. *Appl. Soil Ecol.* 51, 66-78.
- Brazilian Institute of Geography and Statistics - IBGE. 2004. Map of vegetation from Brazil and map of biomes from Brazil. Available online: <http://www.ibge.gov.br>. (accessed on 20 December 2011).
- Brockett, B.F.T., Prescott, C.E., Grayston, S.J., 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western. *Soil Biol. Biochem.* 44, 9-20.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods.* 7, 335-336.
- Carson, J.K., Gleeson, D.B., Clipson, N., Murphy, D.V., 2010. Afforestation alters community structure of soil fungi. *Fungal Biol.* 114, 580-584.

- Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* 18, 117-143.
- Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User Manual/Tutorial*. PRIMER-E, Plymouth.
- DuPont, S.T., Culman, S.W., Ferris, H., Buckley, D.H., Glover, J.D., 2010. No-tillage conversion of harvested perennial grassland to annual cropland reduces root biomass, decreases active carbon stocks, and impacts soil biota. *Agric. Ecosyst. Environ.* 137, 25-32.
- Elsas, J.D., Boersma, F.G.H., 2011. A review of molecular methods to study the microbiota of soil and the mycosphere. *Eur. J. Soil Biol.* 47, 77-87.
- Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M., Ball, A.S., 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *App. Soil Ecol.* 69, 1800-1809.
- Grossman, J.M., O'Neill, B.E., Tsai, S.M., Liang, B., Neves, E., Lehmann, J., Thies, J.E., 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. *Microb. Ecol.* 60, 192-205.
- Hossain, Z., Sugiyama, S., 2011. Geographical structure of soil microbial communities in northern Japan: Effects of distance, land use type and soil properties. *Eur. J. Soil Biol.* 47, 88-97.
- Jesus, E.C., Marsh, T.L., Tiedje, J.M., Moreira, F.M.S., 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. *The ISME J.* 3, 1004-1011.
- Kasel, S., Bennett, L.T., Tibbits, J., 2008. Land uses influences soil fungal community composition across central Victoria, south-eastern Australia. *Soil Biol. Biochem.* 40, 1724-1732.
- Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.* 40, 2407-2415.

Lorenzo, P., Rodríguez-Echeverría, S., Ganzález, L., Freitas, H., 2010. Effect of invasive *Acacia dealbata* link on soil microorganisms as determined by PCR-DDGE. *Appl. Soil Ecol.* 44, 245-251.

Lumini, E., Orgiazzi, A., Borriello, R., Bonfante, P., Bianciotto, V., 2010. Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environ. Microbiol.* 12, 2165-2179.

Macdonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., Singh, B.K., 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with *Pinus radiata*. *Soil Biol. Biochem.* 41, 1642-1651.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Øvreås, L., Reysenbach, A-L., Smith, V.H., Staley, J.T., 2006. Microbial biogeography: putting microorganisms on the map. *Nat. Rev. Microbiol.* 4, 102-112.

Ministry of the Environment - MMA. Brazilian biodiversity - Assessment and identification of areas and priority actions for conservation, sustainable use and benefit sharing of biodiversity across brazilian biomes, 2002. MMA/SBF, Brasília. Available online on: <http://www.mma.gov.br> (accessed on 10 January 2012).

Ministry of the Environment - MMA. Brazilian biodiversity - Priority Areas for the Conservation, Sustainable Use and Benefit Sharing of Brazilian Biological Diversity, 2007; Available online on: <http://www.mma.gov.br> (accessed on 10 January 2012).

Osborne, C.A., Zwart, A.B., Broadhurst, L.M., Young, A.G., Richardson, A.E., 2011. The influence of sampling strategies and spatial variation on the detected soil bacterial communities under three different land-use types. *FEMS Microbiol. Ecol.* 78, 70-79.

- Overbeck, G.E., Müller, S.C., Pillar, V.D., Pfadenhauer, J., 2006. Floristic composition, environmental variation and species distribution patterns in burned grassland in southern Brazil. *Braz. J. Biol.* 66, 29–41.
- Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R., Forneck, E.D., 2007. Brazil's neglected biome: The South Brazilian Campos. *Pers. Plant Ecol. Evol. Syst.* 9, 101–116.
- Pillar, V.P., Müller, S.C., Castilhos, Z.M.S., Jacques, A.V.Á., 2009. Southern Fields - conservation and sustainable use of biodiversity. Ministry of Environment, Brasília.
- Roesch, L.F.W., Vieira, F.C.B., Pereira, V.A., Schünemann, A.L., Teixeira, I.F., Senna, A. J.T., Stefenon, V.M., 2009. The Brazilian Pampa: A Fragile Biome. *Diversity.* 1, 182-198.
- Sambrook, J., Russell, D.W., 2001. *Molecular cloning: a laboratory manual*, third ed. Cold Spring Harbor Laboratory Press, New York.
- Sequerra, J., Marmeisse, R., Valla, G., Normand, P., Capellano, A., Moiroud, A., 1997. Taxonomic position and intraspecific variability of the nodule forming *Penicillium nodositatum* inferred from RFLP analysis of the ribosomal intergenic spacer and random amplified polymorphic DNA. *Mycol. Res.* 101, 465-472.
- Silva, F.C., 2009. *Handbook of chemical analysis of soils, plants and fertilizers*, second ed. Embrapa Information Technology, Brasília.
- Singh, B.K., Munro, S., Potts, J.M., Millard, P., 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *App. Soil Ecol.* 36, 147-155.
- Singh, B.K., Dawson, L.A., Macdonald, C.A., Buckland, S.M., 2009. Impact of biotic and abiotic interaction on soil microbial communities and functions: a field study. *App. Soil Ecol.* 41, 239-248.

- Singh, P., Ghoshal, N., 2010. Variation in total biological productivity and soil microbial biomass in rainfed agroecosystems: Impact of application of herbicide and soil amendments. *Agric. Ecosyst. Environ.* 137, 241-250.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498-2504.
- Summit, M., Baross, J.A., 2001. A novel microbial habitat in the mid-ocean ridge seafloor. *Proc. Natl. Acad. Sci.* 98, 2158-2163.
- Taketani, R.G., Tsai, S.M., 2010. The influence of different land uses on the structure of archaeal communities in Amazonian anthrosols based on 16S rRNA and *amoA* genes. *Microbiol. Ecol.* 59, 734-743.
- Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol. Lett.* 11, 1111-1120.
- Vance, E.D., Brookes, O.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703-707.
- Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. *Soil Biol. Biochem.* 40, 803-813.
- Wallenius, K., Rita, H., Mikkonen, A., Lappi, K., Lindström, K., Hartikainen, H., Raateland, A., Niemi, R.M., 2011. Effects of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. *Soil Biol. Biochem.* 43, 1464-1473.
- Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D., Tilman, D., 2006. Resource availability controls fungal diversity across a plant diversity gradient. *Ecol. Lett.* 9, 1127-1135.

Walker, B., 2001. Tropical savanna. in: Chapin, F.S., Sala, O.E., Huber-Sannwald, E. (Eds.), *Global biodiversity in a changing environment*. Springer, Berlin, pp. 139-156.

Wertz, S., Czarnes, S., Bartoli, F., Renault, P., Commeaux, C., Guillaumaud, N., Clays-Josserand, A., 2007. Early-stage bacterial colonization between a sterilized remoulded soil clod and natural soil aggregates of the same soil. *Soil Biol. Biochem.* 39, 3127-3137.

Zhang, C., Liu, G., Xue, S., Song, Z., 2011. Rhizosphere soil microbial activity under different vegetation types on the Loess Plateau, China. *Geoderma.* 161, 115-125.

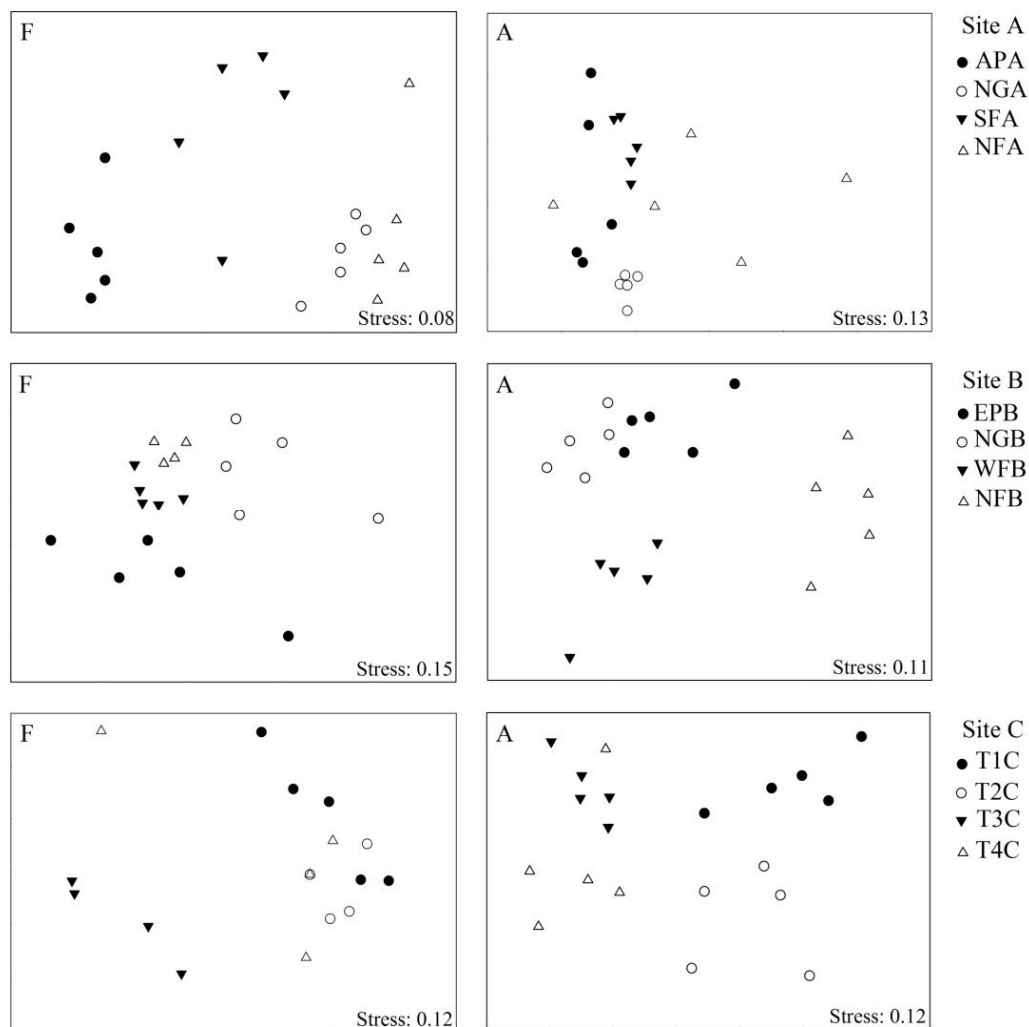


Fig. 1. 2D Nonmetric multidimensional scaling (NMDS) plots of fungal (F) and archaeal (A) communities structure determined by RISA profiles. The stress values for all plots were <math>< 0.2</math> which indicate that these data were well-represented by the two-dimensional representation. APA = *Acacia* plantation; NGA;NGB = Native grassland; SFA = Soybean field; NFA;NFB = Native forest; EPB = *Eucalyptus* plantation; WFB = Watermelon field; T(1:4)C = Toposequence.

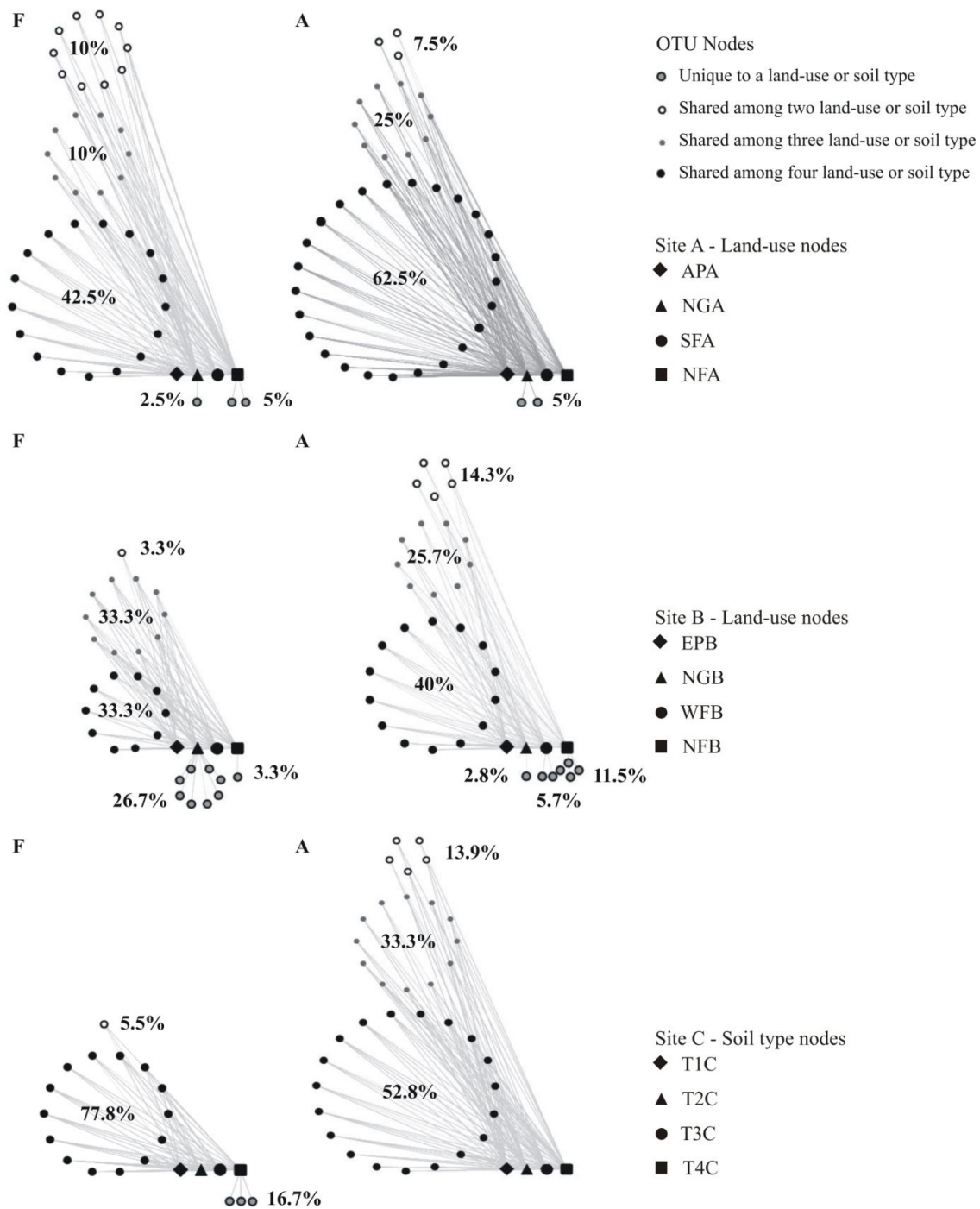


Figure 2. OTU network map showing fungal and archaeal communities OTUs share among land-uses and soil types in three study sites (A, B and C) in Brazilian Pampa biome. The nodes represents land-uses or soil types and fungal or archaeal OTUs shared among four, three or two land-uses or fungal or archaeal OTUs unique to only one land-use or soil type according to the legend. When one OTU is found in only one node (land-uses or soil types) the two nodes are connected with a line (an “edge”) and when one OTU is found in more than one node (land-uses or soil types) the OTU is connected with more than one edge. The numbers show the percentage to total of fungal and archaeal OTUs finding in each site shared

or unique among land-uses or soil types. APA = *Acacia* plantation; NGA;NGB = Native grassland; SFA = Soybean field; NFA;NFB = Native forest; EPB = *Eucalyptus* plantation; WFB = Watermelon field; T(1:4)C = Toposequence.

Table 1. Soil chemical and physical properties of surface soils (0-5 cm) from twelve points encompassing two sites (A and B) with different land-uses and one site (C) with different soil types from Brazilian Pampa biome.

Soil properties	Site A				Site B				Site C			
	APA ¹	SFA	NFA	NGA	EPB	WFB	NFB	NGB	T1	T2	T3	T4
Clay (g kg ⁻¹)	160	150	240	140	160	190	180	100	120	90	140	120
U (%)	20.8	9.7	19.6	7.1	6.3	4.2	10.1	19.1	2.5	2.9	10.4	25.6
pH	6.0	6.1	6.2	5.8	6.9	6.4	6.1	6.9	6.7	6.6	6.5	7.0
Total C (g kg ⁻¹)	10.0	50.2	17.7	14.3	18.2	5.7	7.5	17.1	8.4	5.7	13.7	9.0
Total N (g kg ⁻¹)	0.1	3.9	1.3	1.1	1.3	0.5	0.7	1.4	0.7	0.5	1.0	0.5
P (cmol _c L ⁻¹)	4.5	21.8	32.3	4.5	76.0	9.3	25.8	14.4	6.8	3.7	3.0	18.0
K (cmol _c L ⁻¹)	0.3	0.4	0.2	0.1	0.2	0.4	0.3	0.2	0.2	0.3	0.1	0.1
Al (cmol _c L ⁻¹)	0.4	0.4	0	1.3	0	0.5	0.6	0	0.1	0.4	0.6	0
Ca (cmol _c L ⁻¹)	2.4	1.2	11.3	1.2	4.1	1.5	2.3	1.3	1.8	1.1	1.0	3.7
Mg (cmol _c L ⁻¹)	0.7	0.5	1.4	0.2	1.4	0.7	0.5	0.4	0.7	0.6	0.2	2.3
CEC pH7	7.9	6.8	16.4	6.9	7.3	5.5	7.1	3.5	4.8	4.2	3.8	7.6
BS (%)	43.8	42.6	78.8	20.8	78.0	48.3	44.5	54.4	59.2	47.8	35.0	81.0
Zn (mg L ⁻¹)	1.8	1.6	25.8	1.3	6.3	3.2	2.7	2.8	2.0	1.6	1.6	1.6
Cu (mg L ⁻¹)	3.2	1.2	0.6	1.7	0.6	3.0	0.9	0.7	0.5	0.7	1.7	0.5
S (mg L ⁻¹)	12.0	6.0	9.0	7.2	7.6	7.8	6.5	4.5	6.1	5.4	5.2	4.0
B (mg L ⁻¹)	0.7	0.5	0.6	0.4	0.6	0.6	0.8	0.5	0.3	0.3	0.2	0.5

¹APA = *Acacia* plantation; NGA, NGB = Native grassland; SFA = Soybean field; NFA, NFB = Native forest; EPB = *Eucalyptus* plantation; WFB = Watermelon field; T(1:4)C = Toposequence.

Table 2. Average number of OTUs obtained by RISA from fungal and archaeal communities from different sites, land-uses or soils in the toposequence from Brazilian Pampa biome.

	Site A			Site B			Site C	
	Fungal	Archaeal		Fungal	Archaeal		Fungal	Archaeal
APA ¹	8 c ²	24 ab	EPB	8 b	16 a	T1C	10 b	21 ab
NGA	26 a	30 a	NGB	15 a	17 a	T2C	12 ab	20 b
SFA	15 b	25 ab	WFB	13 a	17 a	T3C	13 a	25 a
NFA	25 a	18 c	NFB	14 a	15 a	T4C	11 ab	28 a

¹APA = *Acacia* plantation; NGA ;NGB = Native grassland; SFA = Soybean field; NFA; NFB = Native forest; EPB = *Eucalyptus* plantation; WFB = Watermelon field; T(1:4)C = Toposequence.

² Different letters in each columns denote a significant mean difference in OTU number between respective microbial community (Tukey; p <0.05).

Table 3. One-way analysis of similarities (ANOSIM) of soil fungal and archaeal data from twelve points encompassing two sites (A and B) with different land-uses and one site (C) with different soil types from Brazilian Pampa biome.

Site A			Site B			Site C		
	Fungal	Archaeal		Fungal	Archaeal		Fungal	Archaeal
Groups ¹	Pairwise <i>R</i>		Groups	Pairwise <i>R</i>		Groups	Pairwise <i>R</i>	
APA, NGA	1.00 ²	0.54	EPB, NGB	0.57	0.34	T1C, T2C	0.53	0.66
APA, SFA	0.87	0.52	EPB, WFB	0.40	0.88	T1C, T3C	0.94	0.95
APA, NFA	1.00	0.38	EPB, NFB	0.42	0.89	T1C, T4C	0.09	0.97
NGA, SFA	0.88	1.00	NGB, WFB	0.68	0.90	T2C, T3C	0.95	0.87
NGA, NFA	0.33	0.48	NGB, NFB	0.48	0.99	T2C, T4C	0.05	0.84
SFA, NFA	0.95	0.38	WFB, NFB	0.67	0.98	T3C, T4C	0.65	0.58

¹APA = *Acacia* plantation; NGA;NGB = Native grassland; SFA = Soybean field; NFA;NFB = Native forest; EPB = *Eucalyptus* plantation; WFB = Watermelon field; T(1:4)C = Toposequence.

² All p values were highly significant ($p < 0.001$).

Table 4. Microbial biomass C (MBC) and metabolic quotient (qCO_2) from twelve points encompassing two sites (A and B) with different land-uses and one site (C) with different soil types from Brazilian Pampa biome.

Sites	Land-uses /soil types	MBC mg kg ⁻¹ of C	qCO_2 mg mg ⁻¹ de C-CO ₂
Site A	APA ¹	33.04 b ²	0.17 bc
	SFA	23.34 c	0.21 a
	NFA	53.35 a	0.11 b
	NGA	28.03 ab	0.08 c
Site B	EPB	90.69 a	0.04 a
	WFB	10.67 c	0.35 a
	NFB	27.52 b	0.09 a
	NGB	11.99 b	0.57 a
Site C	T1C	5.12 b	0.19 a
	T2C	16.45 a	0.28 a
	T3C	17.97 a	0.16 a
	T4C	20.43 a	0.23 a

¹APA = *Acacia* plantation; NGA;NGB = Native grassland; SFA = Soybean field; NFA;NFB = Native forest; EPB = *Eucalyptus* plantation; WFB = Watermelon field; T(1:4)C = Toposequence.

²Statistical analysis was performed for each site and for MBC and qCO_2 separately. Means followed by the same letter are not significantly different at the 5% level (Tukey; $p < 0,05$).

CONCLUSÃO

Com este estudo buscou-se responder uma simples pergunta: Qual fator, tipo de uso da terra ou tipo de solo, é responsável pelas maiores mudanças nas comunidades de fungos e arqueas no bioma Pampa Brasileiro? A estratégia de amostragem e as abordagens moleculares e bioquímicas aplicadas aqui permitiram detectar padrões diferentes nas comunidades de fungos e arqueas impulsionado pela mudança no uso da terra e tipo de solo, mostrando que ambos os fatores são importantes na estruturação da comunidade e atividade microbiana. Florestamentos de acácia e eucalipto apresentaram as maiores diferenças nas comunidades quando comparados com a vegetação natural. No entanto, independentemente do tipo de uso ou tipo de solo, os locais estudados compartilham grande parte das unidades taxonômicas e apenas uma parte da comunidade sofreu alterações causadas pela interferência humana.

REFERÊNCIAS

BERG, G.; SMALLA, K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. **FEMS Microbiology Ecology**, v. 68, p. 1-13, 2009.

CARSON, J.K. et al. Afforestation alters community structure of soil fungi. **Fungal Biology**, v. 114, p. 580-584, 2010.

CHRIST, S. et al. Fungal communities in bulk soil and stone compartments of different forest and soil types as revealed by a barcoding ITS rDNA and a functional laccase encoding gene marker. **Soil Biology & Biochemistry**, v. 43, p. 1292-1299, 2011.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE. Mapa da vegetação do Brasil e Mapa de biomas do Brasil, 2004. Disponível em: <<http://www.ibge.gov.br>>. Acesso em: 20 dez. 2011.

JESUS, E.C. et al. Changes in land use alter the structure of bacterial communities in Western Amazon soils. **The ISME Journal**, v. 3, p. 1004-1011, 2009.

LAUBER, C.L. et al. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. **Soil Biology & Biochemistry**, v. 40, p. 2407-2415, 2008.

MINISTÉRIO DO MEIO AMBIENTE - MMA. Biodiversidade brasileira - Avaliação e identificação de áreas e ações prioritárias para conservação, utilização sustentável e repartição dos benefícios da biodiversidade nos biomas brasileiros. Brasília: MMA/SBF, 2002. Disponível em: <<http://www.mma.gov.br>>. Acesso em: 10 jan. 2012.

MINISTÉRIO DO MEIO AMBIENTE - MMA. Áreas prioritárias para conservação, uso sustentável e repartição de benefícios da biodiversidade brasileira. Brasília: MMA, 2007. Disponível em: <<http://www.mma.gov.br>>. Acesso em: 10 jan. 2012.

OSBORNE, C.A. et al. The influence of sampling strategies and spatial variation on the detected soil bacterial communities under three different land-use types. **FEMS Microbiology Ecology**, v. 78, p. 70-79, 2011.

OVERBECK, G. E., et al. Floristic composition, environmental variation and species distribution patterns in burned grassland in southern Brazil. **Brazilian Journal of Biology**, v. 66, p. 29 – 41, 2006.

OVERBECK, G.E. et al. Brazil's neglected biome: The South Brazilian Campos. **Perspectives in Plant Ecology, Evolution and Systematics**, v. 9, p. 101–116, 2007.

PILLAR, V.P. et al. **Campos Sulinos** - conservação e uso sustentável da biodiversidade. Brasília: MMA, 2009. 403 p.

ROESCH, L.F.W. et al. The Brazilian Pampa: A Fragile Biome. **Diversity**, v. 1, p. 182-198, 2009.

SINGH, B.K. et al. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. **Applied Soil Ecology**, v. 36, p. 147-155, 2007.

SINGH, B.K. et al. Impact of biotic and abiotic interaction on soil microbial communities and functions: a field study. **Applied Soil Ecology**, v. 41, p. 239-248, 2009.