Chapter 2

Land-use changes and soil type are drivers of fungal and archaeal communities in the Pampa biome

Authors

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Abstract

The current study aimed to test the hypothesis that both land-use changes and soil type are responsible for the major changes in the fungal and archaeal communities structure and functioning of the soil in the Brazilian Pampa biome. Soil samples were collected at sites with different land uses (native grassland, native forest, *Eucalyptus* and *Acacia* plantation, soybean and watermelon fields) and in a typical toposequence in Pampa biome formed by Paleudult, Albaqualf and alluvial soils. The structure of the soil microbial community (archaeal and fungal) was evaluated by Ribosomal Intergenic Spacer Analysis and soil functional capabilities were measured by microbial biomass carbon and metabolic quotient. We detected different patterns in microbial community structure driven by land-use changes and soil type, showing that both factors are significant drivers of fungal and archaeal community structure confirming the hypothesis that both land-use changes and soil type are drivers of archaeal and fungal community structure as well as of soil functional capabilities. Fungal community structure was more affected by land use and archaeal community was more affected by soil type. Irrespective of the land use or soil type, a large percentage of Operational Taxonomic Units (OTUs) were shared among the soils. Moreover, we also suggest the existence of a soil microbial core.

Keywords

Intergenic spacer analysis; Microbial community; Afforestation; Soil microbial core

2.1 Introduction

The Pampa biome covers an area shared by Brazil, Argentina and Uruguay in the southern part of South America and is characterized by typical vegetation of native grassland, with sparse shrub and tree formations (Overbeck et al. 2007). In Brazil, this biome occupies part of Rio Grande do Sul State and was officially recognized by the Brazilian Institute of Geography and Statistics in 2004 (IBGE 2007). It presents distinctive characteristics of vegetation, climate and soil types, making it a unique ecosystem on the planet, capable of holding a high plant and animal diversities. Although considered one of the priority areas for flora and fauna conservation (MMA 2002), this biome is the most unknown of the Brazilian's biomes in relation to its biodiversity.

Despite its ecological importance, the Pampa biome is not adequately protected under current conservation policies (Overbeck et al. 2007). In recent years, this biome has undergone severe modifications through land-use changes by the conversion of natural vegetation to agricultural land (rice and soybean) and to large areas with exotic tree plantation (*Acacia* sp., *Eucalyptus* sp. and *Pinus* sp.). It is estimated that half of its original vegetation has been removed and transformed into other types of vegetation (Pillar et al. 2009). Furthermore, another important characteristic is that this biome has a wide specific variety of soil types, formed mainly upon sandy material. These soils are fragile and very susceptible to natural or anthropogenic erosion, which may enhance the effects of land-use changes (for more information see Overbeck et al. (2007) and Roesch et al. (2009)).

Many studies have shown that both land use and the soil type determine the composition of soil bacterial communities with emphasis on the effects of edaphic parameters on this domain (da C Jesus et al. 2009; Wallenius et al. 2011; Osborne et al. 2011). However, there are few reports and little is known about the effect of land-use changes or soil type on soil fungal and archaeal communities (Wakelin et al. 2013; Carson et al. 2010; Taketani & Tsai 2010; Takada Hoshino et al. 2011). According to recent findings, land-use changes and soil type can affect the structure of both fungal and archaeal communities in different ways. While studies show that the fungal community is more affected by vegetation or soil management (Kasel et al. 2008), the modification of the archaeal community structure is more affected by soil types or chemical variables (Chen et al. 2010). In addition to modifying the structure of soil microbial communities, these factors can also influence the soil ecosystem processes and important functions mediated by fungal and archaeal communities (Bissett et al. 2011; Berthrong et al. 2009).

Microbial communities can have a profound influence on biochemical processes in soil. Studying how environmental changes affect these groups could help to predict how biogeochemical cycles will respond to different soil modifications. For example, ammonia-oxidizing archaea are considered to be major contributors for the first step in nitrification, the transformation of ammonium (NH_4^+) to nitrite (NO_2^-) (Leininger et al. 2006; Gubry-Rangin et al. 2010), a key process in the global nitrogen cycle. The soil type is a determinant factor that affects the population and structure of these communities in soil (Chen et al. 2010; Takada Hoshino et al. 2011) and different land uses also influence these communities, especially through changes in soil chemical properties (Taketani & Tsai 2010). Fungal communities are thought to be the main active players in the decomposition, a key process of the carbon cycle, because of their ability to degrade complex polymers, such as cellulose and hemicelluloses, and recalcitrant compounds such as lignin (Schneider et al.

2012). There are indications that soil fungal communities are less correlated with modifications or differences in soil chemical properties (Wakelin et al. 2008), but this group is mainly influenced by litter nutrient content (Schneider et al. 2012). Therefore, the new vegetation type introduced by land-use changes might be the main factor affecting soil fungal structure and function (Lauber et al. 2008). Although several works focus on finding differences in microbial communities in distinct environments, recently more attention has been given to microorganisms widespread across habitats, suggesting the existence of a soil microbial core which harbor microorganisms presents in all environment regardless of soil type or land use (Shade & Handelsman 2012).

Nevertheless, there are no studies in the Brazilian Pampa biome about the effects of land-use changes on soil microbial communities and there is no information about the differences between microbial communities in different soil types of this biome. Due to these factors, we attempted here to address a simple question: which factor, land use or soil type, is responsible for the structuring of the fungal and archaeal communities in the Brazilian Pampa biome? In this study, we hypothesized that both land-use changes (*e.g.*, introduction of agricultural cropland and exotic tree plantations) and soil types in the Pampa biome have a significant effect on the soil archaeal and fungal community structure and functioning. To study the effect of land-use changes on the microbial communities, we sampled two sites with the same soil type (Paleudult) and with different usages (natural grassland, natural forest, agricultural cropland and exotic trees plantation). To verify the effect of different soil types on microbial communities we sampled a typical sequence of soils (toposequence) of this biome under native grassland. Soil microbial community structure was characterized using the Ribosomal Intergenic Spacer Analysis (RISA) approach and the overall status of the microbial community was evaluated by measuring microbial biomass and metabolic activity.

2.2 Materials and methods

2.2.1 Study sites and soil sampling

To analyze the impact caused by land-use changes in 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 away far from site A (the sampling sites can be visualized in Fig. S2.1). To minimize the effect of climate and soil type on the microbial communities at each site, samples were collected at the same day (December 17th, 2010), in adjacent areas and under the same soil type (Paleudult, U.S. Soil Taxonomy; Alisols, WRB-FAO). To analyze the effect of different soil types of the Pampa biome on microbial communities, soil samples were collected in a common toposequence (sequence of related soils which differ from each other, especially due to the topography as a factor soil formation but having the same parent material) in the landscape of this region with uniform vegetation, formed by native grassland, currently used for grazing cattle. The toposequence was denominated site C, located in São Gabriel municipality, approximately 4.5 km far from site B is formed by Paleudult (U.S. Soil Taxonomy; Alisols, WRB-FAO), Albaqualf (U.S. Soil Taxonomy; Planosols, WRB-FAO) and typical alluvial soil.

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 120 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 122 m); and APA - 9-year-old *Acacia* tree plantation (*Acacia mearnsii* Willd.) (30° 00′ 27.5″ S and 54° 50′ 10.2″ W, altitude 120 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-year-old *Eucalyptus* tree 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. This occurs because soils in the region are sandy and very susceptible to erosion.

For microbial community analysis, a total of 60 soil samples were collected following the experimental design proposed by Baker et al. (2009). The samples were taken by drawing five randomly distributed 1 m² plots per land use or point in the toposequence. A composite sample was collected by 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.

For physical, chemical and microbial metabolic activity analyses, soil samples from each land use or point in the landscape, were pooled to make a

single composite sample, resulting in 12 samples. The samples were collected in the same day 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.

2.2.2 Physical, chemical and microbial metabolic activity analyses

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 analyser (Flash EA 1112, Thermo Finnigan, Milan, Italy). Soil moisture content was determined gravimetric method by oven drying soils at 105°C for 24h. 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}, sulfur was extracted with $Ca(H_2PO4)_2$ and boron was extracted with hot water. Physical and chemical data from the sites studied are show in Table S2.1. 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 (Stotzky 1965) and microbial biomass (three repetitions was used to estimate microbial biomass and qCO_2).

2.2.3 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 µl of ultrapure H_2O 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 according to Sequerra et al. (1997) and the archaeal intergenic spacer region was amplified according to Summit and Baross (2001). The gels were stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Molecular Probes) following the manufacturer's instructions. An aliquot of 20 µl PCR products of fungal and archaeal was loaded into 8% non-denaturing polyacrylamide gel 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.

2.2.4 Data analysis

The RISA profiles were used to generate a binary matrix (presence/absence) with Gel-Pro Analyser 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 index) was calculated from the binary matrix with PRIMER 6 (Clarke & Gorley 2006). Changes in relative similarities of each site were measured using ranks of similarity represented by non-metric multidimensional scaling (NMDS) with PRIMER 6, where ordination distance ranking was showed using Kruscal's stress value, which indicates the degree of inconsistency between the similarity matrix and the final configuration of the plots. This value should be smaller than 0.2 to give a good and accurate representation of the similarities between the samples Clarke (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 type, which use a similarity matrix (the same of NMDS) to calculate the R value. R value represent a difference of average rank similarities between and within groups, which can varies between 0 and 1. R values near 0 indicate a true null hypothesis of no difference between groups, whereas those greater than 0 indicate discrimination between groups (reaching a maximum of 1, when similarities within groups are less than any similarities between groups) (Clarke & Gorley 2006). R values smaller than 0.5 indicate that 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 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. 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.

2.3 Results

The average number of OTUs obtained by RISA from fungal and archaeal communities are presented in Fig. 2.1. The number of OTUs from fungal communities differed between land uses in both sites A and B, while the number of OTUs from archaea in site B did not present statistical differences.

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 usages and soil type, the RISA profiles were used to calculate non-metric multidimensional scaling (NMDS) plots associated with analysis of similarities (ANOSIM) of fungal and archaeal communities. These analyzes 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. 2.2).

Changes in land use affected both fungal and archaeal communities at the two sites evaluated (Table 2.1). The global R, which is the average pairwise R for each group and indicates the general similarity of the microbial community analyzed was 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. 2.2).

The highest fungal community similarities at site A were found between the native vegetation (pairwise R = 0.33) and the highest dissimilarities were found between *Acacia* plantation and the two native vegetation (Pairwise R = 1.0) (Table 2.1). The highest archaeal communities' dissimilarity at site A was found between the native grassland and the soybean field (Pairwise R = 1.0) (Table 2.1). 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 close 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 more 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 2.1).

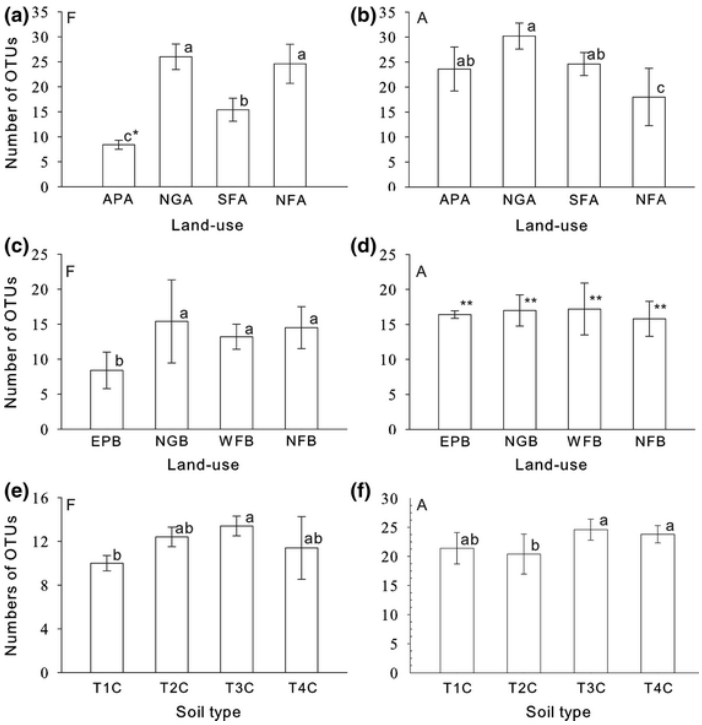

Fig. 2.1 Average number of OTUs obtained by RISA from fungal and archaeal communities from site A (a and b), site B (c and d) and site C (e and f), from Brazilian Pampa biome. APA, *Acacia* plantation; NGA, NGB, Native grassland; SFA, Soybean field; NFA, NFB, Native forest; EPB, *Eucalyptus* plantation; WFB, Watermelon field; T(1:4)C, Toposequence. Bars are mean ± SE, $n = 5$. *Different letters in each columns denote a significant mean difference in OTU number ($p < 0.05$). ** Indicate no difference between numbers of OTUs.

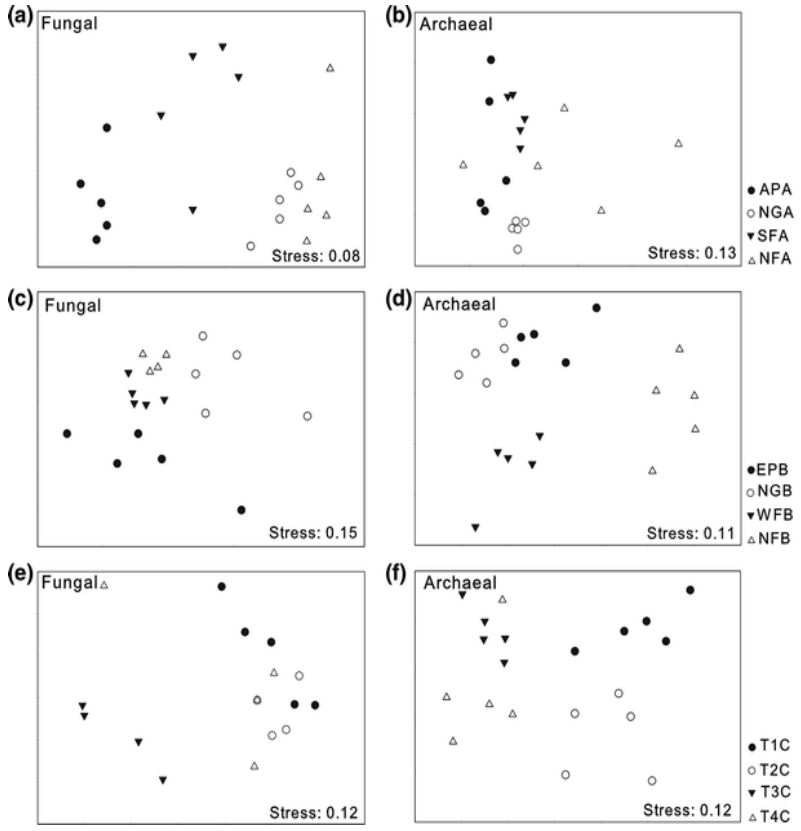

Fig. 2.2 2D Non-metric multidimensional scaling (NMDS) plots of fungal and archaeal communities structure from site A (a and b), site B (c and d) and site C (e and f) determined by RISA profiles. The stress values for all plots were <0.2 which indicate that these data were well-represented by the two-dimensional 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.

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. 2.2). The ANOSIM test (Table 2.1) 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, T4C) while the highest similarities were observed between closer soil samples (T1C, T2C and T3C, T4C).

Table 2.1 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
Group[a]	Pairwise *R*		Group	Pairwise *R*		Group	Pairwise *R*	
APA, NGA	1.00[b]	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

[a]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.[b] All *p*-values were highly significant ($p < 0.001$).

Even although changes in land use have caused significant alterations in community structure of soil fungal and archaeal, showed by NMDS (Fig. 2.2) and ANOSIM test (Table 2.1), the overall network analysis (Fig. 2.3) emphasizes the existence of fungal and archaeal taxa broadly distributed among all soil samples but also reveals the presence of taxa restricted to a particular environment. The OTUs that occurred in only one land use were mainly associated with the native vegetation, especially native grassland (NGA and NGB) and between different soil types. Only the alluvial soil (T4C) presented unique fungal OTUs which probably is due to the high moisture content of the soil (Table 2.1), which is the lowest elevation of the slope.

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 2.2). 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, in sites A and B, 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 2.2), where the highest values were observed in annual crops. 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 altitude a progressive increase in the MBC was observed, although this increment was not statistically different at 5% probability. A positive relationship can be found between soil moisture and MBC (Table S2.1 and 2.2).

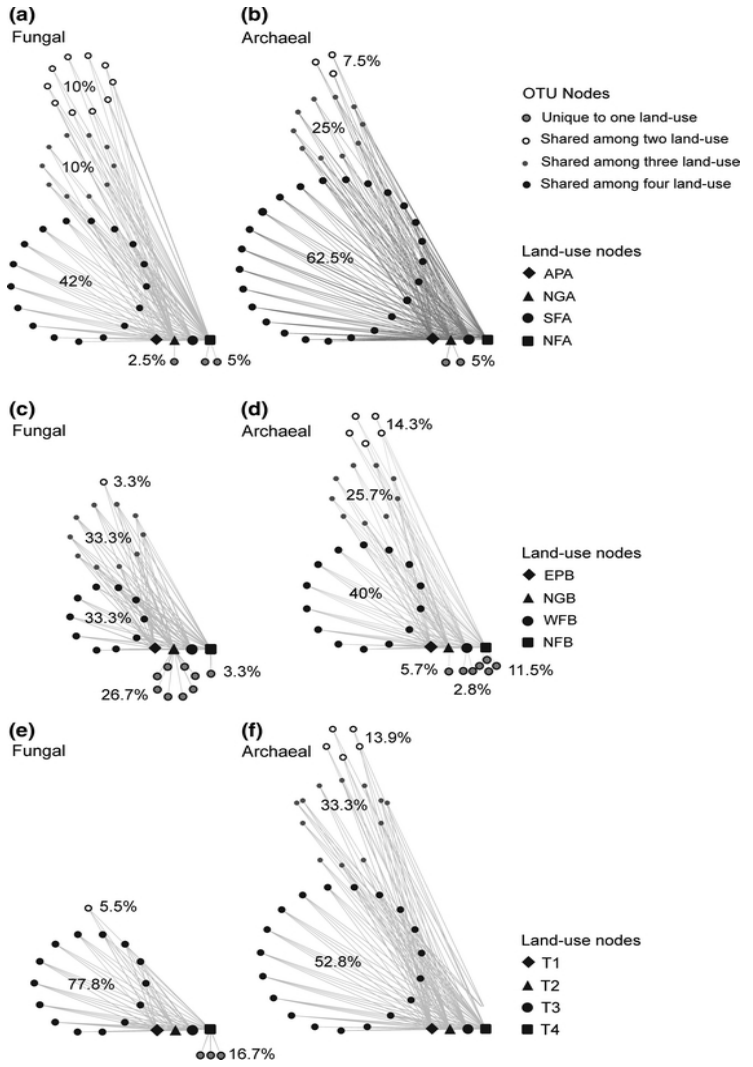

Fig. 2.3 OTU network map showing fungal and archaeal communities and OTUs shared among site A (a and b), site B (c and d) and site C (d and f) in Brazilian Pampa biome. The nodes represent land uses or soil types and fungal or archaeal OTUs shared among four, three or two land uses or fungal or archaeal OTUs unique to only one land use or soil type according to the legend.

When one OTU is found in only one node (land use or soil type) the two nodes are connected with a line (an "edge") and when one OTU is found in more than one node (land use or soil type) 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), Toposequence.

Table 2.2 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

Site	Land use /soil type	MBC mg $kg^{-1}$of C	qCO_2 mg mg^{-1} 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

[1]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. [2]Statistical 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).

2.4 Discussion

The fungal and archaeal communities structures were assessed by RISA (Ribosomal Intergenic Spacer Analysis) profiles. The RISA method is considered a highly reproducible fingerprint technique that allows for an easy comparison between samples (van Elsas & Boersma 2011). The method involves PCR amplification of the intergenic spacer region located between the small and large subunit rRNA genes in the rRNA operon. The intergenic spacer is extremely variable in both sequence and length (ranging from 50 bp to more than 1.5 kb) among different taxa allowing for a rapid assessment of the genetic structure of complex communities in soil environments (Borneman & Triplett 1997). It should be mentioned however that, as a molecular technique that relies upon total community DNA extraction and PCR amplification, RISA is subject to the usual systematic biases introduced by these procedures. For these reasons, any conclusions regarding the relative abundance of microbial communities represented in the RISA profiles should be carefully made. In this regard, we used the RISA profiles as a rapid survey technique in conjunction with the measurement of the overall microbial activity (microbial biomass and metabolic quotient) to verify the effect of land use change and soil type on the archaeal and fungal communities.

Changes in land use are common in many landscapes in the world and are among the factors that affect the soil archaeal and fungal community structure (Kasel et al. 2008). Another important factor that influences the microbial community is soil type, mainly by the difference in physicochemical properties (Wakelin et al. 2008; Takada Hoshino et al. 2011). In this study, we hypothesized that soil type can harbor distinct fungal and archaeal communities and land-use change alter significantly these microbial communities in the Pampa biome. To confirm our hypothesis we used two approaches to analyze the alteration in the soil fungal and archaeal

communities caused by land-use change and soil type. One approach was based on the microbial community fingerprinting, and the other on the microbial biomass and metabolic activity.

To our knowledge, this is the first report about the soil fungal and archaeal communities structure across different land uses and soil types in the Pampa biome. Our results clearly revealed that land-use changes exerted a strong influence on the community structure of soil fungal and archaeal communities in the sites analyzed. This was consistent with previous findings (Singh et al. 2009; Berthrong et al. 2009; Wakelin et al. 2008) showing that alteration in land use causes a strong effect on soil fungal and archaeal communities. The modification on the fungal community structure is mostly associated with effects of dominant vegetation type and management practices (Wakelin et al. 2008) while the shifts in archaeal community structure can be mainly associated with modification of bulk soil chemical properties caused by different land uses (Navarrete et al. 2011). We attributed these land use effects directly to the actual dominant vegetation, which 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 influences on microbial communities (Lorenzo et al. 2010) among other effects. Different plants in native environments release large amount of metabolites through root that might positively affect the soil microbial population (Zhang et al. 2011). In addition to direct effects of litter on microbial resources, land-use changes can indirectly influence soil microbial composition through plant-mediated changes in soil properties like aggregation, density, infiltration and evaporation of water and bioavailability of nutrients (Singh et al. 2009). Soil management practices used for the establishment of exotic forests and annual crops can modify the physicochemical properties of soil and consequently alter the soil microbial communities and ecological functions (Bissett et al. 2011; Lumini et al. 2009).

We also observed that the fungal community showed more similarity in samples of native vegetation than of introduced vegetation and the archaeal community structure showed major dissimilarity between native grassland and soybean field. These results are similar to other findings, where fungal and archaeal communities tend to be more similar in soils under natural vegetation than in arable soils, because the latter soils are exposed to frequent human disturbances strongly affecting the soil microbial community (Hossain & Sugiyama 2011; Navarrete et al. 2011).

The effect of land-use changes on fungal and archaeal communities varied according to the site analyzed. The fungal community was more influenced by land use in site A; contrary, the archaeal community structure was more altered by land use in site B. This result showed that the environmental and edaphic factors of each location could have a specific impact on the fungal and archaeal communities (Kasel et al. 2008). The low number of OTUs found in our study in *Acacia* and *Eucalyptus* afforestation show that these land usages had a greater impact on the soil fungal community than on archaeal community. Previous studies have shown that introduction of *Eucalyptus* and *Acacia* plantations in areas under natural vegetation can modify soil fungal microbial communities, mainly causing changes in the decomposer fungal community (Lauber et al. 2008; Carson et al. 2010; Lorenzo et al. 2010). The fungal communities presented a higher response due to the vegetation modification than other soil microorganisms because the characteristics of the litter can act as selective pressure towards fungal species capable of degrading specific substratum (Macdonald et al. 2009).

We found that fungal community structure was less affected by soil type than archaeal community. Similarly, previous findings reported that soil type might be less important as a primary driver of fungal community structure than other factors like soil management or land use (Wakelin et al. 2008). Apparently, the fungal communities are mainly associated with the

degradation of plant residues, suffering more intensely by the effects of shifts in vegetation type than by edaphic properties (Lorenzo et al. 2010). Fundamental differences in archaeal and fungal physiology and ecology would suggest that each group would be influenced by different factors. The archaeal communities are mainly affected by soil types and by physicochemical properties (Taketani & Tsai 2010). Chen et al. (2010) found that the population size and community structure of ammonia-oxidizing archaea is mainly determined by the soil types in flooded paddy soils, irrespective of the presence or absence of vegetation. Similarly, Takada Hoshino et al. (2011) found that the archaeal communities are strongly influenced by soil type, mainly by differences in soil physicochemical properties even under distinct management systems.

Although specific impacts on fungal and archaeal communities structure were found, different land uses and soil types shared a large number of OTUs, suggesting the existence of a core in soil fungal and archaeal communities that did not suffer significant changes related to land use changes or soil type. Microbial communities that do not change might present some degree of resistance or resilience, maintaining its structure unchanged after disturbance (Allison & Martiny 2008). Our results suggest the prevalence of a resilient core microbial community that does not suffer any change related to land-use changes, soil type or edaphic conditions. In addition to detecting the presence of a soil microbial core, we additionally found unique OTUs in natural vegetations, in sites A and B, mainly associated with the native vegetation, suggesting that land-use changes and the introduced plant species might cause the loss of some microbial communities (Carson et al. 2010). In the landscape (site C), only the alluvial soil showed unique OTUs from fungal community. This soil is located in the lower part of the toposequence and presents a moisture content above the others and can accommodate a fungal community adapted to higher water availability and reduced availability of oxygen.

To investigate the effect of land-use change and soil type on the overall microbial conditions we measured the microbial biomass and metabolic quotient at each sampling site. The results demonstrated that different land use and soil type also exerted influence on the microbial biomass carbon and the metabolic quotient. The microbial biomass controls many important functions in soil and can be used as an indicator of environmental disturbance cause by land-use changes. As well as changes found in fungal and archaeal communities in sites A and B, significant differences were also detected in the microbial biomass, indicating microbial biomass affected by the land use changes. It is well documented that the soil biomass and microbial activity (soil respiration and metabolic quotient - qCO_2) are largely determined by the most abundant and diverse group of microbes in soil *i.e.,* bacterial communities, which may be the largest contributor for biogeochemical process, such as decomposition and CO_2 efflux in soil (Hofmann & Illmer 2015; Goberna et al. 2011). Fungal communities are also actively involved in biogeochemical processes, thereby contributing significantly to soil respiration (Kant et al. 2010). The fungal abundance is positively related to microbial biomass, contributing from 52% in forest soils up to 80% in arable soils to total biomass (Susyan et al. 2011). The rate of soil respiration is highly correlated to fungal population in soil and shifts in respiration are often attributed to differences in the structure of fungal community (Aanderud et al. 2013). Although archaeal community are important in CH_4 and N dynamics (Taylor et al. 2012), the contribution of this group to soil biomass and soil respiration is less studied and largely unknown. Comparison among archaeal structure and total biomass and soil respiration have to made carefully because this group may represent only a small proportion of the community (Esperschütz et al. 2007; Ushio et al. 2013), and it is expected that this group contribute less to the soil respiration than bacterial and fungal groups. Most part of the studies so far showed no correlation of archaea with shifts in biomass or soil respiration (Goberna et al.

2011). However, the archaeal community may be important to soil respiration process at certain conditions. Han (2013) suggested that the increase in soil respiration is a process determined by the increased proliferation of microbial populations, including archaea.

Areas under natural conditions (grassland and natural forest) presented the greatest amount of MBC in both sites. This effect has already been detected in grassland and native forests (Bissett et al. 2011; DuPont et al. 2010) and might be attributed to the high plant diversity and consequent high litter deposition and release of metabolites through root turnover that might positively affect the soil microbial community (Zhang et al. 2011). Furthermore, many factors are associated with the decreased microbial biomass in croplands: soil tillage, chemical inputs (Treseder 2008), herbicide application (Singh & Ghoshal 2010), reduced inputs from plants through root exudation and rhizodeposition (Waldrop et al. 2006).

In addition, we found that soil type also can influence the MBC, because a positive correlation was found between soil moisture and MBC in site C. The results are in agreement with Bissett et al. (2011), who suggested that moisture is probably the most important factor in dry land soils determining the size of the microbial biomass. Soil water content is essential for all soil biogeochemical processes and an important factor determining microbial activity (Brockett et al. 2012). The metabolic quotient (qCO_2) is an index that expresses soil quality, representing the efficiency by which organisms use ecosystem resources (Zhang et al. 2011). High qCO_2 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 here and natural grassland showed the lower metabolic quotient, indicating better soil quality. The change in

metabolic quotient may be due to modifications of the community of microorganisms influenced by different land uses (Bissett et al. 2011). Even with changes in fungal and archaeal communities and microbial biomass in different land uses in site B and different soil types in site C, the qCO_2 was not significantly different ($p < 0.05$). Changes in microbial composition might not affect the ecosystem process because the old and the new community can carry out the same processes even having a different microbial structure (Allison & Martiny 2008) or because the core community can maintain some of the roles played by microorganisms in the soil, since the changes detected occurred in only part of the microbial community.

2.5 Conclusion

With this study we attempted to address a simple question: Which factor, land use or soil type is responsible for the major changes in the fungal and archaeal communities in the Brazilian Pampa biome? The sampling strategy and the molecular and biochemical approaches applied here allowed us to conclude that both land-use changes and soil types are significant drivers of microbial community richness, structure as well as of soil functional capabilities. The unique OTUs occurred mainly associated with the native vegetation, suggesting that land use changes might cause the loss of some microbial groups. Nevertheless, irrespective of the land use or soil type, a large percentage of OTUs were shared among the soils, which can be indication of the existence of a soil microbial core.

2.6 Supplementary material

Table S2.1 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 property	Site A				Site B				Site C			
	APA[1]	SFA	NFA	NGA	EPB	WFB	NFB	NGB	T1	T2	T3	T4
Clay (g kg^{-1})	150	150	180	120	130	135	135	85	105	95	100	90
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	5.2	6.1	5.4	4.3	6.3	5.1	4.5	6.4	5.4	5.0	5.2	5.9
Total C (%)	2.2	1.0	5.8	2.1	6.2	1.8	2.8	1.9	1.2	1.3	2.5	1.3
Total N (%)	0.2	0.1	0.5	0.2	0.5	0.2	0.2	0.1	0.1	0.1	0.2	0.1
P (mg L^{-1})	5.7	21.8	33.5	4.5	76.0	11.4	26.9	14.0	8.1	5.7	6.9	16.2
K (cmol$_c$ L^{-1})	0.4	0.4	0.2	0.1	0.2	0.5	0.4	0.2	0.3	0.3	0.3	0.2
Al (cmol$_c$ L^{-1})	0.3	0.4	0.1	1.2	0.0	0.3	0.5	0.0	0.1	0.3	0.2	0.0
Ca (cmol$_c$ L^{-1})	2.2	1.2	10.2	1.0	4.3	1.4	2.0	1.2	1.7	1.0	1.4	2.4
Mg (cmol$_c$ L^{-1})	0.6	0.5	1.3	0.2	1.6	0.7	0.5	0.4	0.7	0.6	0.7	1.4
CEC pH7	8.6	6.8	16.9	8.9	8.0	5.4	8.7	3.4	5.1	5.2	5.2	5.5
BS (%)	38.0	42.6	69.7	15.3	77.7	47.6	34.8	55.0	53.5	38.7	46.1	67.2
Zn (mg L^{-1})	2.6	1.6	27.2	1.3	6.9	3.6	2.8	3.0	2.1	2.1	2.1	1.6
Cu (mg L^{-1})	2.6	1.2	0.6	1.4	0.6	2.6	0.7	0.5	0.4	0.7	0.6	0.4
S (mg L^{-1})	12.2	6.0	11.5	9.2	9.3	7.9	10.4	6.2	6.8	8.2	7.5	5.0
B (mg L^{-1})	0.5	0.5	0.4	0.3	0.5	0.5	0.5	0.4	0.2	0.2	0.2	0.3

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

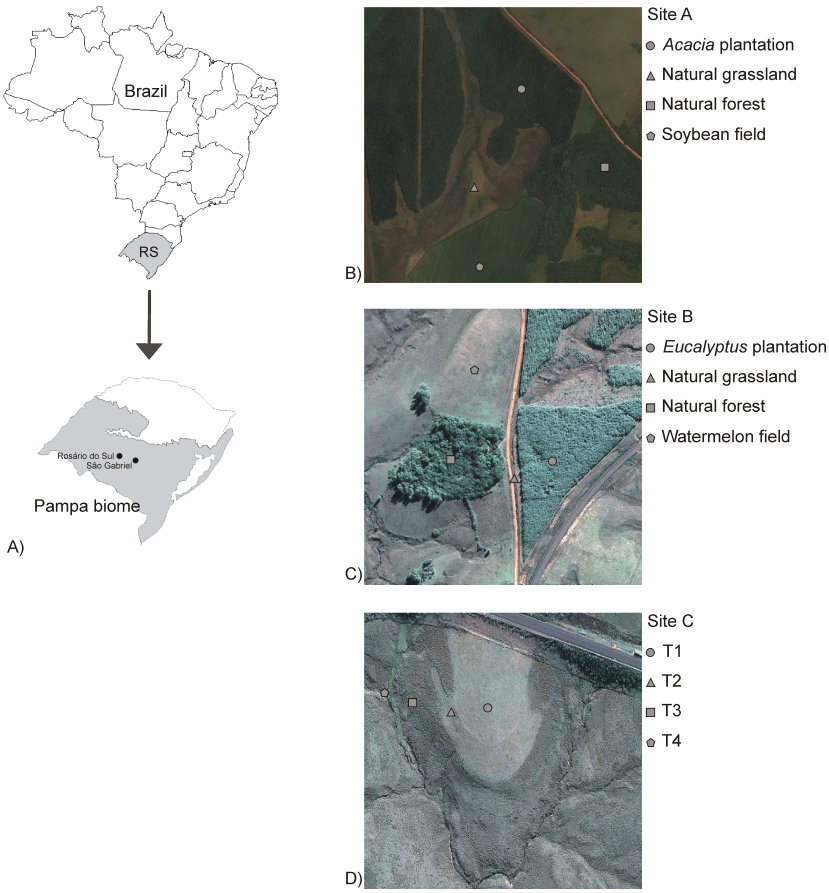

Fig. S2.1 (A) Map of Brazil and the state of Rio Grande do Sul, showing the location of Rosário do Sul and São Gabriel municipalities in the Pampa biome. (B) Site A, located in Rosário do Sul; (C) Site B, located in São Gabriel; (D) Site C, located in São Gabriel. The markers indicate points of soil sampling in different land uses and soil types.

2.7 References

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