UNIVERSIDADE FEDERAL DO PAMPA
CAMPUS SÃO GABRIEL
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

A RECENTE MUDANÇA NO USO DA TERRA PROVOCA POUCAS ALTERAÇÕES NA COMUNIDADE BACTERIANA DO SOLO

DISSERTAÇÃO DE MESTRADO

Afnan Khalil Ahmad Suleiman

São Gabriel, RS, Brasil
2012

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Dissertação apresentada ao Programa de Pós-Graduação Strictu Sensu em Ciências Biológicas da Universidade Federal do Pampa — UNIPAMPA, *Campus* São Gabriel, como requisito parcial para obtenção do Título de Mestre em Ciências Biológicas.

Orientador: Dr. Luiz Fernando Wurdig Roesch

São Gabriel

2012

SULEIMAN, Afnan Khalil Ahmad

A recente mudança no uso da terra provoca poucas alterações na comunidade bacteriana do solo/ Afnan Khalil Ahmad Suleiman. 2012.

47 f.

Dissertação (Mestrado) Universidade Federal do Pampa, 2012.

Orientação: Luiz Fernando Wurdig Roesch.

1. Qualidade Ambiental. 2. Ecologia Molecular. 3. Diversidade Microbiana. I. Roesch, Luiz Fernando Wurdig. II. Doutor.

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Área de Concentração: Qualidade Ambiental

Dissertação defendida e aprovada em: 02 de Agosto de 2012.
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Dedico essa dissertação aos meus pais,
Khalil e Aida, pelo apoio e incentivo.

AGRADECIMENTO

A Allah por me oferecer força e perseverança para a realização desse trabalho.

Aos meus pais Khalil e Aida e aos meus irmãos Ghaith, Mohamad e Hamzah pelo amor, compreensão, paciência, apoio e motivação dedicados a mim.

Ao Prof. Dr. Luiz Fernando Wurdig Roesch pela orientação desde a minha iniciação científica e pela paciência, descontração e incentivo nos momentos difíceis ao longo da execução desse trabalho. Vou sentir falta do “Tudo Pronto?”.

A Manoeli Lupatini pela ajuda nas análises desse trabalho, incentivo e conversas divertidas.

Aos colegas do Programa de Pós-Graduação em Ciências Biológicas, principalmente as minhas amigas Graziele Stürmer e Lilian Machado pela amizade e pelos momentos compartilhados que serão sempre lembrados.

A CAPES pela bolsa concedida.

A todos que mesmo não citados, sabem que de forma direta ou indireta, colaboraram na execução do trabalho.

RESUMO

A interação entre plantas, solo e microrganismos é considerada o maior condutor das funções do ecossistema e qualquer modificação na cobertura vegetal e/ou propriedades do solo pode afetar a estrutura microbiana, que por sua vez, poderá influenciar os processos ecológicos. Supondo-se que as propriedades do solo são os principais fatores que governam a diversidade e a estrutura das comunidades bacterianas do solo, dentro do mesmo tipo de solo, pode a retirada de cobertura vegetal causar mudanças significativas na composição da comunidade bacteriana do solo? Para abordar esta questão foi utilizado o pirosequenciamento do gene 16S para detectar diferenças na diversidade, composição e/ou abundância relativa dos táxons bacterianos a partir de uma área coberta por floresta nativa e de uma pastagem de oito anos de idade cercada por essa floresta. Após a remoção da floresta natural, a comunidade bacteriana do solo não sofreu uma grande diferenciação. Sessenta e nove por cento das Unidades Taxonômicas (UTs) foram compartilhadas entre os ambientes. Em geral, as amostras da floresta e as amostras de pastagem apresentaram a mesma diversidade e as análises de agrupamento não mostram a ocorrência de comunidades bacterianas muito distintas entre os ambientes. No entanto, foram detectadas onze UTs em maior abundância estatisticamente significativa nas amostras da floresta, mas em menor abundância nas amostras de pastagem e doze UTs em maior abundância estatisticamente significativas nas amostras de pastagens, mas em menor abundância nas amostras florestais. Esses resultados mostraram que a história recente do uso da terra provocou poucas mudanças na comunidade bacteriana do solo.

Palavras-chave: Pirosequenciamento; Diversidade Bacteriana; Agricultura; Desmatamento.

ABSTRACT

The interaction among plants, soil and microorganisms is considered to be the major driver of the ecosystem functions, and any modification on plant cover and/or soil properties might affect the microbial structure, which, in turn, will influence the ecological processes. Assuming that soil properties are the major drivers of soil bacterial diversity and structure, within the same soil type, can the plant cover cause significant shifts on soil bacterial community composition? To address this question we used 16S rRNA pyrosequencing to detect differences in diversity, composition and/or relative abundance of bacterial taxa from an area covered by pristine forest and eight years old grassland surrounded by this forest. After removing the natural forest, the soil bacterial community did not suffer a great alteration or at least, the community responded to disturbance and then returned to its initial state. Sixty nine percent of the operational taxonomic units (OTUs) were shared between environments. Overall, forest samples and grassland samples presented the same diversity and the clustering analysis did not show the occurrence of very distinctive bacterial communities between environments. However, we detected eleven OTUs in statistically significant higher abundance in the forest samples but in lower abundance in the grassland samples and twelve OTUs in statistically significant higher abundance in the grassland samples but in lower abundance in the forest samples. Those results illustrate that the recent history of land use buffers against the shifts in soil bacterial community.

Keywords: Pyrosequencing; Bacterial Diversity; Agriculture; Deforestation

SUMÁRIO

1 INTRODUÇÃO

O solo é provavelmente o ambiente microbiano mais complexo do ambiente terrestre com relação à riqueza de espécies e tamanho da comunidade. As atividades humanas têm modificado os ecossistemas levando a preocupações de que o funcionamento dele pode ser negativamente afetado pela perda de biodiversidade. As mudanças de uso da terra, como a substituição de paisagens naturais para usos antrópicos tem como resultado a aquisição dos recursos naturais muitas vezes à custa da degradação desses ambientes. Assim, a mudança de uso da terra pode provocar declínio na biodiversidade através da perda, modificação e fragmentação de habitats e degradação do solo.

O bioma Pampa, localizado no Estado do Rio Grande do Sul, apresenta solos bastante utilizados para a agricultura. Ele é caracterizado por apresentar campos nativos caracterizados principalmente pela presença de gramíneas, árvores esparsas e arbustos. Além disso, esse bioma representa um clima típico para o desenvolvimento de ecossistemas florestais. No entanto, o bioma Pampa vem sofrendo ao longo dos anos um intenso processo de degradação ambiental motivado, principalmente, por fatores naturais, como o vento e a água, porém ampliado pela adoção de práticas de uso do solo, principalmente para fins econômicos. A degradação dos solos nessa região é resultado da presença de extensas áreas arenosas, fruto da intensificação das atividades agropecuárias e do estabelecimento de florestas com espécies exóticas. Essas modificações podem ter fortes impactos sobre os microrganismos do solo, incluindo tanto os relacionados à biomassa e atividade, bem como aqueles relacionados com a composição da comunidade. Provavelmente, esses ambientes acabam exercendo pressão de seleção sobre os microrganismos do local sob as condições impostas pelo ambiente.

As modificações causadas pelas atividades agrícolas podem provocar a degradação das propriedades do solo e mudanças nas comunidades microbianas tanto em relação a capacidade dos microrganismos de resistir a uma perturbação ou estresse e/ou a capacidade de se recuperar de uma perturbação. Por isso que a degradação das terras agrícolas e a consequente perda da biodiversidade do solo e produtividade são de grande preocupação e um entendimento claro dos impactos das mudanças nesses diferentes fatores sobre o desenvolvimento de comunidades microbianas do solo irão ser benéficos para a restauração de ambientes degradados.

Vários fatores ambientais podem provocar mudanças na diversidade microbiana do solo afetando a sobrevivência e estrutura da comunidade microbiana, como os bióticos (por exemplo, predação e competição) e abióticos (por exemplo, temperatura, pH ou disponibilidade de substrato) provocando mudanças fisiológicas em seus organismos. Isso ocorre porque os microrganismos medeiam muitos processos que são essenciais para a produtividade agrícola dos solos, como ciclagem de nutrientes de plantas, manutenção da estrutura do solo e degradação dos produtos agroquímicos e poluentes. Desse modo, o conhecimento sobre as alterações nas comunidades microbianas pode fornecer informações importantes para a sua gestão e avaliação do impacto ambiental provocado pelo uso do solo.

Como a diversidade microbiana não pode ser estimada apenas pelo uso de meios de cultura, ferramentas moleculares vêm reduzindo as limitações relacionadas à abordagem de comunidades de microrganismos e demonstrando a real diversidade destes em amostras ambientais. Assim há a possibilidade de compreender sua composição e sua variabilidade nos ecossistemas, em resposta aos distúrbios e atividades de manejo. No entanto, a maioria dos estudos de diversidade microbiana empregaram métodos tradicionais moleculares, tais como sequenciamento de Sanger ou métodos baseados na “impressão digital” das comunidades microbianas. Uma nova perspectiva tem sido a aplicação de técnicas de sequenciamento mais abrangentes, chamadas de sequenciamento de nova geração, para uma análise mais refinada das comunidades bacterianas e detecção mais precisa de membros abundantes e raros da comunidade microbiana, resolvendo o problema de limitação das técnicas tradicionais de clonagem.

Tendo em vista o que foi apresentado acima, esse trabalho teve como objetivo avaliar quais foram as mudanças que ocorreram na comunidade bacteriana do solo causada pela mudança de uso da terra provocada pela recente retirada da cobertura vegetal de uma floresta nativa do bioma Pampa para a implantação de uma pastagem.

2 RECENT HISTORY OF LAND USE BUFFERS AGAINST SHIFTS IN SOIL BACTERIAL COMMUNITY

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2.1 Abstract

The interaction among plants, soil and microorganisms is considered to be the major driver of the ecosystem functions, and any modification on plant cover and/or soil properties might affect the microbial structure, which, in turn, will influence the ecological processes. Assuming that soil properties are the major drivers of soil bacterial diversity and structure, within the same soil type, can the plant cover cause significant shifts on soil bacterial community composition? To address this question we used 16S rRNA pyrosequencing to detect differences in diversity, composition and/or relative abundance of bacterial taxa from

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an area covered by pristine forest and eight years old grassland surrounded by this forest. After removing the natural forest, the soil bacterial community did not suffer a great alteration or at least, the community responded to disturbance and then returned to its initial state. Sixty nine percent of the operational taxonomic units (OTUs) were shared between environments. Overall, forest samples and grassland samples presented the same diversity and the clustering analysis did not show the occurrence of very distinctive bacterial communities between environments. However, we detected eleven OTUs in statistically significant higher abundance in the forest samples but in lower abundance in the grassland samples and twelve OTUs in statistically significant higher abundance in the grassland samples but in lower abundance in the forest samples. Those results illustrate that the recent history of land use buffers against the shifts in soil bacterial community.

Keywords: Pyrosequencing; Bacterial Diversity; Agriculture; Deforestation

2.2 Introduction

Soils are considered to be the most diverse microbial habitats on Earth. However, little is known on how the environmental changes affect the microbiota and it's functions [19, 40]. Land use and agricultural management are major causes of biodiversity loss with negative consequences to the environment [3, 15, 47]. Changes in composition or species diversity from aboveground communities can effect the composition and function of belowground communities and vice versa [28]. Particularly, changes in the aboveground vegetation affect size, activity and composition of soil microbial communities [31, 48]. Nevertheless, agricultural practices not always deplete soil bacterial diversity. Shifts in microbial diversity and structure caused by land uses may present a positive, negative or neutral impact [54].

According to Jangid et al. [30], microbial communities in relatively pristine deciduous forest and long-term mowed grassland soils were very similar despite major differences in soil properties and vegetation.

It has been reported that soil properties are important drivers of soil bacterial community structure. On a large scale, soil pH appears to be the major factor influencing the soil microbiome [18, 37, 51]. In this regard, land use indirectly affects the bacterial community structure by modification of soil properties [31]. Although continuous cropping can be responsible for deterioration of soil quality, recent changes in soil cover do not cause significant differences between properties of certain soil types under natural vegetation and soils put under cultivation. In eight years of land-use change [11] no differences were found in the soil physical and chemical properties. Only after 15 years of continuous cultivation the authors found significantly lower physical and chemical soil properties in soils under cultivation compared with soils under natural vegetation. In agreement, Geissen et al. [21] studying the effect of land-use change after 15 years did not detect chemical soil degradation but detected severe compaction of soils under permanent pasture. In certain ecosystems, the effect of agricultural practices on soil properties (e.g. forestland replaced by cropland and pasture) was clearly detected only in the past 50 years after the land use change [34].

Although changes in soil properties due to continuous cultivation appear to be a slow process, any land use change can possibly cause a disturbance, which in turn might affect the soil microbial communities. According to Allison and Martiny [1], there are three potential impacts caused by disturbance. After the disturbance, the microbial composition might be resistant and not change, might be altered and rapidly return to the original composition (resilient) or remain altered implying in a functional change. Assuming that recent changes in plant cover would not cause major changes in soil properties and that soil properties are the major drivers of soil bacterial diversity and structure, we hypothesize that the soil bacterial

community from a pristine forest will not be dramatically different from the soil bacterial community from a cultivated grassland surrounded by the forest in the first years of cultivation. Instead, in the initial years the plant cover change will promote a selection pressure leading to a sub-community formation in which a sub-community of the forest will compose the microbial community from the grassland.

Within this context, the aim of this work was to investigate bacterial communities from the same soil type but with different soil covers and address the following question: What is the contribution of plant community composition on bacterial community patterns in the first years of land use change? We analyzed an area covered by pristine forest soil and eight years old grassland surrounded by this forest. The area presented low human activity; no inputs of fertilizers (except for the manure added by animal activity) and a very low animal influence being ideal for testing the effect of plant cover removal under soil bacterial communities. Attempting to detect differences in diversity, composition and/or relative abundance of taxa, bacterial communities were characterized by high throughput pyrosequencing of 16S rRNA genes amplified from DNA extracted directly from the soil samples.

2.3 Material and methods

2.3.1 Site description and soil sampling

The sampling site is located within the Pampa biome, which has both subtropical and temperate climates with four well-characterized seasons. Grasslands, with sparse shrub and tree formations, are the dominant vegetation. The soil in the major part of the region has an extremely sandy texture due to its sedimentary rook origin [50, 58]. Because of the natural

grasslands, livestock production is one of the main economic activities, however, the natural fragility of the soil, combined with the climatic conditions and inappropriate human activities have lead to losses of both biodiversity and socio-economic opportunities.

The site consisted of pristine forest and eight years old grassland surrounded by this forest (Table 1). The grassland resulted from the deforestation of a small area (5.500 m^2) inside the forest for a non-commercial cattle settlement. No fertilizers, except for the manure added by animal activity, have been added to the soil and no exotic plants were introduced. Bulk soil samples were collected following the experimental design proposed by Baker et al. [2]. The soil samples were taken by drawing four randomly distributed 1m² plots per land-use. From each plot, a composite sample was collected by taking samples in every corner of the square. Bulk soil samples were collected taking 5 cm diameter, 0-10 cm depth cores during the spring of 2010. Equal amounts of sub-samples removed from cores were pooled and mixed to form four composite samples from the native forest and four composite samples from the grassland. Soil samples were put on ice upon collection and stored at -18°C until DNA extraction and chemical analyses were performed. For the soil chemical analyses, the four replicates from each land use were combined. The soil pH was determined in water (1:1 soil to water ratio) and the concentrations of Ca, Mg, Al, K, Na, P, Total nitrogen, NH_4^+, NO_3^- +NO_2^- and total organic carbon (TOC) were quantified according to Embrapa [16]. The dissolved organic carbon (DOC) was quantified according to Bartlett and Ross [4].

DNA was isolated from at least 1g of soil using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. After the DNA extraction, samples were purified with the DNeasy Tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions and the total DNA concentration were quantified using the NanoVue spectrophotometer (GE Healthcare, Harriet, USA).

2.3.2 16S rRNA amplification and pyrosequencing

The 16S rRNA gene fragments were sequenced using 454 GS FLX Titanium (Lib-L) chemistry for unidirectional sequencing of the amplicon libraries. Barcoded primers allow for combining amplicons of multiple samples to one amplicon library and furthermore enable the computational separation of the samples after the sequencing run. To do this, 8-base barcodes were added to the 5'-end of the reverse primers using the self-correcting barcode method of Hamady et al. [25]. The primers were attached to the GS FLX Titanium Adaptor A-Key (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG -3') and Adaptor B-Key (5'-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG-3') sequences, modified for use with GS FLX Titanium emPCR Kits (Lib-L) and a two-base linker sequence was inserted between the 454 adapter and the 16S rRNA primers to reduce any effect the composite primer might have on PCR efficiency. Eight independent PCR reactions were performed for each composite soil sample with the universal primers 338R and 27F for the amplification of the V1-V2 region of the 16S rRNA gene. PCR was performed with the GoTaq PCR core system (Promega, Madison, WI, USA). The mixtures contained 5 µl of 10X PCR buffer, 200 mM dNTPs, 100 mM of each primer, 2.5 U of Taq polymerase and approximately 100 ng of DNA template in a final volume of 50 µl. The PCR conditions were 94°C for 2 minutes, 30 cycles of 94°C for 45s; 55°C for 45s; and 72°C for 1 min extension; followed by 72°C for 6 minutes. The PCR products were purified and combined in equimolar ratios with the quantitative DNA binding method (SequalPrep Kit, Invitrogen, Carlsbad, CA, USA) to create a DNA pool that was further used for pyrosequencing from the A-Key adaptor. All raw sequences were submitted to the NCBI Sequence Read Archive (SRA) under the accession number SRA013204.1.

2.3.3 Processing of pyrosequencing data and statistical analyses

The raw sequences obtained were processed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit [12] with default parameters. Briefly, bacterial sequences were firstly quality trimmed by removing short sequences (< 200bp), sequences that presented low average quality scores (< 25), sequences that did not present a perfect match to the sequence barcode and primer, sequences that presented more than two undetermined bases, or that did not match at least 60% a previously determined 16S rRNA gene sequence [25]. Additionally, to identify potentially chimeric sequences, the dataset were subject to the ChimeraSlayer implemented in Mothur [52]. After removing low quality sequences, the multiplexed reads were assigned to the corresponding soil samples based on their barcodes. Bacterial sequences were grouped into OTUs using a 97% identity threshold and the most abundant sequence from each OTU was selected as a representative sequence for that OTU. After that, the sequences were taxonomically classified using the RDP naïve Bayesian rRNA Classifier [59], which assigns complete taxonomic information from domain to species to each sequence in the database with 80% taxonomy confidence and an e-value of 0.001. For each taxonomic level (Phylum, Class, Order, Family and Genus) and at 97% similarity cutoff Good's coverage was calculated [24]. The representative set of sequences was also used for aligning the sequences against a reference database and to build a phylogenetic tree necessary for downstream measurements. These taxonomic assignments were used to build an OTU table, which is a matrix of OTU's abundance for each sample with meaningful taxonomic identifiers for each OTU. The total number of sequences obtained from the native forest and the grassland are shown in Table 2.

2.3.4 Measures of differences among the soil bacterial communities

Principal Coordinate Analysis (PCoA) was performed to find clusters of similar groups of bacterial communities from the soil samples. PCoA is an ordination method based on multivariate statistical analysis that maps the samples in different dimensions and reflects the similarity of the biological communities. A matrix using the UniFrac metric (weighted and unweighted) for each pair of environments was calculated. The distances were turned into points in space with the number of dimensions one less than the number of samples. The first three principal dimensions, which usually contain most of the variation found in the samples, were used to plot a three-dimensional graph that illustrate the distribution of soils according to their similarity. To test whether the results were robust to sample size we used a sequence-jackknifing technique in which the PCoA clusters were regenerated using a subset of 600 sequences randomly selected from each soil for 100 replicate trials and drawing a graph (Fig. 2) made up by the mean values obtained. Around the mean values, ellipses that represent the IQRs (measure of statistical dispersion obtained by sequencing jackknifing) were drawn. If the ellipses are small, the same result would likely to be achieved with a different set of sequences from the same environment, but if the ellipses are large a different result might be expected. Furthermore, a hierarchical cluster tree was constructed on the basis of the distance matrix calculated by unweighted UniFrac algorithm. To assess the uncertainty in hierarchical cluster analysis we computed 1,000 bootstrap re-samplings. The Jackknifed PCoA and the hierchical cluster analysis were performed using QIIME [12].

To compare the similarity between bacterial communities from the soil samples we estimated the diversity of each sample using Shannon-Wiener index (Shannon and Weaver, 1949) and Faith's index of phylogenetic diversity [17]. For these measurements we calculated

the diversity metrics for a randomly selected subset of 12,393 sequences per soil, as alpha diversity indexes are correlated with the number of sequences collected [38].

To find which OTUs were abundantly different between the two environments an exact Chi-square test (based on 50,000 Monte Carlo iterations) was calculated to obtain a p-value for the null hypothesis that there was no difference between all possible pairwise combinations of soil samples from the native forest and the grassland. The p-values (≤ 0.01) were ordered and processed to find a false discover rate (FDR) less or equal than 1%. The test was performed using the OTU table summarized at the genus level with the sub-sampled number of sequences (12,392 sequences) for each sample obtained with QIIME and running an R script implemented in PANGEA [22].

2.4 Results

2.4.1 Vegetation and soil chemical analysis

The most common native tree species that were found in the forest belong to the families of Boraginaceae, Euphorbiaceae, Fabaceae, Lauraceae, Malvaceae, Meliaceae, Myrtaceae, and Rutaceae. The most dominant grass species found in the grassland belong to the Poaceae family. The number of plant families indicates greater plant diversity in the forest and a dominance of a single family in the grassland.

The location, altitude and soil chemical analysis are presented in Table 1. The pH and sodium content did not differed between soils from the native forest and from the grassland. All the other variables measured presented higher contents in the native forest than in the grassland exempting K that was greater in the grassland than in the forest. Some nutrients like P, Ca+Mg, NH_4^+, $NO_3^- + NO_2^-$ were found to be at least 1.5-fold higher in the native forest

then in the grassland. The total organic carbon was 2.2-fold higher in the native forest than in the grassland.

2.4.2 Assessment of taxon distribution and bacterial diversity

After filtering the 454 reads by base quality and removing reads smaller than 200 bases, a total of 170,046 sequences were obtained from the eight soil samples collected in the native forest and the grassland from the Pampa biome. Across all eight samples, 127,238 (74.83%) were classified below the domain level. The number of high quality sequences per sample varied from 12,393 to 37,225 and the average number of sequences per sample was 21,256 (Table 2). The classified sequences were affiliated to 20 bacterial phyla but only eight phyla were found in relative abundance greater than 1% (Fig. 1A). They were: *Proteobacteria* (26.9%), *Acidobacteria* (24.7%), *Actinobacteria* (10.8%), *Bacteroidetes* (3.5%), *Gemmatimonadetes* (2.3%), *Verrucomicrobia* (1.6%), *Nitrospira* (1.4%) and *Firmicutes* (1%). Within the *Proteobacteria* the more abundant classes were *Alphaproteobacteria* (14.7%), *Deltaproteobacteria* (4.1%), *Betaproteobacteria* (4.0%) and *Gammaproteobacteria* (1.6%). The phyla considered rare (relative abundance smaller than 1%) included *Chloroflexi*, *Cyanobacteria*, *OD1*, *OD10*, *OD11*, *Planctomycetes*, *SR1*, *Spirochaetes*, *TM7* e *WS3* (Fig. 1B).

The relative abundance of phyla presented minor variations between soils from native forest and grassland. The *Nitrospira* was found to be more abundant in the forest but less abundant in the grassland while *Firmicutes* was found to be more abundant in the grassland but less abundant in the forest. The more abundant phyla found in the forest were: *Proteobacteria* (28.05 ± 4.47%), *Acidobacteria* (23.47 ± 5.18%), *Actinobacteria* (11.325 ± 6,26%), *Bacteroidetes* (3.175 ± 1.91), *Nitrospira* (2.5 ± 0.83), *Gemmatimonadetes* (2.35 ±

0.67) and *Verrucomicrobia* (1.1 ± 0.16). The more abundant phyla from the grassland were: *Acidobacteria* (25.85 ± 5.14), *Proteobacteria* (25.75 ± 2.72), *Actinobacteria* (10.2 ± 4.28), *Bacteroidetes* (3.9 ± 1.67), *Gemmatimonadetes* (2.27 ± 0.76), *Verrucomicrobia* (2.02 ± 0.55) and *Firmicutes* (1.8 ± 1.35).

To identify shifts related to bacterial diversity between the forest and the grassland, two diversity indexes, Shannon–Weaver index and the Phylogenetic diversity (PD), were calculated. For the calculations a random subset of sequences (12,392 per sample) was sampled to correct the differences between samples related to the sampling coverage (Table 2). Overall, forest samples and grassland samples presented the same diversity. The Shannon index ranged from 10.01 to 10.58 for samples from the forest and from 10.18 to 10.53 for samples from the grassland. The average Shannon diversity index revealed no significant differences between environments according to the Tukey's range test at 5% error probability. The Phylogenetic diversity index ranged from 145.37 to 172.23 for samples from the forest and from 141.84 to 165.02 for samples from the grassland. Although the average PD index was higher in the samples from the forest, the Tukey's range test at 5% error probability revealed no significant differences between environments.

2.4.3 Similarity among communities based on membership and structure

A simple comparison of the number of OTUs between groups can lead to misinterpretations due to undersampling or due to variability between individuals rather than between groups. To better explore the similarities and the differences between the two environments tested, Jacknifed Principal Coordinates Analysis (PCoA) (Fig. 2) and a Hierarchical clustering analysis (Fig. 3) were performed to find clusters of similar groups of samples. To test whether the results were robust to sample size we used a sequence-

jackknifing technique in which the PCoA clusters were regenerated using a subset of 600 sequences randomly selected from each soil for 100 replicate trials. To assess the uncertainty in hierarchical cluster analysis we computed 1,000 bootstrap re-samplings.

Although a clustering pattern of samples from the forest and the grassland could be observed, the weighted and unweighted PCoA analyses (Fig.2A and 2B) did not show the occurrence of very distinctive groups of soil bacterial communities using both weighted distance metric, which accounts for changes in the relative abundance of taxa, and unweighted distance metric, which accounts for presence/absence of taxa.

In addition, the analysis of microbial communities using hierarchical cluster analysis showed that the bacterial communities from the same environment (forest or grassland) are more similar to each other than are bacterial communities in different environments as observed by the two highly supported clusters made up of samples from the forest soil and the grassland soils (Fig. 3). However those clusters were grouped at a very low Unifrac distance (< 0.05) indicating that although clustered apart, the soil samples presented similar structure.

2.4.4 Co-occurrence of OTUs among soil samples

An important component of this analysis is to identify those bacteria that were responsible for the differences observed between forest and grassland soil samples. To determine the OTUs statistically different between environments an exact chi-square test was performed. On the basis of the test, only eleven OTUs were found to be in higher abundance in the forest samples but in lower abundance in the grassland samples (Table 3). Among those, *Nitrospira*, a nitrite-oxidizing bacterium, was seven times more abundant in the forest than in the grassland. On the other hand, twelve bacterial taxa were found to be in statistically significant higher abundance in the grassland samples but in lower abundance in the forest

samples. Among those taxa *Bacillus*, *GP1* and *Rhodospirillales* presented the greatest difference relative to the forest (7.2, 3.4 and 3 fold difference, respectively). As we applied a rigid criterion to find the closest bacterial relatives, some sequences were identified at Domain level only with the confidence level used. Those unclassified Bacteria also presented different abundances between the environments. Seven unclassified OTUs presented higher abundances in the native forest while two unclassified OTUs presented higher abundance in the grassland. Among those sequences that could not be classified to known taxa, the OTUs 5084, 6116 and 4424 presented the greatest difference between forest and grassland.

The analysis of the OTUs that were partitioned between samples showed that most of the taxa were shared between forest and grassland (69%). However, 16.4% of the taxa were found only in the forest soil and 14.6% only in the grassland. The exclusive OTUs from each environment are shown in Table S1. The abundance of genus was analyzed statistically to provide support for the analysis of shared OTUs. The OTUs found exclusively in the forest samples belonged to the genus *Sphingobium*, *Methylotenera* and *Pedobacter* and to the phyla *WS3*. The OTUs found exclusively in the grassland samples belonged to the genus *Dechloromonas*, *Zoogloea* and *Geobacter*.

2.5 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 [13, 33]. 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 might directly affect the functioning of ecosystems, like biogeochemical cycles [8].

With this work we tested differences of diversity, composition and/or relative abundance of bacterial taxa form bulk soil samples collected in two environments: pristine forest and eight years old grassland resulted from the deforestation of a small area inside the forest. The area chosen for sampling was ideal for testing the effect of removing plant cover under soil bacterial communities since it presented low human activity, no inputs of fertilizers and a very low animal influence. As our samples were taken in one single period of time (during the spring) it is important to mention that the results obtained represent a picture of the microbial community status and variations in plant growth cycles and clime are not considered in our study. Our approach was based in pyrosequencing of the 16S rRNA genes amplified from microbial DNA extracted directly from four soil samples from each environment. This approach is considered to present high levels of robustness and resolution [41, 42, 49]. It should be mentioned however that, other factors, not assessed in this study, like the biases at the steps of DNA extraction, PCR amplification, primers choice and pyrosequencing, might present some degree of interfere in the results obtained. Sequencing errors could artificially inflate diversity estimates. In our study this problem was circumvented by using proper statistic analysis and following the suggestions of Kunin et al. [35] applying stringent quality-based trimming and clustering thresholds no greater than 97% identity. In this regard these biases are unlikely to have missed so many taxa that our main findings could be incorrect. The selection of primers is still in debate among researchers. No primer is truly "universal" and the choice of reference database and taxonomy can have a dramatic impact on the resulting classification accuracy [56]. The most widely used PCR primer sets spans hypervariable regions V1-V3 however *in silico* testing reveled that primers designed for amplification of this region underestimate the richness because they neglect Candidate Divisions [60]. On the other hand, *in silico* predictions may not reflect the real performance of the primers. The V3-V4 region, for example, presented high-simulated

accuracy and good classification consistency but the set of primers designed to amplify this region proved to produce bias caused by amplification artifacts arising from the combination of these two specific V3-forward and V4-reverse primers [14]. Although we are aware that our primer choice excludes some phyla during amplification, mainly Verrucomicrobia [6], and that the barcoded primers used might be a source of bias [7] we opted for amplify the DNA with the 27F and 338R primers and perform the sequencing through the reverse end because this set of primers do not generate PCR artifacts and the sequences produced by this primers set generally provides relatively good cluster recovery even for short ($\leq$ 250 bases) pyrosequencing reads [41]. Thus PCR primers rarely amplify all bacterial members of a community and any PCR-based approach is likely to miss some bacterial groups or at least under estimate the abundance of some bacterial taxa. Although microbial surveys are always limited by those practical problems, when the data analysis is conducted in a consistent manner it is possible to obtain robust comparison across samples [5, 26]. According to Liu et al. [41], community comparison methods such as UniFrac provide robust results irrespective of the 16S region sequenced.

To detect relevant bacterial patterns within our samples, the datasets of 16 rRNA sequences were analyzed using phylogenetic and taxon-based approaches. The methods based on phylogeny are useful to explore similarities and differences based on a phylogenetic tree [27] while OTU-based approaches need a rigid OTU definition based on a cutoff distance. Since there are no accepted dissimilarity cutoffs for the different microbial taxonomic levels we used the clustering threshold proposed by Kunin et al. [35] of 3% dissimilarity. According to the authors, diversity estimates is grossly overestimated when clustering threshold are higher than 97% identity. Therefore, phylogeneticaly parental sequences can be grouped differently than those based on OTU identification. In this regard we applied two different metrics to calculate bacterial diversity among samples: Shannon diversity index (*H*') and

Phylogenetic diversity index (PD). Shannon's index is an OTU-based analysis and measures the average degree of uncertainty in predicting to what species an individual chosen at random from a collection of *S* species and *N* individuals will belong. The value increases as the number of species increases and as the distribution of individuals among the species becomes even [43]. The Phylogenetic Diversity is defined and calculated as the sum the branch-lengths of the minimal subtree connecting the taxa in the subset [17]. This evaluation is based on a single phylogenetic tree and is sensitive to the quality of the branch length and topology. Another problem associated with measures of microbial diversity through diversity indexes is related with uneven sequence sampling. Diversity index values increase with sample size making normalization of the number of sequences in all samples crucial. Within this work the calculations of both diversity indexes mentioned above were performed with sub-samples of 12,393 sequences. This reduced the bias associated to the sample size and allowed for a better comparison between the samples. Using OTU-based and phylogenetic-based indexes we did not detect any change in bacterial diversity after eight years of afforestation.

The interaction among plants, soil and microorganisms is the driver of the ecosystem functions and any modification of this relationship might affect the microbial structure, which, in turn, will influence the ecological processes [54]. The most significant finding of this work was the discovery that after eight years of afforestation, the soil bacterial community did not suffer a great differentiation or at least, the community responded to disturbance and then leveled off to its initial state. In our experiment the disturbance was constant but relatively recent (eight years). Before removing the plant cover, we expected to find the same soil microbial diversity and structure. According to Miki et al. [45], a change in the composition of plant community leads to a change in the litter quality altering in turn the nutrient cycling process and soil conditions. Due to differences in vegetation composition, a

clear discrimination between the microbial diversity and community structure from the forest and the grassland soils would be expected [46]. However, we found a large overlap (69% of shared OTUs) within both microbial communities leading to no clear discrimination between them. Analysis of shared OTUs would be reasonable only when the sequencing coverage were enough to detect most of the OTUs present (90% or more) since the power for detecting overlapping species from multiple environments is strongly related to the sequencing intensity [38]. To circumvent the problem associated with detection of overlapping taxa, we first calculated how well each sample was representative of the bacterial community. The data summarized at the genus level provided reasonable coverage (greater than 99%) therefore the analysis of bacterial genus that were either unique or shared by specific soil samples was sensitive enough to detect the changes in the number of sequences as well as the presence/absence of taxonomic units.

Recently, Fierer and Lennon [20] revised the generation and maintenance of diversity in microbial communities. According to authors, one factor that influences richness is microbial dormancy. Dormancy "refers to an organism's ability to enter a reversible state of low metabolic activity when faced with unfavorable environmental conditions" [39] and it may work as a microbial seed bank that help maintain the high levels of microbial biodiversity that are observed in nearly all ecosystems [32]. In fact, only a fraction of bacteria recovered from environmental samples appear to be metabolically active. Evaluating the ecological and evolutionary implications of dormancy, Lennon and Jones [39] found that the proportion of inactive bacterial cells from soils ranged from 61 to 96% (six articles were analyzed). As our approach is not sensitive to microbial activity the metabolic status of our samples is unclear. However following the concept of seed bank proposed by Lennon and Jones [39] the similarity of our soil samples may reflect a reservoir of biodiversity can potentially be resuscitated in the future under different environmental conditions.

In agricultural systems with low vegetative diversity and high inputs of xenobiotics, overall species diversity may be reduced to a bottleneck, from which species diversification is possible, albeit from a limited number of phyla [49]. Agricultural practices like tillage, application of pesticides and nutrients, traffic of machinery used for the establishment and management of exotic forests and annual crops modify the physical and the chemical properties of soil and consequently alter the soil microbial communities and ecological functions [9, 42]. Hossain and Sugiyama [29] studying 32 sites under different land uses found that microbial community structures were more similar between forests and grasslands (two natural undisturbed environments) than between agricultural soils. They suggested that soils that are exposed to frequent human disturbances might present modification or reduction of the soil microbial community.

According to Martiny et al. [44] the present-day community structure may have been driven by historical events (e.g. prevalence of any type of vegetation, weather conditions) that might influence present-day community structure. Our results suggest the prevalence of a resilient core microbial community that did not suffer any change related to land use, soil type or edaphic conditions. Although the soil bacterial community did not suffer great alteration after removing the natural forest, we were able to detect shifts related to specific bacterial groups. Eleven OTUs were found in statistically significant higher abundance in the forest samples but in lower abundance in the grassland samples. The *Nitrospira* genus, for example, was found in statistically greater numbers in the forest than in the grassland. On the other hand, twelve bacterial taxa were found in statistically significant higher abundance in the grassland samples but in lower abundance in the forest samples. This observation might be indicative that the soil bacterial communities under the influence of environmental change will gradually being replaced by another community composed of different species that survive better with the new conditions. The bacterial community structure might change with

time and without any significant changes in soil properties, the plant cover will be the major driver of bacterial diversity and structure as proposed by Mitchell et al. [46]. Even having a different structure, the new bacterial community may be functionally equivalent to the original one [1].

The results obtained by other researches indicate, that for the soil tested in this work, a greater degree of disturbance would be necessary to cause major shifts in microbial diversity and structure. This disturbance may involve changes in soil features like physical and chemical degradation, nutrient depletion and pollution from over-use of pesticides [10, 23, 36, 51, 54, 57]. Our results suggest the prevalence of a resilient microbial community less influenced by plant cover in which the recent history of land use buffers against the shifts in soil bacterial community.

Acknowledgements

AKAS and ML were supported by the Coordination for the Improvement of Higher Education Personnel (CAPES - Brazil). This work was supported by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS process nº 1012030) and by the National Council for Scientific and Technological Development (CNPq – Brazil process nº 476762/2010-30).

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Figure 1 - Relative abundance of phylum for each soil library. (A) relative abundance greater than 1%. (B) relative abundance smaller than 1%.

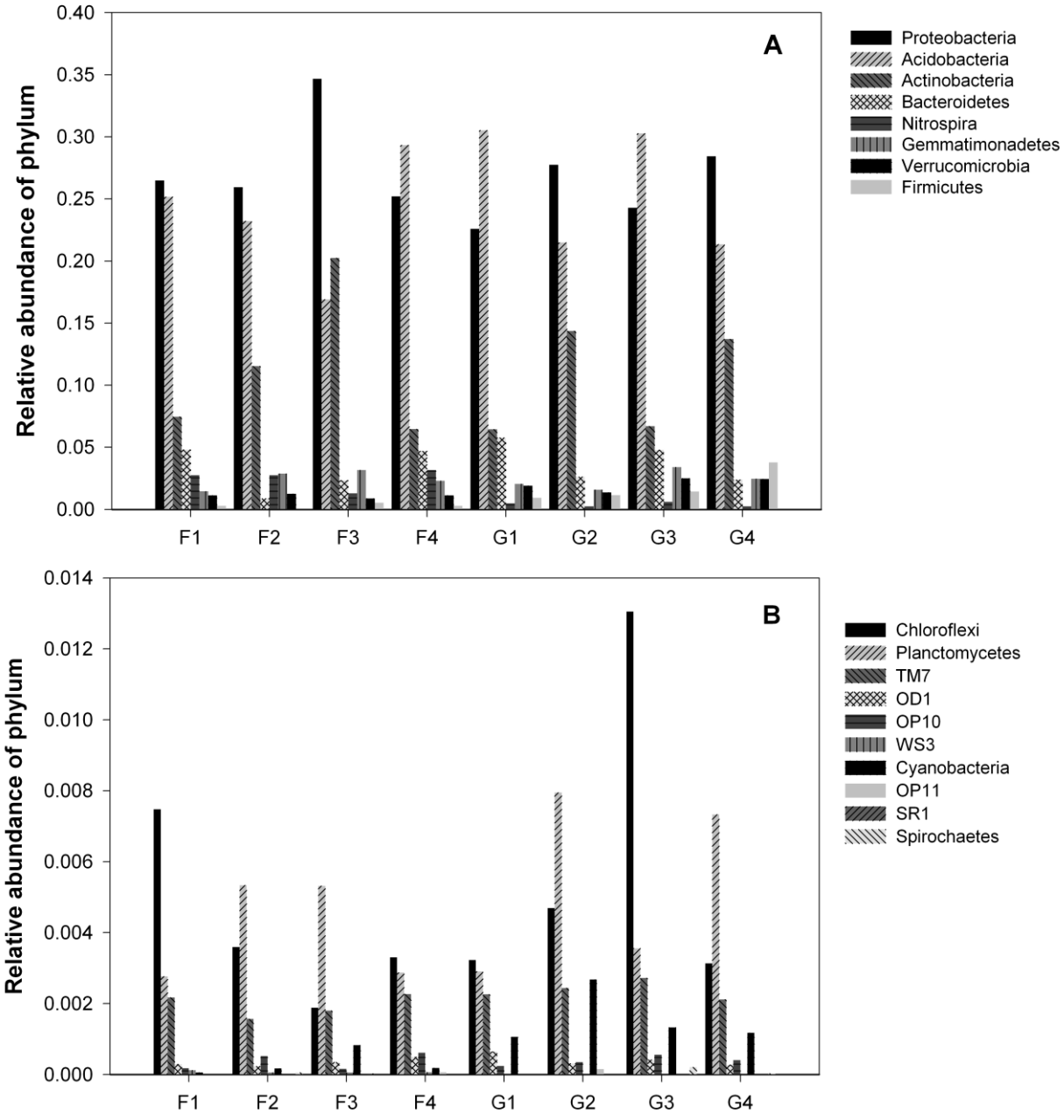

Figure 2 - Jackknifed PCoA plots with (A) unweighted UniFrac distance metric, which accounts for presence/absence of taxa and (B) weighted UniFrac distance metric, which accounts for changes in the relative abundance of taxa. The clusters were generated using a subset of 600 sequences from each environment for 100 replicate trials. The positions of the points are the average for the jackknife replicates and are displayed with a network around the points representing the statistical dispersion in each axis. Dark grey – Grassland; Light grey – Natural Forest.

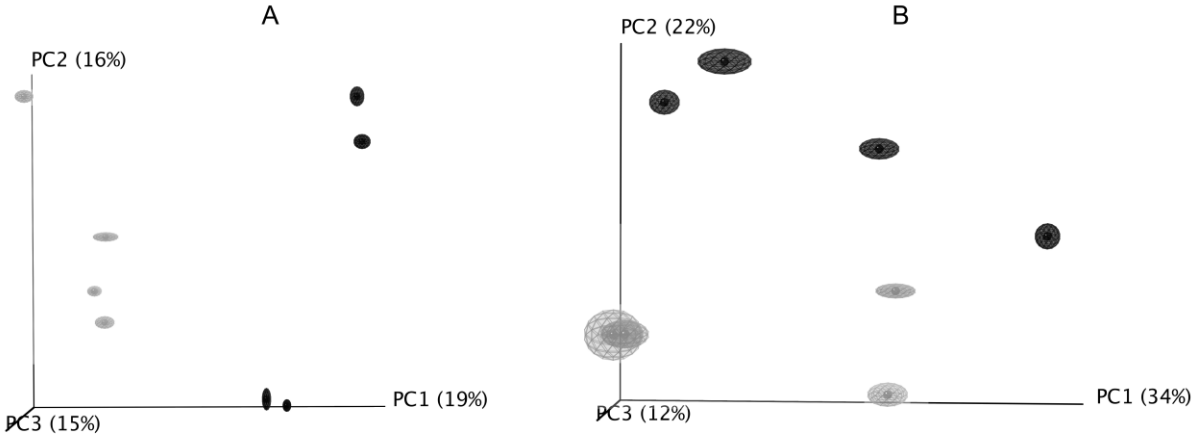

Figure 3 - Hierarchical cluster tree constructed on the basis of the distance matrix calculated by unweighted UniFrac algorithm. Numbers at branch points indicates the percentage of 1,000 bootstrap re-samplings.

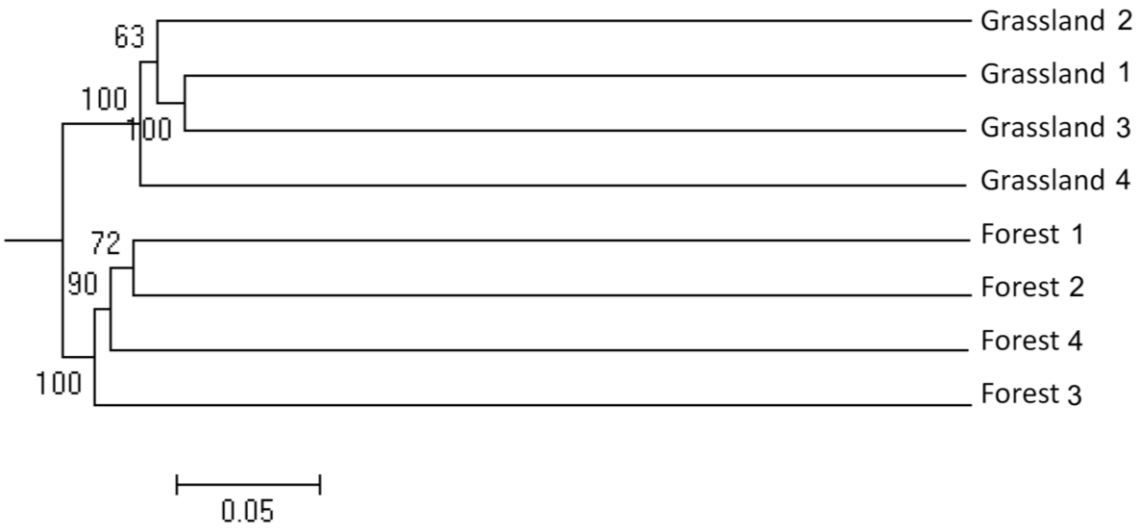

Table 1. Location, altitude and soil chemical analysis of native forest and grassland from Brazilian Pampa Biome.

	Native Forest	**Grassland**
Coordinates	30º 24' 09.3" S 53º 52' 59.1" W	30º 24' 08.9" S 50º 53'05.9" W
Altitude (m)	616	616
pH	5.8	5.6
Ca+Mg (cmolc kg^{-1})	39.0	23.2
Al (cmolc kg^{-1})	0.50	0.13
Na (cmolc kg^{-1})	0.014	0.014
K (cmolc kg^{-1})	0.6	1.0
P (cmolc kg^{-1})	39	12
Total N (%)	0.76	0.40
NH_4^+ (mg kg^{-1})	180	120
NO_3^-+NO_2^- (mg kg^{-1})	102	30.8
Total Organic Carbon (%)	7.3	3.3
Dissolved Organic Carbon (g kg^{-1})	0.24	0.21
Humin (g kg^{-1})	60.3	24.4
Humic acid (g kg^{-1})	7.3	5.5
Fulvic acid (g kg^{-1})	4.9	4.1

Table 2. Total number of sequences, calculations of good's coverage and diversity indexes.

	Forest				Grassland			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Total n° of sequences	16,337	16,994	37,225	17,240	12,393	14,328	25,797	29,732
Sequence coverage (%)								
Phylum	99.98	99.99	99.99	99.98	100	100	100	100
Order	99.96	99.96	99.99	99.97	99.97	99.99	99.99	99.98
Class	99.93	99.95	99.98	99.95	99.97	99.97	99.98	99.97
Family	99.85	99.91	99.96	99.91	99.90	99.87	99.96	99.95
Genus	99.67	99.76	99.85	99.77	99.69	99.69	99.86	99.87
3% dissimilarity cutoff	85.36	83.05	90.95	85.55	84.77	82.39	89.75	90.50
Diversity indexes								
***Phylogenetic diversity**	154.5	172.3	145.4	148.0	141.8	165.0	143.8	144.2
Shannon	10.3	10.6	10.0	10.6	10.2	10.5	10.4	10.2

*All samples were normalized to 12,393 sequences prior to diversity indexes calculations. The average phylogenetic diversity for the forest samples was 155.0 and for the grassland samples was 148.7. The average Shannon diversity index for the forest samples was 10.4 and for the grassland samples was 10.3. The means did not differ statistically between the forest samples and the grassland samples by the Tukey test at 5% probability error.

Table 3**.** List of the closest bacterial relative of Operational Taxonomic Unities (OTUs) whose abundances differ statistically ($p \leq 0.01$; FDR ≤ 0.01) between forest and grassland soils.

***Classifiable OTUs with greater abundance in the native forest**	**% of all Forest sequences**	**% of all Grassland sequences**	**Fold Difference**
Acidobacteria; Acidobacteria; Gp6; Gp6	15.26	11.26	1.4
Acidobacteria; Acidobacteria; Gp22; Gp22	0.61	0.28	2.2
Actinobacteria; Actinobacteria; Acidimicrobiales	0.59	0.21	2.8
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae	5.11	2.88	1.8
Actinobacteria; Actinobacteria; Actinomycetales; Mycobacteriaceae; Mycobacterium	0.71	0.41	1.8
Actinobacteria; Actinobacteria; Actinomycetales; Propionibacteriaceae; Microlunatus	1.24	0.82	1.5
Nitrospira; Nitrospira; Nitrospirales; Nitrospiraceae; Nitrospira	3.91	0.57	6.9
Proteobacteria	5.02	3.57	1.4
Proteobacteria; Alphaproteobacteria	5.44	3.42	1.6
Proteobacteria; Alphaproteobacteria; Rhizobiales	14.57	10.70	1.4
Proteobacteria; Deltaproteobacteria	4.36	3.37	1.3
Classifiable OTUs with greater abundance in the Grassland			
Acidobacteria; Acidobacteria; Gp1; Gp1	1.73	5.83	3.4
Acidobacteria; Acidobacteria; Gp4; Gp4	8.48	10.36	1.2
Actinobacteria; Actinobacteria; Actinomycetales	4.56	5.91	1.3
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Chitinophagaceae; Terrimonas	1.14	1.87	1.6
Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae	0.51	0.78	1.5
Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus	0.38	2.74	7.2
Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae	0.26	0.42	1.6
Proteobacteria; Alphaproteobacteria; Rhodospirillales	0.15	0.45	3.0
Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae	0.41	0.69	1.7
Proteobacteria; Betaproteobacteria	2.63	3.76	1.4
Verrucomicrobia; Spartobacteria; Spartobacteria; genera; incertae sedis	0.58	1.04	1.8
Verrucomicrobia; Subdivision3; Subdivision3; genera; incertae sedis	0.62	1.36	2.2
Unclassified Bacteria			
OTUs with greater abundance in the native forest			
Bacteria 1137	0.081	0.006	1.3
Bacteria 3541	0.020	0.002	1.0
Bacteria 4424	2.058	0.056	3.6
Bacteria 5084	0.141	0.002	7.0
Bacteria 5735	0.061	0.004	1.5
Bacteria 5785	0.121	0.010	1.2
Bacteria 6116	0.101	0.002	5.0
OTUs with greater abundance in the Grassland			
Bacteria 2294	0.726	0.107	1.5
Bacteria 2768	0.020	0.008	4.0

* Each OTU were classified at the highest taxonomic level with 80% taxonomy confidence and an e-value of 0.001. The unclassified Bacteria correspond to an OTU that did not match any of the sequences in the database according to the criteria mentioned above.

Table S1. List of the closest bacterial relative of Operational Taxonomic Unities (OTUs) exclusively found in native forest or grassland.

Native Forest	Grassland
Actinobacteria, Actinobacteria, Actinomycetales; Segniliparaceae; Segniliparus	Firmicutes; Clostridia
Bacteroidetes; Bacteroidia; Bacteroidales	Proteobacteria; Gammaproteobacteria; Xanthomonadales
Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae	Firmicutes; Bacilli; Bacillales; Bacillaceae; Lysinibacillus
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Thermomonas	Proteobacteria; Deltaproteobacteria; Bdellovibrionales; Bacteriovoracaceae; Bacteriovorax
Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Hylemonella	Actinobacteria; Actinobacteria; Actinomycetales; Promicromonosporaceae; Xylanimonas
SR1; SR1_genera_incertae_sedis	Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Azohydromonas
Actinobacteria; Actinobacteria; Actinomycetales; Nocardiaceae; Nocardia	Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae; Anaerolinea
Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; Pirellula	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Azospirillum
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas	Actinobacteria; Actinobacteria; Actinomycetales; Streptosporangiaceae; Sphaerisporangium
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Sinobacteraceae; Nevskia	Proteobacteria; Alphaproteobacteria; Rhizobiales; Methylobacteriaceae; Methylobacterium
Actinobacteria; Actinobacteria; Actinomycetales; Bogoriellaceae; Georgenia	Proteobacteria; Alphaproteobacteria; Rhizobiales; Bradyrhizobiaceae; Balneimonas
Firmicutes; Clostridia; Clostridiales; Ruminococcaceae	Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Pantoea
Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Cellvibrio	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Magnetospirillum
Actinobacteria; Actinobacteria; Actinomycetales; Cellulomonadaceae	Proteobacteria; Deltaproteobacteria; Desulfuromonadales
Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae	Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadacea
Proteobacteria; Deltaproteobacteria; Myxococcales; Phaselicystidaceae; Phaselicystis	Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae
Proteobacteria; Betaproteobacteria; Methylophilales; Methylophilaceae; Methylophilus	Proteobacteria; Deltaproteobacteria; Desulfobacterales; Desulfobulbaceae; Desulfobulbus
Actinobacteria; Actinobacteria; Actinomycetales; Promicromonosporaceae; Promicromonospora	Actinobacteria; Actinobacteria; Actinomycetales; Actinosynnemataceae; Lechevalieria
Actinobacteria; Actinobacteria; Actinomycetales; Nocardiaceae; Williamsia	Actinobacteria; Actinobacteria; Actinomycetales; Geodermatophilaceae; Modestobacter
Proteobacteria; Deltaproteobacteria; Myxococcales;	Actinobacteria; Actinobacteria; Actinomycetales;

Nannocystaceae; Nannocystis	Propionibacteriaceae; Friedmanniella
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Cytophagaceae; Dyadobacter	Proteobacteria; Alphaproteobacteria; Rhizobiales; Methylobacteriaceae; Microvirga
Firmicutes; Bacilli	Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingosinicella
Proteobacteria; Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae; Devosia	Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Chitinimonas
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Dokdonella	Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Roseateles
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Dokdonella Verrucomicrobia; Opitutae	Proteobacteria; Deltaproteobacteria; Myxococcales; Myxococcaceae
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae; Rugosimonospora	Actinobacteria; Actinobacteria; Actinomycetales; Geodermatophilaceae; Blastococcus
Actinobacteria; Actinobacteria; Actinomycetales; Nocardiaceae; Smaragdicoccus	Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Zoogloea
Actinobacteria; Actinobacteria; Actinomycetales; Cryptosporangiaceae; Cryptosporangium	Acidobacteria; Acidobacteria_Gp19; Gp19
Actinobacteria; Actinobacteria; Actinomycetales; Streptomycetaceae; Streptacidiphilus	Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; Gemmata
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Cytophagaceae; Emticicia	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Defluviicoccus
Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae; Longilinea	Firmicutes; Bacilli; Bacillales; Paenibacillaceae; Brevibacillus
Proteobacteria; Betaproteobacteria; Burkholderiales; Comamonadaceae; Comamonas	Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Desulfosporosinus
Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Kaistia
Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Paracoccus	Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Pannonibacter
Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Pseudoxanthomonas	Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Serratia
Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae; Hyalangium	Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Luteolibacter
Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae; Aminobacter	Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Roseomonas	Cyanobacteria; Cyanobacteria
Chloroflexi; Chloroflexi	Chloroflexi; Thermomicrobia; Sphaerobacterales; Sphaerobacteraceae; Sphaerobacter
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Pedobacter	Actinobacteria; Actinobacteria; Actinomycetales; Nocardioidaceae; Actinopolymorpha
WS3; WS3_genera_incertae_sedis	Actinobacteria; Actinobacteria; Actinomycetales;

	Nocardiaceae; Rhodococcus
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Cytophagaceae; Cytophaga	Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae; Planosporangium
Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae; Sphingobium	Actinobacteria; Actinobacteria; Actinomycetales; Nakamurellaceae; Nakamurella
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Chitinophagaceae; Filimonas	Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae; Dechloromonas
Acidobacteria; Acidobacteria_Gp9; Gp9	Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae; Geobacter
Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae; Haliangium	Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Haloferula
Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Janthinobacterium	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Skermanella
Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Azorhizobium	Proteobacteria; Alphaproteobacteria; Sphingomonadales; Erythrobacteraceae
Bacteroidetes; Sphingobacteria; Sphingobacteriales; Flammeovirgaceae	Firmicutes; Bacilli; Bacillales; Bacillales_incertae_sedis; Solibacillus
Actinobacteria; Actinobacteria; Actinomycetales; Micromonosporaceae; Catellatospora	
Acidobacteria; Holophagae; Holophagales; Holophagaceae	
Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Chryseobacterium	
Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae; Stigmatella	
Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Telmatospirillum	
Proteobacteria; Betaproteobacteria; Methylophilales; Methylophilaceae; Methylotenera	

3 CONSIDERAÇÕES FINAIS

A partir dos resultados obtidos conclui-se que as modificações nas propriedades químicas assim como a mudança na cobertura vegetal do solo causaram poucas alterações na estrutura das comunidades microbianas. Assim, mesmo com a observação de grupos bacterianos distintos para ambos os ambientes, a retirada da floresta não causou uma grande diferenciação à comunidade bacteriana do solo já que a maior parte das Unidades Taxonômicas estava compartilhada entre a floresta e a pastagem com apenas algumas mudanças relacionadas a grupos específicos de bactérias.

Como a técnica do pirosequenciamento indica apenas a presença ou ausência e a abundância dos microrganismos, não se pode relacionar esses resultados com o papel que os microrganismos estão desempenhando no solo. Assim, embora tenham sido verificadas poucas alterações em alguns grupos de bactérias, não se sabe no que isso pode refletir em termos funcionais do solo. Desse modo, para dar continuidade a esse trabalho, as próximas questões que precisam ser respondidas são: Após quantos anos de implantação da pastagem terá uma grande mudança na diversidade bacteriana do solo? Os grupos bacterianos que sofreram alterações podem provocar mudanças na diversidade funcional do solo?