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CENTRO DE ENERGIA NUCLEAR NA AGRICULTURA

ALEXANDRE PEDRINHO

Effect of land-use change and soil abandonment on microbial communities in Eastern Amazon Rainforest

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"*Man seems to insist on ignoring the lessons available from history*."

Norman Borlaug

ABSTRACT

PEDRINHO, A. **Effect of land-use change and soil abandonment on microbial communities in Eastern Amazon Rainforest**. 2018. 55 p. Dissertação (Mestrado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2018.

Land-use change threatens soil biodiversity in the Amazon Region. Over the last 40 years, the Amazon rainforest has lost a remarkable portion of its original cover. Forest has been converted into pasture through slash-and-burn process causing irreversible loss of plants and animals. However, the impact of land-use change on the soil microbial community and ecosystem functioning is still poorly understood. Here, we hypothesized that land-use change in Amazon region would affect soil physicochemical properties and, consequently, microbial composition and functions. We used DNA shotgun metagenome sequencing approach to assess soil microbial communities of three land-use systems, namely primary forest, pasture, and secondary forest in the Amazon region at the wet and dry seasons. Our data showed that the microbial community was influenced by the alterations in soil properties, with Al, Al saturation, water holding capacity, and pH significantly correlated to overall community structure and most of microbial phyla. Pasture was the most distinct site and presented the highest taxonomic and functional diversity in comparison with forest sites. Taxonomic changes were followed by functional changes in the community, with pasture presenting high abundance of sequences related to the metabolism of carbohydrates and stress response; primary forest soil hosted a high number of sequences related to the nitrogen metabolism; while secondary forest soil included abundant genes related to respiration and sulfur-metabolism. Although taxonomic structures were very distinct between the three sites, we observed a recovery of the functional profile in secondary forest after pasture abandonment. This observation was evidenced by network analysis, where the two forest sites presented similar key microbial groups dominating the core correlations.

Keywords: Tropical rainforest. Microbial ecology. Land-use change. Metagenome. Soil properties.

RESUMO

PEDRINHO, A. **Efeito da mudança do uso da terra e do abandono do solo em comunidades microbianas na Amazônia Oriental.** 2018. 55 p. Dissertação (Mestrado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2018.

As mudanças no uso da terra ameaçam a biodiversidade do solo na região Amazônica. Nos últimos 40 anos, a Floresta Amazônica perdeu grande parte da sua cobertura. Áreas de floresta nativa vêm sendo convertidas em pastagem através do corte e queima da vegetação natural, causando perdas irreversíveis de plantas e animais. No entanto, o impacto da mudança do uso da terra na comunidade microbiana do solo e no funcionamento do ecossistema ainda é pouco compreendido. Neste trabalho, temos como hipótese que a mudança no uso da terra na região Amazônica afeta as propriedades físico-químicas do solo e, consequentemente, a composição e as funções dos microorganismos. Utilizamos a técnica de sequenciamento do DNA metagenômico para avaliar as comunidades microbianas de três usos da terra, floresta primária, pastagem e floresta secundária na região Amazônica nas estações seca e úmida. Nossos dados mostraram que a comunidade microbiana foi influenciada pelas alterações nas propriedades do solo, com saturação por Al, Al, capacidade de retenção de água e pH significativamente correlacionados com a estrutura geral da comunidade e com a maioria dos filos microbianos. A pastagem foi a área mais distinta e apresentou a maior diversidade taxonômica e funcional em comparação as áreas de floresta. Mudanças taxonômicas foram acompanhadas por mudanças funcionais na comunidade, com pastagem apresentando alta abundância de sequências relacionadas ao metabolismo dos carboidratos e resposta ao estresse; solo de floresta primária apresentou um alto número de seqüências relacionadas ao metabolismo de nitrogênio; enquanto o solo da floresta secundária apresentou alta abundância genes relacionados à respiração e ao metabolismo do enxofre. Embora as estruturas taxonômicas fossem muito distintas entre os três locais, observamos uma recuperação do perfil funcional na floresta secundária após o abandono da pastagem. Esta observação foi evidenciada pela análise de network, onde as duas florestais apresentaram grupos de microorganismos semelhantes dominando as principais correlações.

Palavras-chave: Floresta tropical. Ecologia microbiana. Mudança do uso da terra. Metagenoma. Propriedades do solo.

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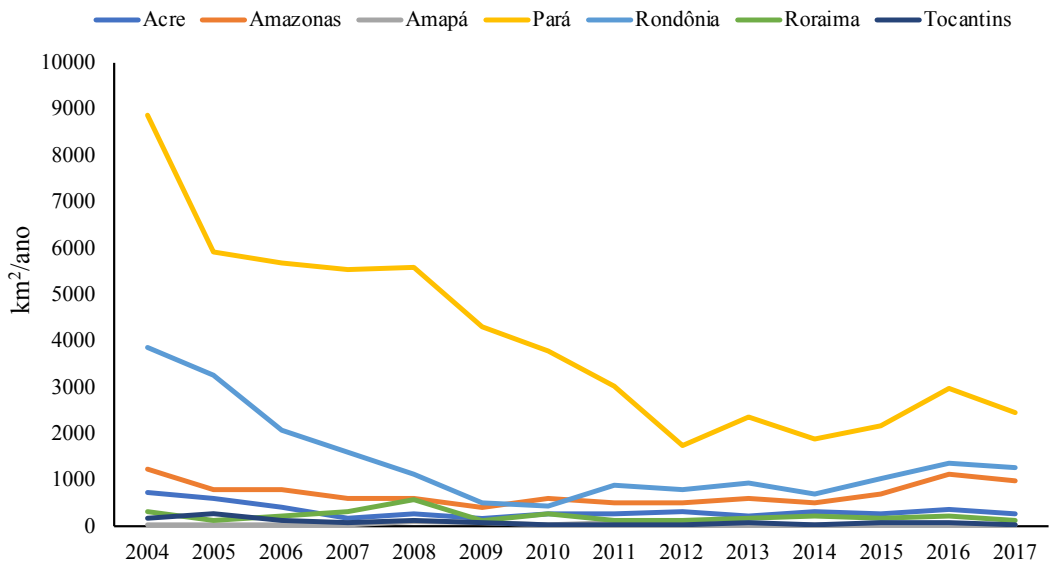
1 INTRODUCTION

The Amazon Rainforest is considered the largest *hotspot* of biodiversity on earth, hosting a great diversity of plants, animals, and microorganisms (LEWINSOHN; PRADO, 2005; NAVARRETE et al., 2015). The Amazon Rainforest also performs a great variety of ecosystem services, including the carbon sequestration, maintenance of freshwater supplies, control of temperature and precipitation, and the stabilization of biogeochemical processes (DAVIDSON et al., 2012; PAULA et al., 2014). However, the rainforest has been under constant threat of destruction by anthropogenic activities (PAULA et al., 2014).

During the 1960s, a governmental policy stimulated land use and occupation in the Amazon region. Cheap land and subsidized credit have brought large numbers of migrants to the region (VIERA; TOLEDO; HIGUCHI, 2018). National surveys showed that the population of the Amazon region increased fivefold between 1960 and 2010, reaching 25 million, and it is likely that in 2018 it will reach more than 27 million people (IBGE, 2018). As consequence, Amazon Rainforest lost a remarkable portion of its original cover. Data from Satellite Monitoring Project of the Brazilian Amazon Rainforest (PRODES) show that in the last 14 years the Amazon region lost more than 143,037 km^2 of forest, and Pará state presented the highest rate of deforestation, 56,172 km^2 (Figure 1) (INPE, 2018).

Figure 1 - Annual deforestation rates in Amazon Region (2004-2017)



Fonte: INPE, 2018

According to Mendes et al. (2015a) and Soares-Filho et al. (2009) large part of these deforested areas (560,000 km^2) were transformed into pasture. Studies have shown that in the early 1960s, Pará state represented 1.5% of the national herd, and in 1990 this share reached 4.2%, corresponding to a growth rate of 10.6% per year (IBGE, 2016; SANTOS et al., 2018). This growth rate was much higher than the national rate for the same period, which was 2.6% per year (IBGE, 2016b). However, between 1990 and 2015, the herd growth rate of Pará state decreased to 5.5% per year (IBGE, 2016). This reduction was associated to a greater pressure on environmental regularization (SANTOS et al., 2018) and many problems caused by deforestation.

Forest-to-pasture conversion in Amazon region typically occurs through a process of selective logging of valuable timber, followed by burning of the remaining vegetation, and seeding of grasses (*Urochloa* or *Panicum* genera) to establish pastures for cattle ranching (MEYER et al., 2017; NAVARRETE et al., 2015). In the short-term, this process helps to increase soil pH and nutrient availability in the soils, supporting pasture formation and the initial production of biomass (MELO et al., 2017). However, in the long-term, slashing-and-burning of tropical forests followed by mismanagement of pastures affects negatively soil physical, chemical, and biological properties (BRAZ et al., 2013). These practices cause progressive disturbances in the soil leading to reduction of soil organic matter, which is accompanied by the emission of greenhouse gases to the atmosphere, especially CH_4, CO_2, and N_2O (KNORR et al., 2005). Moreover, these practices also cause significant increase of soil erosion, changes in carbon and nitrogen content, shift in soil moisture and in microbial communities (BRAZ et al., 2013; KURAMAE et al., 2012; MENDES et al., 2015a).

Studies have shown that land-use change causes biodiversity losses of animals (CARDINALE et al., 2012) and plants (FEELEY; SILMAN, 2009) in the tropical region. More recently, with the advance of culture-independent methods (*i.e.* next-generation DNA sequencing), studies have shown that microbial diversity losses also occur in tropical forests converted to pastures (JESUS et al., 2009; PAULA et al., 2014; RODRIGUES et al., 2013; TAKETANI; TSAI, 2010). These microbiological studies have shown that the conversion of Amazon rainforest affects the microbial communities by altering their structure and composition in the soils. As an example of that, Jesus et al. (2009) and Rodrigues et al. (2013) have shown that bacterial communities from pasture soils were significantly different from those of forest soils. They observed that after forest-to-pasture conversation the phyla Acidobacteria, Nitrospirae, and Gemmatimonadetes decreased. Contrary to that, the phyla

Firmicutes and Chloroflexi increased in response to conversion. More recently, researchers have linked these alterations in microbial communities to the changes in soil physicochemical properties, which are caused by the forest-to-pasture conversion (MENDES et al., 2015a).

The effects of land-use change in the ecological processes remain unknown. There is still a lack of information regarding the effects of land-use change on the microbial diversity and its consequence on ecosystem functioning. So far only few studies have shown that land-use can alter both, the diversity and functionality of soil microorganisms, resulting in loss of species and a reduction of microbial functions (MENDES et al., 2015b; NAVARRETE et al., 2015). In many cases, it is difficult to assess functionality of soil microorganisms due to functional redundancy. This is because different microbial species may have the same function in the ecosystem, thus the loss one or more species does not necessarily alter ecosystem functioning (PHILIPPOT et al., 2013). In this manner, it becomes important not only focuses on the taxonomical composition but also on functional traits and the relationship between microbial communities and external drivers, such as environmental factors (*i.e.,* soil physicochemical properties) (LANGENHEDER et al., 2010; MENDES et al., 2015b).

Several molecular methods can be used to explore the taxonomic and functional profile of soil microbial communities (LANGILLE et al., 2013). Among them, shotgun metagenomics can reveal the taxonomic and the functional potential of the microbiota through the analysis of the DNA from environmental samples (CARVALHAIS et al., 2012); and it is a crucial technique to compare different environments and treatments (BLAGODATSKAYA; KUZYAKOV, 2013). Considering the importance of microbial ecology studies in tropical soils and the necessity of gathering more information about microbial community in Amazon region (MENDES et al., 2015a), this dissertation was elaborated to provide a better understanding not only about the diversity, but also about microbial functional traits. Thus, we sought to understand the response of microbial taxonomic and functional groups to the effect of land-use change in Amazon soils.

2 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

We hypothesize that land-use change will affect both taxonomic and functional structures of microbial communities. Moreover, we expect that land-use change will alter the abundance of specific taxonomic and functional groups, as well as their correlations with soil physicalchemical properties. Lastly, we predict that microbial communities will show some resilience and return to pre-disturbance community structure and functionality when pasture is abandoned, allowing the recovery of the secondary forest.

2.2 Objetives

2.2.1. General objective

The main objective of this dissertation was to evaluate the effects of land-use change in Amazon region on the soil microbial community structure and taxonomic and functional composition.

2.2.2 Specific objectives

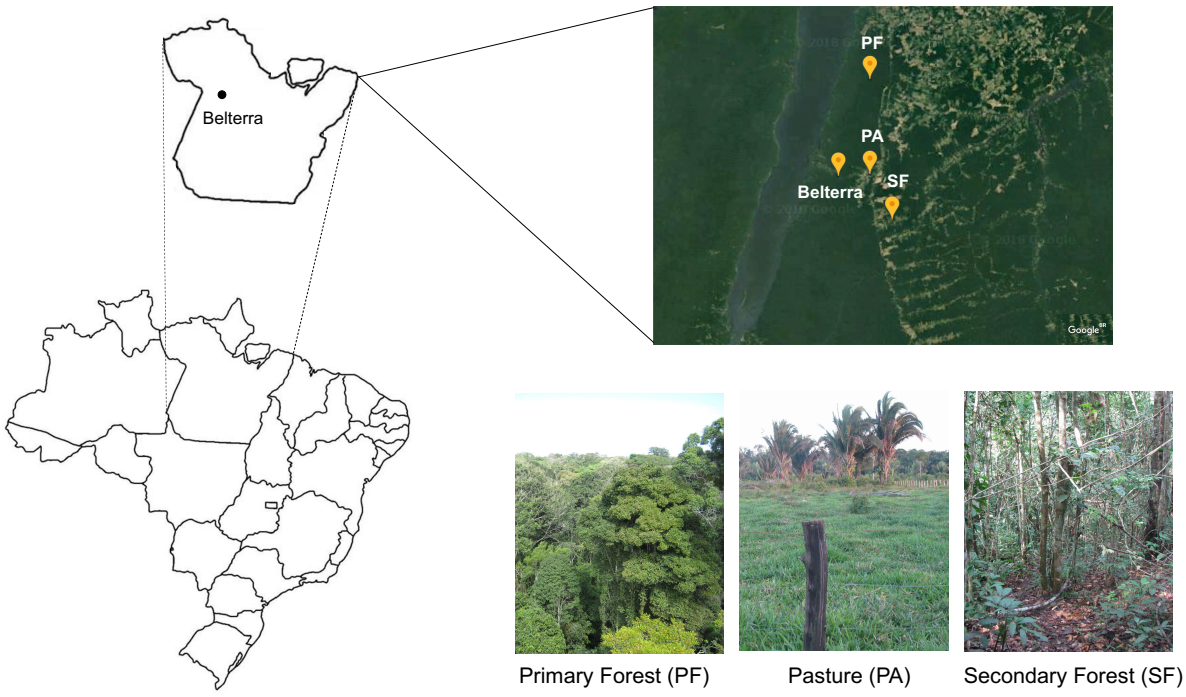
- Assess how taxonomic and functional diversities of microbial communities are altered by land-use change.
- Assess what are the dominant taxonomic groups in each land-use system.
- Assess what are the core functions predominant in each land-use system.
- Assess what are the main soil physicochemical properties that correlate with microbial groups.

3 MATERIAL AND METHODS

3.1 Site description and soil collection

Soil samples were collected at the Tapajós National Forest, a protected environment with more than 6000 km^2 (Figueiredo et al., 2018), and its adjacent areas in the Belterra municipality, in the State of Pará, Brazil (Figure 2).

Figure 2 - Sampling sites at Tapajós National Forest and its adjacent areas in the Belterra municipality, in the State Pará, Brazil. Yellow dots indicate the sampling points. PF = Primary Forest, PA = Pasture, SF = Secondary Forest



The sampling areas were selected according to land-use, as follows: Primary Forest (PF - 2°51'23.9"S, 54°57'28.4"W), a well-preserved primary forest with no signs of logging or previous fire regimes; Pasture (PA - 3°07'52.9"S, 54°57'28.1"W), an area covered with the grass *Urochloa brizantha* and being used for cattle production; and Secondary Forest (SF - 3°15'47.9"S, 54°53'36.0"W), an area previously deforested for logging and cattle ranching with 13-15 years of abandonment and subsequent natural re-colonization by forest plants.

The site selection criteria were based on previous exploratory visits and the history of land-use and management acquired through interviews with farmers and regional experts. Pasture and Secondary Forest areas were originally deforested more than 20 years ago. Pasture conversion occurred through a process of selective logging of valuable timber, followed by slash-and-burn deforestation of the remaining vegetation, and finally mechanical seeding of non-native, fast growing grass *Urochloa* sp. (former *Brachiaria*). Pastures may be burned periodically to control the invasion of weeds. Secondary forest formation occurred as consequence of pasture abandonment after becoming non-productive.

The climate of the region is classified as Am (Köppen classification), tropical monsoon, with an average air temperature of 26 °C and mean annual precipitation of 2150 mm. The soil type is classified as Oxisol (Typic Haplustox) (SOIL SURVEY STAFF, 2014). Soil samples were collected in May and November 2016, comprising the wet and dry seasons, respectively. The wet season is characterized by high humidity and monsoonal rains, while dry season is known by warm and dry sunny days. A total of 24 soil samples were collected (3 sites × 2 sampling periods × 4 replicates). At each location, a 200 m transect with four equally spaced sampling points (50 m apart) was established. First, the litter layer was removed, and then, soil samples were collected from 0 to 10 cm depth. These included (1) an undisturbed soil core for determining soil physical properties, (2) 50 g of loose soil, handled with sterile techniques for molecular analysis, and (3) 500 g loose soil for chemical analysis. Soil samples were transported to the Cell and Molecular Biology Laboratory at Center for Nuclear Energy in Agriculture (CENA/USP, Piracicaba, Brazil) on ice. Samples for molecular analysis was stored at - 80 °C, while samples for physicochemical analysis were stored at 4°C.

3.2 Soil physicochemical analysis

Soil chemical properties were determined for each sample based on 500 g of soil, whereas soil physical properties were determined for each sample based on undisturbed soil core. Analyses were performed at the Laboratory of Soil Analysis at ‘Luiz de Queiroz’ College of Agriculture (ESALQ/USP, Piracicaba, Brazil). Soil pH was measured in a 1:2.5 soil/water suspension. Exchangeable Al, Ca and Mg were extracted with 1 M KCl. Calcium and Mg were determined by atomic absorption spectrometry and Al by acid–base titration. Phosphorus and K were extracted by ion-exchange resin. Phosphorus was determined by colorimetry, and K by flame photometry. Potential acidity (H+Al) was estimated by an equation based on the pH determined in Shoemaker-McLean-Pratt buffer

solution. Total C, H, N and S were extracted and determined by the combustion catalytic oxidation method in a total organic carbon analyzer. Some of the results allowed the calculation of other parameters such as exchangeable bases (EB), the sum of Ca, Mg and K; cation exchange capacity (CEC), the sum of Ca, Mg, K, Al and H; base saturation (V%), the percentage relation between exchangeable bases and cation exchange capacity; and Al saturation (m%), the percentage relation between exchangeable Al and cation exchange capacity. Soil texture was determined using a Bouyoucos densimeter, after shaking the soil vigorously with 1 M NaOH as dispersant. The gravimetric moisture was obtained as a percentage, through the difference between the weight of the sample at the moment of sampling and its dry weight after 48 h in an incubator at 105°C. Soil density was measured by Kopecky's ring method. Total porosity was calculated through the saturation method. Microporosity was obtained by the tension table method. Macroporosity was calculated by difference, deducting the microporosity from the total porosity.

3.3 Soil DNA extraction and sequencing of shotgun metagenomic libraries

Total DNA extraction from 250 mg of soil sample was carried out using the DNeasy Power Lyzer Power Soil DNA Isolation Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA quality and concentration were measured using NanoDrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, EUA) and 1% sodium boric acid (Brody and Kern, 2004) agarose gel electrophoresis. In total, 24 DNA samples libraries (3 sites × 2 sampling periods × 4 replicates per site) were prepared using the Hiseq Reagent Kit v4 (Illumina, San Diego, CA, USA), according to the manufacturer's protocol for shotgun metagenomic sequencing in a Illumina HiSeq 2500 platform (2 x 100 bp paired-end).

3.4 Annotation of metagenomic data and analysis

Raw sequences were sorted based on assigned barcodes, followed by filtering to discard sequences with low-quality bases (quality score lower than 20) under default parameters in Hiseq software (Illumina). The remaining paired-end sequences were initially assembled (R1 and R2) using PEAR (ZHANG et al., 2014) and the remaining not merged reads R1 were included within the output. Sequences below 50 nucleotides length and under Q20 were removed. Pair-ended DNA sequences were annotated with Metagenomics Rapid Annotation Server (MG-RAST) pipeline version 4 (MEYER et al., 2008). The taxonomic and

functional profiles were assessed using Refseq (O'LEARY et al., 2016) and SEED (AZIZ et al., 2008) databases, respectively (default parameters, maximum *E*-value cutoff of $E < 1 \times 10^{-5}$; minimal identity cutoff of 60%; and minimum alignment length of 15 bp). Once the data matrices were generated, reads annotated as eukaryotes and viruses were manually removed from the table. For each metagenome library, the data was standardized using the proportion of reads of each taxon compared to the total. The shotgun metagenome data are available at MG-RAST under the project 'NSF-Dimensions: Amazon Biodiversity' (mgp83361).

3.5 Statistical analysis

All statistical analyses were performed comparing the land-uses and seasonal effects. Data were presented together when the effect of season was negligible. The comparison of soil physiochemical parameters was performed using R (R CORE TEAM, 2013). Data were checked for normal distribution (Shapiro-Wilks test) and homogeneity of variances (Levene's test) before data analysis. One-way ANOVA was used to determine the significance of the differences between sampling areas. The comparison of sites was based on Tukey *post hoc tests* for pairwise comparisons ($P < 0.05$). The normalized matrices from taxa (Refseq database) and functional subsystems (SEED database) generated from MG-RAST were used for downstream analyses. In order to identify the main environmental drivers of microbial taxonomic assembly and functional potential profile, we performed distance-based redundancy analysis (RDA) of Bray–Curtis dissimilarity matrices. Forward selection (FS) and the Monte Carlo permutation tests were applied with 1000 random permutations to verify the significance of soil physicochemical properties upon a microbial community. Additionally, we used permutational multivariate analysis of variance (PERMANOVA; ANDERSON, 2001) to test whether sample categories harbored significantly different community structures. RDA plots were generated using the software Canoco 4.5 (Biometrics, Wageningen, The Netherlands) and PERMANOVA using the PAST software, v.3.0 (HAMMER, 2001). Richness and Shannon's alpha-diversity diversity were calculated based on the taxonomic richness matrix at the genus level and the functional potential matrix at subsystem Level 3, using PAST software v.3.0.

To determine statistical differences among soil samples, the statistical analysis of metagenomic profiles (STAMP) software package was used (PARKS; BEIKO, 2010). A table of frequency of hits of taxa and functional profiles for each metagenome was generated from MG-RAST and used as input. P-values were calculated using the two-sided Welch's t test (WELCH, 1947), and correction was made using the Benjamini-Hochberg false discovery rate (BENJAMINI; HOCHBERG, 1995). Spearman's rank correlation coefficients were calculated to explore the relationship between relative abundance of microbial groups and physicochemical properties according to the different soil sites using the 'multtest' package in R (R CORE TEAM, 2013) and the correction was made using Benjamini-Hochberg false discovery rate.

In addition, network analyses were performed to assess the complexity of the interactions among microbial taxa in each land-use. Non-random co-occurrence analyses were carried out using the Python module 'SparCC' (FRIEDMAN; ALM, 2012). For this, a table of frequency of hits affiliated at genus level was used for analysis. For each network, it was calculated SparCC correlations between microbial taxa and selected only strong (SparCC > 0.9 or < -0.9) and highly significant ($P < 0.01$). The nodes in the reconstructed network represent taxa at genus level, whereas the edges represent significantly positive or negative correlations between nodes. The network graphs were based on a set of measures, as number of nodes, number of edges, modularity, number of communities, average node connectivity, average path length, diameter and cumulative degree distribution. Networks visualization and properties measurements were calculated with the interactive platform Gephi.

4 RESULTS

4.1 Soil physicochemical characteristics

Land-use change resulted in several alterations in soil physicochemical properties (Table 1 and 2). In general, primary forest soil was characterized by higher N, aluminum (Al), potential acidity (H+Al), cation exchange capacity (CEC), macroporosity, total porosity, water holding capacity (WHC), and clay content ($P < 0.05$). The change from primary forest to pasture caused a significant increase in soil pH, base saturation (V%), exchangeable bases (EB), calcium (Ca), magnesium (Mg), and soil density ($P < 0.05$). No differences in the total C, C/N ratio, phosphorus (P), organic matter (OM) content, and microporosity were observed across the land-uses in both sampling periods ($P > 0.05$). After the abandonment of pasture and the recovery to a secondary forested landscape, soil pH, V%, EB, Ca, and Mg become similar to primary forest levels. When comparisons were performed between sampling periods, H+Al, and Al concentration were higher for all land-uses in wet season ($P < 0.05$).

Table 1 - Soil chemical characteristics from 0- to 10 cm topsoil layer in the primary forest, pasture, and secondary forest during the wet and dry season in Belterra - PA, Brazil

Chemical Properties	Primary Forest	Pasture	Secondary Forest	Primary Forest	Pasture	Secondary Forest
	---------- Wet ----------			---------- Dry ----------		
C-total (g Kg^{-1})	27.68 ±4.67 a	22.67 ±1.88 a	23.14 ±3.00 a	27.32 ±6.95 A	21.52 ±0.76 A	21.63 ±4.39 A
N-total (g Kg^{-1})	2.61 ±0.61 a	1.71 ±0.30 b	1.88 ±0.30 b	2.55 ±0.43 A	1.52 ±0.43 B	1.79 ±0.21 B
C/N	10.56 ±1.28 a	13.27 ±1.89 a	12.28 ±0.93 a	10.84 ±2.71 A	14.16 ±2.40 A	12.26 ±3.43 A
O.M (g Kg^{-1})	47.66 ±8.05 a	39.01 ±3.24 a	39.88 ±5.16 a	47.11 ±11.96 A	37.6 ±2.07 A	37.21 ±7.56 A
pH	3.60 ±0.20 b	4.66 ±0.16 a	3.86 ±0.18 b	3.32 ±0.19 B	4.56 ±0.35 A	3.72 ±0.19 B
PO_4^{2-} (mg Kg^{-1})	6.00 ±0.71 a	5.81 ±1.48 a	5.43 ±0.55 a	7.41 ±1.67 A	8.01 ±3.16 A	6.83 ±1.30 A
K^+ ($mmol_c$ Kg^{-1})	0.56 ±0.17 a	0.44 ±0.13 ab	0.30 ±0.10 b	0.88 ±0.19 A	1.46 ±1.13 A	0.60 ±0.12 A
Ca^{2+} ($mmol_c$ Kg^{-1})	5.84 ±5.16 b	13.61 ±3.36 a	3.20 ±0.44 b	6.02 ±5.66 B	24.05 ±9.54 A	3.21 ±0.45 B
Mg^{2+} ($mmol_c$ Kg^{-1})	4.24 ± 1.41 b	8.45 ±2.70 a	4.21 ±1.22 b	3.21 ±1.22 B	9.27 ±3.90 A	4.24 ±2.39 B
Al^{3+} ($mmol_c$ Kg^{-1})	21.10± 5.29 a	2.23 ±1.73 c	14.44 ±2.30 b	10.65 ±1.67 A	1.64 ±0.89 B	9.43 ±2.61 A
H+Al ($mmol_c$ Kg^{-1})	137.66 ±32.50 a	42.89 ±9.12 c	83.61 ±15.83 b	94.22 ±24.93 A	30.63 ±6.06 C	66.81 ±6.26 B
EB ($mmol_c$ Kg^{-1})	10.36 ±6.43 b	22.44 ±5.76 a	6.10 ±1.48 b	9.48 ±7.12 B	34.66 ±13.53 A	7.40 ±2.40 B
CEC ($mmol_c$ Kg^{-1})	147.96 ±29.60 a	65.24 ±6.61 b	89.70 ±16.37 b	103.68 ±24.93 A	65.26 ±8.39 B	74.40 ±7.28 B
V (%)	7.41 ±5.94 b	35.10 ±9.27 a	6.82 ±1.79 b	9.22 ±6.30 B	51.81 ±14.41 A	10.02 ±2.91 B
m (%)	67.61 ±17.78 a	9.43 ±9.68 b	70.42 ±6.77 a	54.44 ±15.19 A	5.47 ±4.56 B	56.02 ±13.76 A

Mean values and standard deviations are shown (n = 4). The different lowercase letters indicate significant differences among the areas during the wet season ($P < 0.05$). The different uppercase letters indicate significant differences among the areas during the dry season ($P < 0.05$).

Table 2 - Soil physical characteristics from 0- to 10-cm topsoil layer in the primary forest, pasture, and secondary forest during the wet and dry season in Belterra - PA, Brazil

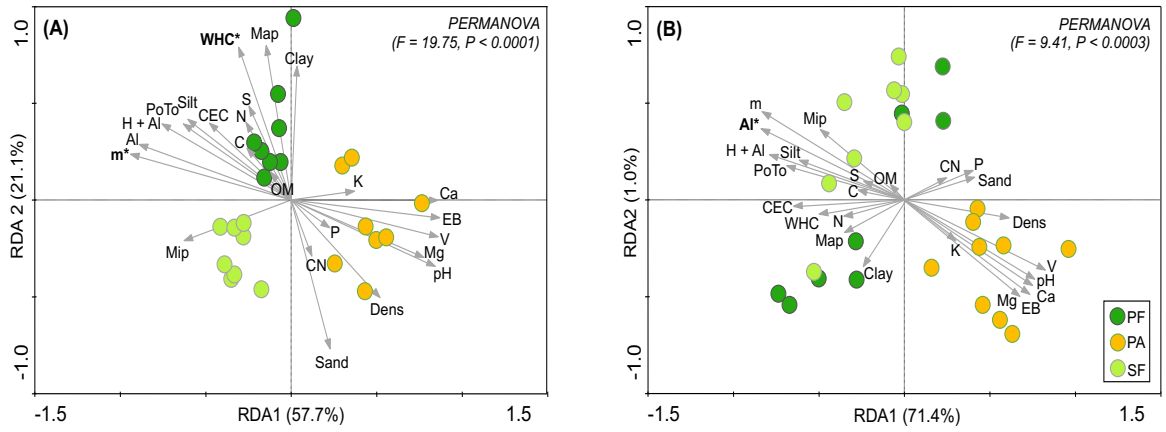
Physical Properties	Primary Forest	Pasture	Secondary Forest	Primary Forest	Pasture	Secondary Forest
	---------- Wet ----------			---------- Dry ----------		
Macropres (cm^{-3} cm^{-3})	0.45 ±0.02 a	0.29 ±0.03 b	0.29 ±0.05 b	0.45 ±0.02 A	0.30 ±0.04 B	0.29 ±0.05 B
Micropores (cm^{-3} cm^{-3})	0.19 ±0.06 a	0.21 ±0.07 a	0.27 ±0.06 a	0.21 ±0.06 A	0.15 ±0.09 A	0.27 ±0.06 A
Total pores (cm^{-3} cm^{-3})	0.64 ±0.05 a	0.50 ±0.04 b	0.56 ±0.04 b	0.67 ±0.03 A	0.45 ±0.05 C	0.56 ±0.04 B
Water Holding Capacity (cm^{-3} cm^{-3})	0.43 ±0.04 a	0.25 ±0.01 b	0.25 ±0.03 b	0.42 ±0.03 A	0.24 ±0.04 B	0.25 ±0.05 B
Soil density (g cm^{-3})	0.81 ±0.11 c	1.33 ±0.11 a	1.09 ±0.01 b	0.80 ±0.09 C	1.34 ±0.12 A	1.10 ±0.10 B
Sand (g Kg^{-1})	36.20 ±10.06 c	462.00 ±34.14 b	529.60 ±50.83 a	29.60 ±7.54 B	461.81 ±34.00 B	541.59 ±10.16 A
Silt (g Kg^{-1})	145.20 ±43.04 a	67.20 ±19.65 b	96.80 ±8.58 b	165.00 ±45.53 A	67.15 ±19.65 B	94.80 ±17.68 B
Clay (g Kg^{-1})	818.60 ±37.23 a	470.80 ±3.36 b	371.60 ±0.44 b	805.40 ±5.66 A	471.04 ±9.54 B	363.61 ±0.45 B

Mean values and standard deviations are shown (n = 4). The different lowercase letters indicate significant differences among the areas during the wet season ($P < 0.05$). The different uppercase letters indicate significant differences among the areas during the dry season ($P < 0.05$).

4.2 Microbial community structure

The redundancy analysis (RDA) was used to evaluate the taxonomic and functional structure of microbial communities and relate it to the soil physicochemical properties of the sites (Figure 3). Regardless the sampling period, samples were clustered according to the land-use system. The first and second axes of the plots explained more than 70% of the data variation. Primary forest, pasture, and secondary forest were markedly different from each other, as confirmed by PERMANOVA for taxonomy ($F = 19.75$; $P < 0.0001$). In contrast, microbial functions in the primary and secondary forest soils were very similar between each other and different from pasture ($F = 9.41$, $P < 0.0003$). According to RDA followed by Monte Carlo analysis, aluminum (Al) ($F = 23.56$, $P = 0.001$), Al saturation (m%) ($F = 23.03$, $P= 0.001$), and water holding capacity (WHC) ($F = 9.86$, $P = 0.001$) showed significant correlation with general community structure.

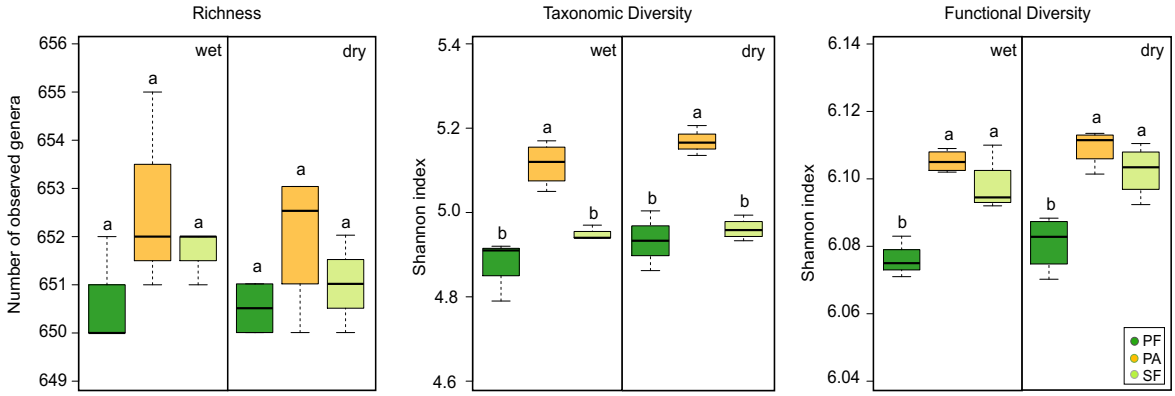
Figure 3 - Redundancy analysis (RDA) of microbial community patterns and soil physicochemical properties from samples of primary forest, pasture, and secondary forest soil. a) Taxonomic analysis using relative abundance based on Refseq database at genera level. b) Functional analysis using relative abundance based on SEED database at subsystem level 3. Arrows indicate correlation between soil physicochemical properties and microbial functional profile. The significance of these correlations was evaluated via the Monte Carlo permutation test and is indicated by asterisk ($P < 0.05$). Analysis of permutation (PERMANOVA) is indicated in the upper right of each graph. *WHC* water holding capacity, *Map* macropores, *Mip* micropores, *PoTo* total pores, *Dens* soil density



4.3 Microbial community diversity and composition

The richness of genera did not differ across land-uses in both sampling periods ($P > 0.05$). On the other hand, the taxonomic and functional diversity changed after land-use change ($P < 0.05$). It was observed higher taxonomic diversity in pasture soils compared to primary and secondary forest soils in both sampling periods (Figure 4). Regarding the functional profile, pasture and secondary forest soils were characterized by the highest value of diversity compared to primary forest soil, which featured the lowest value of functional diversity.

Figure 4 - Diversity measurements of microbial communities in soils from primary forest, pasture, and secondary forest for the two sampling periods: wet and dry. Taxonomic diversity is based on genera level (Refseq database) and functional diversity based on subsystem level 3 (Seed database). Error bars represent the standard deviation of four independent replicates. Different lower-case letters refer to significant differences between treatments based on Tukey's HSD test ($P < 0.05$)



Approximately 342 million of sequences were obtained from 24 soil samples using a shotgun metagenomic approach. A list with the number of sequences generated per sample, as well as the number of hits to each database for each sample is presented in the Table 3. On average, 99.09% of the shotgun metagenome reads were assigned to prokaryotes with the majority assigned to bacteria (98.14%) and a small fraction to archaea (0.95%). The remaining reads were assigned to Eukaryota (0.89%) and to viruses (0.02%) and were removed from the analysis. Interestingly, the proportion of Archaea and Bacteria sequences was altered by land-use, with a decreased proportion of Archaea in secondary forest soil.

The microbial community was composed by 33 phyla with 28 belonging to Bacteria and five to Archaea based on of the Refseq database. Proteobacteria (44.61%) followed by Actinobacteria (31.27%), Acidobacteria (6.59%), Firmicutes (4.71%), Planctomycetes (2.28%), Chloroflexi (2.16%), Cyanobacteria (1.82%), Verrucomicrobia (1.73%), and Bacteroidetes (1.37%) were the most abundant phylum for all samples, and together represented about 96.54% of the microbial community (Figure 5). The same patterns were observed when comparing samples collected in both seasons. When focusing on the most abundant phylum Proteobacteria, the dominant class was Alphaproteobacteria (59.5% of the sequences affiliated to Proteobacteria), followed by Betaproteobacteria (16.3%), Gammaproteobacteria (12.2%), and Deltaproteobacteria (11.3%), with clear differences among the three sites (Figure 6).

Table 3 - Number of sequencing reads, base pairs, GC content, predicted protein, identified protein, and functional categories on MG-RAST pipeline for Primary Forest (PF), Pasture (PA), and Secondary Forest (SF) during the wet and dry season

MG-RAST ID	Area	Season	Number of sequences	Average size (bp)	GC content (%)	Number of sequences	Average size (bp)	GC content (%)	Protein prediction	Identified protein	Functional categories
			Before quality control			After quality control					
4775027.3	FP	wet	10,045,202	108 ± 22	62 ± 9	9,768,244	107 ± 22	62 ± 9	8,243,120	1,687,084	1,290,863
4775025.3	FP	wet	13,419,185	107 ± 21	62 ± 9	13,067,587	106 ± 21	62 ± 9	10,807,476	2,161,858	1,642,508
4775026.3	FP	wet	13,078,589	107 ± 21	63 ± 9	12,728,855	106 ± 21	63 ± 9	10,536,194	2,193,416	1,666,934
4775024.3	FP	wet	12,157,568	107 ± 20	62 ± 9	11,784,611	106 ± 20	62 ± 9	9,837,494	1,940,207	1,475,495
4775293.3	SF	wet	14,536,862	107 ± 21	62 ± 8	14,130,588	106 ± 21	62 ± 8	12,131,496	2,609,605	2,005,574
4775298.3	SF	wet	15,092,767	107 ± 21	62 ± 9	14,702,771	106 ± 21	62 ± 9	12,840,620	2,967,419	2,284,686
4775292.3	SF	wet	14,139,119	106 ± 20	62 ± 8	13,732,282	105 ± 20	61 ± 8	11,296,866	2,253,894	1,714,086
4775300.3	SF	wet	15,318,094	106 ± 20	61 ± 8	14,933,627	106 ± 20	61 ± 8	12,938,749	2,744,833	2,105,323
4775294.3	PA	wet	16,874,804	113 ± 27	66 ± 9	16,444,038	112 ± 27	66 ± 9	14,502,107	3,712,876	2,854,986
4775428.3	PA	wet	12,718,939	107 ± 21	65 ± 9	12,427,948	107 ± 21	65 ± 9	10,867,979	2,565,765	1,963,154
4775405.3	PA	wet	14,607,852	107 ± 21	64 ± 9	14,338,333	106 ± 21	64 ± 9	12,918,199	3,060,708	2,352,603
4775414.3	PA	wet	16,093,495	109 ± 23	65 ± 9	15,786,540	109 ± 24	65 ± 9	14,295,883	3,468,067	2,671,816
4775424.3	FP	dry	15,622,101	108 ± 22	62 ± 9	15,302,218	108 ± 22	62 ± 9	13,246,604	2,864,484	2,213,723
4775430.3	FP	dry	16,253,338	110 ± 25	61 ± 9	15,840,131	110 ± 25	62 ± 9	13,515,653	2,866,049	2,191,857
4775419.3	FP	dry	14,225,828	106 ± 20	62 ± 9	13,874,889	106 ± 20	62 ± 9	11,348,036	2,249,154	1,707,640
4775969.3	FP	dry	16,225,105	106 ± 20	62 ± 9	15,767,589	106 ± 20	62 ± 9	13,572,137	2,837,047	2,010,380
4775407.3	SF	dry	14,292,339	108 ± 22	62 ± 9	13,993,610	108 ± 22	62 ± 9	12,291,049	2,784,356	2,140,031
4775406.3	SF	dry	10,792,277	107 ± 21	62 ± 8	10,557,380	107 ± 21	62 ± 8	9,049,720	2,050,769	1,548,703
4775400.3	SF	dry	13,107,613	109 ± 23	61 ± 8	12,817,213	108 ± 23	61 ± 8	10,554,126	2,158,871	1,638,418
4775410.3	SF	dry	15,374,050	111 ± 26	61 ± 8	15,028,400	111 ± 26	61 ± 8	13,069,562	3,024,548	2,335,386
4775411.3	PA	dry	13,253,481	112 ± 26	65 ± 9	12,907,785	111 ± 26	65 ± 9	10,910,752	2,678,254	2,056,317
4775409.3	PA	dry	16,059,057	113 ± 28	65 ± 9	15,675,558	113 ± 29	65 ± 9	13,799,009	3,491,166	2,698,052
4775402.3	PA	dry	14,462,397	113 ± 28	64 ± 9	14,123,812	113 ± 28	64 ± 9	12,464,959	3,122,565	2,371,977
4775398.3	PA	dry	14,166,512	110 ± 25	64 ± 9	13,857,926	110 ± 25	64 ± 9	12,458,871	3,001,047	2,312,851

Figure 5 - Heat maps showing the differencial abundance of phyla between primary forest, pasture, and secondary forest soil for the two sampling periods: wet and dry. Asterisk indicates the overrepresented phyla/function compared to the other land-use system (P < 0.05 after Benjamini–Hochberg correction). The color key relates the heat map colors to the standard score (z-score), i.e., the deviation from row mean in units of standard deviations above or below the mean

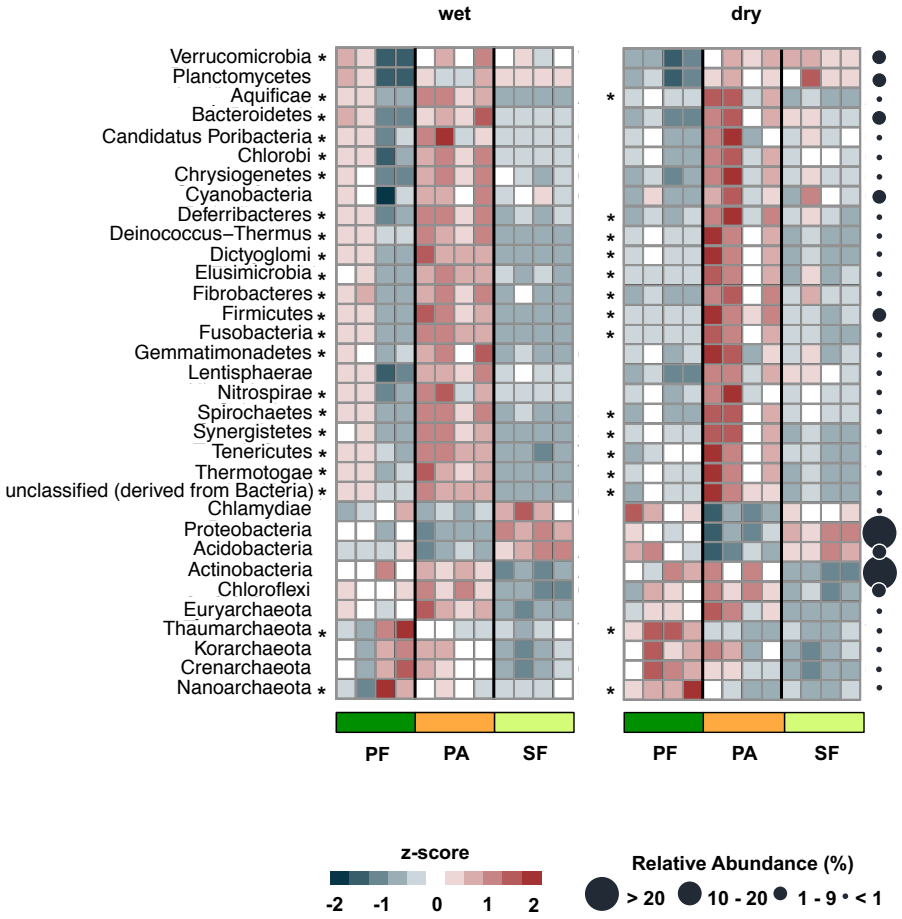
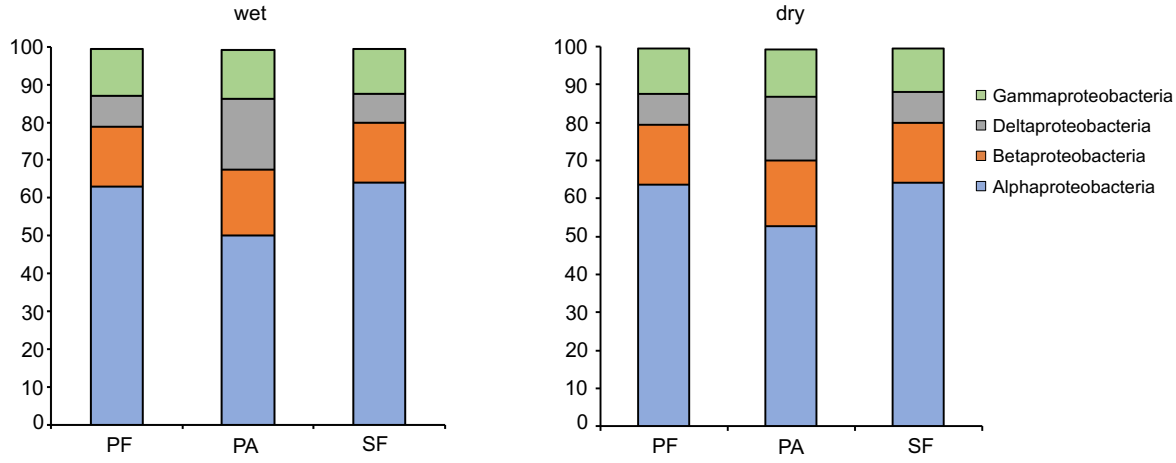


Figure 6 - Distribution of sequences affiliated to Proteobacteria classes for the primary forest, pasture, and secondary forest during the wet and dry seasons

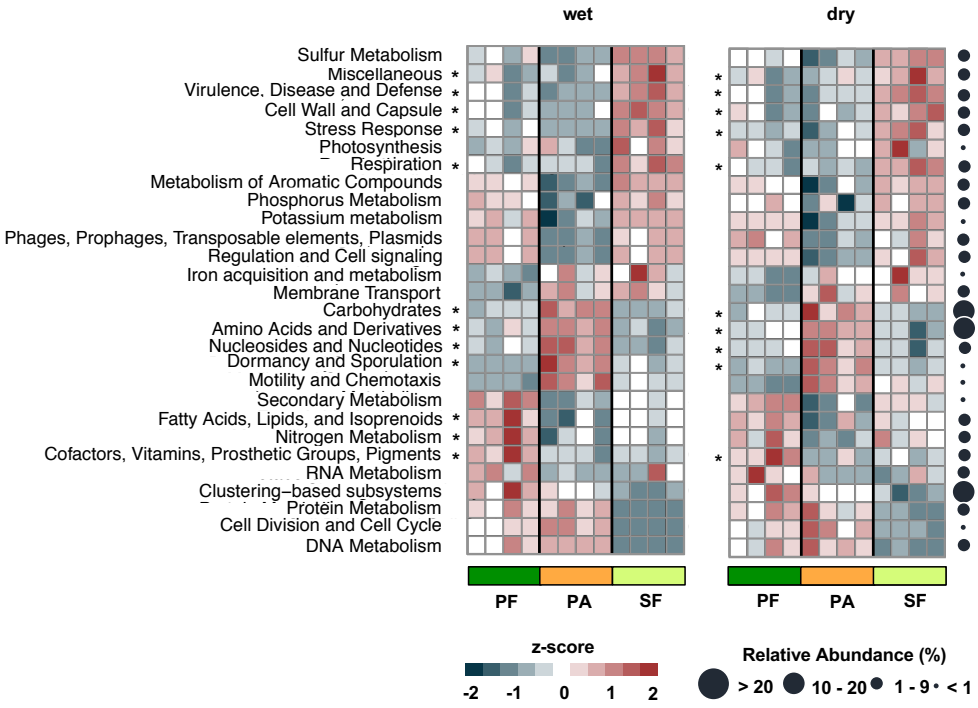


The phylum abundance was very distinct among the land-uses, with primary forest soils hosting higher abundance of Thaumarchaeota and Crenarchaeota, whereas secondary forest site presented high abundance of Proteobacteria. Pasture was the most distinct area, with 22 microbial groups, with higher abundance in comparison to primary and secondary forest, particularly Firmicutes, Chloroflexi, Bacteroidetes, Deinococcus-Thermus, and Chlorobi.

4.4 Microbial functional profile

Soil metagenomes were assigned to 28 different functional subsystems (Figure 7). Several categories were significantly altered after land-use change; however, no seasonal effects were observed. The most prevalent core of functions in primary forest soils was related to nitrogen metabolism, ‘cofactors, vitamins, prosthetic groups, pigments’, secondary metabolism, and clustering-based subsystems. Pasture soils hosted higher abundance of genes related to the metabolism of carbohydrates, amino acids and derivatives, nucleosides and nucleotides, cell division and cell cycle, dormancy and sporulation, and ‘motility and chemotaxis’. On the other hand, the secondary forest ecosystem presented higher abundance of sequences affiliated with respiration, miscellaneous, ‘cell wall and capsule’, ‘virulence, disease and defense’, stress response, photosynthesis, metabolism of aromatic compounds, and sulfur metabolism.

Figure 7 - Heat maps showing the differencial abundance of functional categories (Subsystem Level 1) between primary forest, pasture, and secondary forest soil for the two sampling periods: wet and dry. Asterisk indicates the overrepresented phyla/function compared to the other land-use system (P < 0.05 after Benjamini–Hochberg correction). The color key relates the heat map colors to the standard score (z-score), i.e. the deviation from row mean in units of standard deviations above or below the mean.



4.5 Correlation between bacterial community and soil properties

In order to analyze the correlation between individual microbial phyla and soil physicochemical properties we calculated all possible Spearman's rank correlations (Table 4). The soil factors that correlated with the most microbial phyla were Al (27 phyla in total), followed by base saturation (23), Al saturation (m%) (22), magnesium (22), exchangeable bases (21), H+Al (20), and soil pH (20). The phyla that correlated with the highest number of soil properties were Bacteroidetes and Fibrobacteres (13 factors in total), followed by Chlamydiae (12), Deferribacteres (12), Lentisphaerae (12), Spirochaetes (12), Chlorobi (11), Chrysiogenetes (11), and Fusobacteria (11).

Table 4 - Spearman's rank correlation coefficients and statistical significance between phyla abundance relative to soil physicochemical properties

Phyla	Soil Factors																
	pH	Ca	Mg	Al	H+Al	V%	m%	CEC	N	Map	Mip	PoTo	WHC	Dens	Sand	Silt	Clay
Acidobacteria	-0.64	**-0.80**	**-0.75**	0.59		**-0.76**	**0.75**										
Actinobacteria											-0.60						
Aquificae	0.59	0.65	0.62	-0.65		0.62	-0.65										
Bacteriodetes	**0.83**		**0.77**	**-0.74**	**-0.79**	0.68	-0.67	**-0.69**		**-0.69**		**-0.81**	**-0.74**	**0.79**		**-0.86**	
C. Poribacteria	**0.71**		0.61	-0.65	-0.65							-0.59					
Chlamudiae	-0.68	**-0.71**	-0.67	**0.80**	**0.81**	**-0.81**	**0.78**	**0.74**				**0.74**		**-0.70**		**0.75**	
Chlorobi	**0.77**		0.63	**-0.72**	**-0.75**	0.60		-0.65		-0.61		**-0.73**	-0.66	**0.69**		**-0.71**	
Chloroflexi		**0.74**		-0.62		0.68	-0.68				-0.59						
Chrysiogenetes	**0.85**		**0.74**	-0.65	-0.69	0.63	-0.59					**-0.72**	-0.61	**0.69**		**-0.74**	
Crenarchaeota																	**0.69**
Cyanobacteria	**0.69**			**-0.69**	-0.68							-0.62		0.61		-0.61	
Deferribacteres	**0.77**	0.60	**-0.70**	**-0.79**	**-0.80**	**0.72**	**-0.69**	**-0.70**				**-0.70**		0.68		**-0.75**	
D-Thermus		**0.82**	0.65	**-0.69**		**0.75**	**-0.78**										
Dictyoglomi		**0.76**	0.62	**-0.73**	-0.63	**0.74**	**-0.77**										
Elusimicrobia	**0.69**		**0.73**	**-0.71**	-0.62	0.65	**-0.71**									-0.62	
Euryarchaeota		**0.77**		-0.60		0.65	-0.67										
Fibrobacteres	**0.81**		**0.77**	**-0.77**	**-0.80**	**0.69**	-0.68	**-0.71**	-0.60			**-0.76**	-0.65	**0.74**		**-0.79**	
Firmicutes	**0.74**	**0.75**	**0.71**	**-0.65**	**-0.63**	**0.69**	**-0.66**					-0.56				-0.62	
Fusobacteria	**0.73**	0.67	**0.69**	-0.68	-0.68	0.65	-0.63					-0.64		-0.61		**-0.70**	
Gemmatimonadetes	0.59		0.68	-0.63		0.61	-0.66										
Lentisphaerae	**0.77**		**0.70**	**-0.74**	**-0.82**	0.65	-0.62	**-0.72**		-0.68		**-0.80**	**-0.75**	**0.79**		**-0.82**	
Nanoarchaeota									0.59	**0.69**			0.68		-0.61		0.68
Nitrospirae	**0.72**		0.61	-0.65	-0.64							-0.59				-0.58	

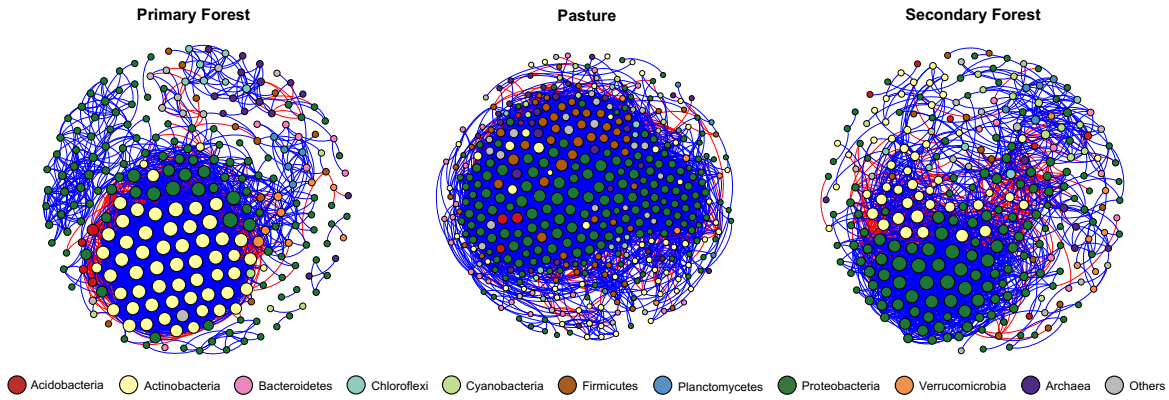
Phyla	Soil Factors																
	pH	Ca	Mg	Al	H+Al	V%	m%	CEC	N	Map	Mip	PoTo	WHC	Dens	Sand	Silt	Clay
Planctomycetes	0.62			-0.59	**-0.69**			-0.59		-0.63		**-0.70**	-0.68	**0.71**		-0.64	
Proteobacteria		**-0.70**	-0.60			-0.63	0.66				0.61						
Spirochaetes	**0.81**	0.61	**0.71**	**-0.75**	**-0.78**	**0.71**	-0.67	-0.66				**-0.74**		**0.72**		**-0.77**	
Synergisteles		**0.75**		-0.68	-0.59	**0.69**	**-0.72**										
Tenericutes		**0.79**	**0.70**	-0.62	-0.60	**0.75**	**-0.73**										
Thaumarchaeota												0.65		**-0.69**		0.68	
Thermotogae		**0.80**	0.59	-0.66		**0.70**	**-0.71**										
Unclassified	0.63	**0.80**	**0.69**	**-0.72**	-0.64	**0.77**	**-0.77**										
Verrucomicrobia	0.63			-0.64	**-0.73**			**-0.72**	**-0.71**	**-0.78**		**-0.69**	**-0.78**	0.68		**-0.74**	

*Only significant levels ($P < 0.05$) for the Spearman's rank correlation are shown. The P-value was corrected by using Benjamini–Hochberg false discovery rate. Bold values indicate significant levels of $P < 0.01$.*WHC* water holding capacity, *Map* macropores, *Mip* micropores, *PoTo* total pores, *Dens* soil density, *C. Poribacteria* Candidate Poribacteria, *D-Thermus* Deinococcus-Thermus.

4.6 Network structure of microbial community

We then used co-occurrence network analysis to investigate the complexity of connections in the microbial community of the different land-use systems (Figure 8). In general, pasture showed the highest number of significant correlations (7025) in comparison to primary (3110) and secondary forests (2136). All land-uses presented a higher number of total positive correlations compared to the negative ones. The network of the primary forest presented larger diameter (13), higher modularity (1.17), average path length (3.88), and average clustering (0.71) in comparison with the other land-use systems (Table 5). On the other hand, the complexity of the network in the pasture was evidently higher than those measured for the primary and secondary forests. This is because pasture network presented higher number of nodes (429) and edges (7025), and average degree (32.75).

Figure 8 - Network co-occurrence analysis of microbial communities of primary forest, pasture, and secondary forest soil samples. A connection stands for SparCC correlation with magnitude > 0.9 (positive correlation–blue edges) or < − 0.9 (negative correlation–red edges) and statistically significant (P < 0.01). Each node represents taxa affiliated at genus level, and the size of node is proportional to the number of connections (that is, degree). Each node was labeled at phylum level



Two phyla, Actinobacteria and Proteobacteria, had a higher number of positive correlations between themselves in the primary forest network. When primary forest was converted to pasture, it was observed a change in the network. The pasture network had a high number of correlations for Proteobacteria, Firmicutes, and Archaea (Euryarchaeota), which were strongly connected to one another. Lastly, after pasture abandonment and the establishment of secondary forest, co-occurring phyla were altered again. The taxa with more connections in the secondary forest network were Proteobacteria followed by Actinobacteria.

Table 5 - Topological characteristics of the networks along the different land-use

Network properties	Primary Forest	Pasture	Secondary Forest
Number of nodes[a]	259	429	275
Number of edges[b]	3110	7025	2136
Positive edges[c]	2117	4996	1616
Negative edges[d]	993	2029	520
Modularity[e]	1.17	1.11	0.82
Number of communities[f]	20	30	26
Network diameter[g]	13	12	11
Average path length[h]	3.88	3.27	3.76
Average degree[i]	24.01	32.75	15.53
Average clustering coefficient[j]	0.71	0.63	0.62

[a]Microbial taxon (at genus level) with at least one significant ($P < 0.01$) and strong (SparCC > 0.9 or < -0.9) correlation;
[b]Number of connections/correlations obtained by SparCC analysis;
[c]SparCC positive correlation (> 0.9 with $P < 0.01$);
[d]SparCC negative correlation (< -0.9 with $P < 0.01$);
[e]The capability of the nodes to form highly connected communities, that is, a structure with high density of between nodes connections (inferred by Gephi);
[f]A community is defined as a group of nodes densely connected internally (Gephi);
[g]The longest distance between nodes in the network, measured in number of edges (Gephi);
[h]Average network distance between all pair of nodes or the average length off all edges in the network (Gephi);
[i]The average number of connections per node in the network, that is, the node connectivity (Gephi);
[j]How nodes are embedded in their neighborhood and the degree to which they tend to cluster together (Gephi).

5 DISCUSSION

The microbial diversity has been recognized as an important factor that affects the functioning of soil ecosystems, and a drastic biodiversity alteration and loss could lead to negative effects on the environment and sustainability (MENDES et al., 2017). Due to their importance in soils, much attention has been given to the effects of land-use change on microbial communities in the last years (NESME et al., 2016). In this study, we investigated the impacts of land-use change in microbial community composition and function in three different areas in the Amazon region. Our results concur with previous studies that showed that land-use change is accompanied by significant alterations in the soil physicochemical properties and the microbial community (JESUS et al., 2009; NAVARRETE et al., 2015; MENDES et al., 2015a; 2015b). Consistent with other studies, we observed that the slash-and-burn of natural vegetation and establishment of pasture leads to an increase of soil pH and the exchangeable cations (Ca, Mg, and K) (MELO et al., 2017; MENDES et al., 2015a). Moreover, forest-to-pasture conversion causes significant changes in soil density, temperature, water content, and nutrients availability (MENDES et al., 2015a; SOUZA et al., 2016). However, when pasture is abandoned, due to low productivity and mismanagement, secondary forest is allowed to form, and soil pH, Ca, and Mg levels becomes similar to those observed in primary forest soil (CENCIANI et al., 2009).

Despite these differences, each land-use presented a distinct microbial community structure. The redundancy analysis based on taxonomical data showed that samples were grouped according to land-use system, with primary forest, pasture and secondary forest being clustered apart from each other. Although the taxonomic data clustered each land-use system apart from each other, the functional data showed that primary and secondary forest samples were clustered together, being separated from the pasture samples. This result indicates that some functional traits might be recovered after pasture abandonment with the secondary forest growth. In a study about the effects of land-use change in the functional diversity, Paula et al. (2014) suggested that secondary forest communities are in an intermediate stage between primary forest and pasture, indicating a progressive re-establishment of ‘forest-like’ functions following pasture abandonment. Our data revealed that the overall microbial community structure was influenced by aluminum (Al), Al saturation (m%), and water holding capacity (WHC). Similar results were observed by Jesus et al. (2009) and Paula et al. (2014) in previous studies performed in the Amazon region. Although several studies have

recognized soil pH as the most important chemical property explaining the variation of microbial communities in soils (FIERER; JACKSON, 2006; LAUBER et al., 2009; MENDES et al., 2015b), we did not find a direct correlation between them. Despite that, we suggest that low soil pH in our studied areas may have an indirect effect in the microbial structure, because pH has a strong influence on abiotic properties, such as nutrient availability and metals solubility in the soils (FIERER; JACKSON, 2006; KEMMITT et al., 2006; MENDES et al., 2017). In this sense, low soil pH may have increased Al content in the soil, strongly influencing microbial community structure. Our results showed that aluminum, m%, and soil pH were the soil properties that presented the correlation with high number of microbial phyla, i.e. 27, 22, and 20 phyla, respectively. This observation is similar to other studies that have shown Al, m%, and soil pH to be generally correlated with the structure and composition of the microbial communities across different land-uses in the Amazon region (JONER et al., 2005; MENDES et al., 2015a). Lastly, the effect of WHC in the microbial community structure can be explained by its direct influence in the water and nutrient availability (UHLÍŘOVÁ et al., 2005). Several studies have shown that microbial composition and activity are sensitive to change in soil water content (MA et al., 2015; ZHAO et al., 2016). In our data, we observed that pasture soils presented lowest WHC values and macropores content, which consequently lead to reduced water availability. In Amazon region, pasture soils are compacted due to cattle trampling and mechanical management (MARTINEZ; ZINCK, 2004).

Overall, our data confirms that forest-to-pasture conversion lead to changes in microbial diversity, with pasture presenting highest taxonomic diversity compared to primary and secondary forests. This same pattern was observed in previous studies that assessed taxonomy diversity after forest-to-pasture conversion in the Amazon region, based on 16S rRNA pyrosequencing (JESUS et al., 2009; RODRIGUES et al., 2013), and taxonomical and functional metagenomic (MENDES et al., 2015b; NAVARRETE et al., 2015). In our study, we observed that Proteobacteria, followed by Actinobacteria, Acidobacteria, and Firmicutes were the top four most abundant phyla in all land-use systems, which were similar to previous studies performed in the Amazon region (KROEGER et al., 2018; MENDES et al., 2015a; 2015b; RODRIGUES et al., 2013). We also observed that different land-uses were characterized by distinct patterns of microbial abundance and functions. Primary forest samples hosted a high abundance of sequences affiliated to Thaumarchaeota and Crenarchaeota phyla. Thaumarchaeota and Crenarchaeota are widely distributed in terrestrial

and marine habitats (HERSHBERGER et al., 1996; STAHL et al., 2012). Both phyla are considered mesophilic and play important roles in the cycling of nutrients such as carbon and nitrogen (KERFAHI et al., 2018). To date, most of their taxa members whose genomes have been analyzed are apparently involved in the ammonia-oxidation (KEMNITZ et al., 2007; KERFAHI et al., 2018). Moreover, according to Isobe et al. (2018) NH_3-oxidizing archaea, rather than heterotrophic nitrifiers and NH_3-oxidizing bacteria, are the main responsible for nitrification in the tropical and subtropical forests. Lastly, we observed a significant reduction in the number os sequences assigned to Thaumarchaeota phylum in pasture soil. According to Subbarao et al. (2009) the type of grasses used in Brazilian cattle ranching (*Urochloa*) have been shown to secrete brachialactone, a biological nitrification inhibitors (BNIs), that inhibits both ammonia-oxidizing archaea and bacteria.

Among our studied areas, we observed that pasture soil had the most distinct microbial composition. In pasture soil, we found an overrepresentation of sequences affiliated to Firmicutes and Chloroflexi. This high abundance of Firmicutes and Chloroflexi can be explained by their preference for environments where nutrients are highly available (RODRIGUES et al., 2013). In this manner, forest-to-pasture conversion through slash-and-burn provided good conditions to their growth. This is because the incorporation of ash into the soil results in a direct input of nutrients and helps to increase soil pH (GIARDINA et al., 2000; NAVARRETE et al., 2015). Supporting this idea, we observed high positive correlation between these phyla with Ca, Mg, exchangeable bases, and soil pH. Moreover, Firmicutes and Chloroflexi members are also known to resist stressing conditions including high temperature variation throughout the day and desiccation (BATTISTUZZI; HEDGES, 2009), which are very common in our pasture areas. Lastly, Firmicutes have been reported as dominant group in gut microbiota of ruminants (JAMI et al., 2014), animals commonly found in pasture, which could contribute to high abundance of this phylum in this soil (MENDES et al., 2015a).

In the same manner, we found an overrepresentation of sequences affiliated to Proteobacteria in secondary forest soil. This phylum is considered one of the largest within prokaryotes and account for the vast majority of the known Gram-negative bacteria (KAZAKOV et al., 2009; ZHANG et al., 2013). Moreover, this group of microorganisms encompass a very complex assemblage of phenotypic and metabolic features including many chemolithotrophs, heterotrophs, and phototrophs (KAZAKOV et al., 2009). Members of this phylum are important to global carbon, nitrogen, and sulfur cycling (MENDES et al., 2015b). In our study, we observed few correlations between Proteobacteria and soil physiochemical

properties. In general, Proteobacteria were positively correlated with m%. Within Proteobacteria, secondary forests presented higher abundance of Alphaproteobacteria class compared to pasture. Alphaproteobacteria comprise most of phototrophic microorganisms, several are capable to metabolize simple carbon compounds (C1-compounds), and also plants symbionts (KHAN et al., 2013). Lastly, Alphaproteobacteria members are involved in the breakdown and recycling of organic compounds (GOODFELLOW; HAYNES, 1984) and play an important role in the sulfur-oxidation (ZHAO et al., 2017).

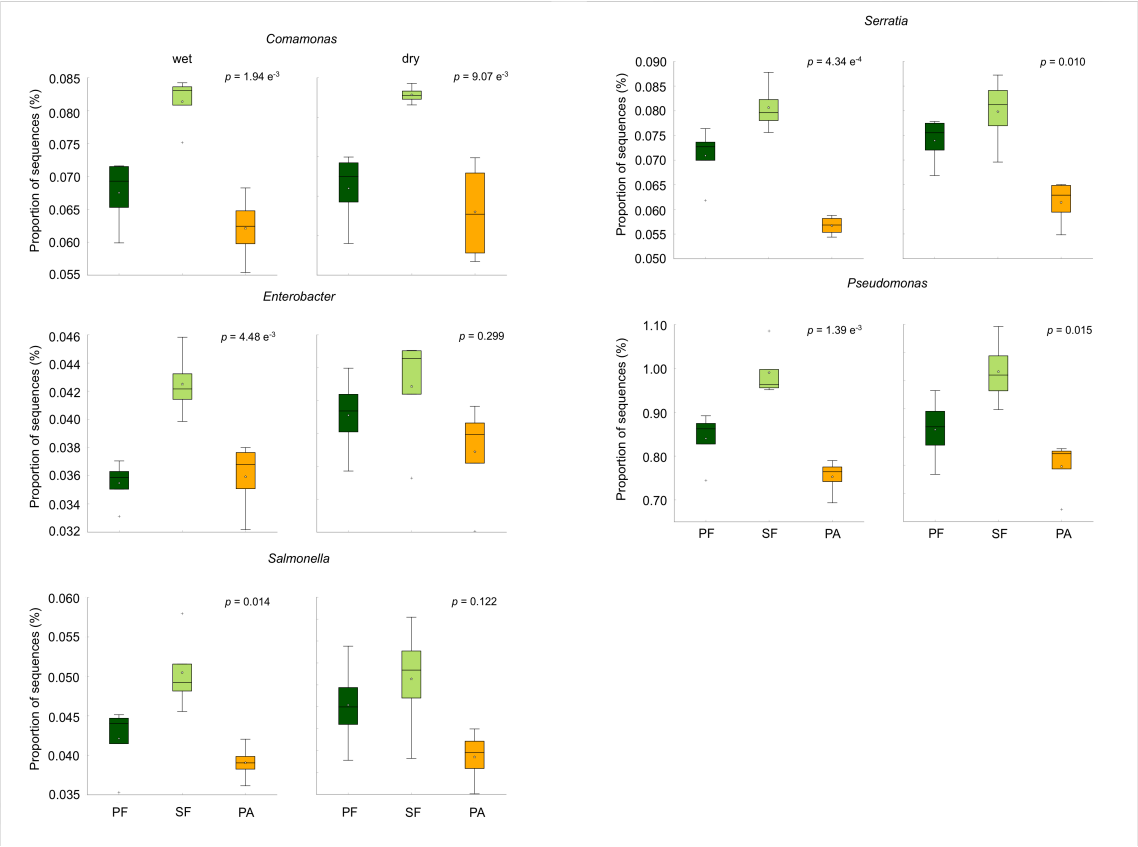
Metagenomic studies allowed us to better understand soil microbial communities, indicating not only the taxonomic groups, but also the potential metabolic functions (SOUZA et al., 2016). Regarding the functional diversity, primary forest presented the lowest values when compared to the other areas. Studies have suggested that in an environment in equilibrium, such as primary forest, the ecosystem functioning is maintained based on low levels of diversity, but high abundance of microorganisms. On the other hand, environments under stress, such as pasture and secondary forest, would present an increased diversity leading to a higher functional diversity and, consequently, the maintenance of essential ecosystem functions (ARAUJO et al., 2018; MENDES et al., 2017). Overall, primary forest presented a dominance of sequences related to nitrogen metabolism. According to Camenzind et al. (2017) tropical forest soils are highly weathered and nutrient impoverished. Its fertility is highly dependent on the cycling of a thin layer of organic matter associated with the large amount of plant litter material (HALL; MATSON, 2003; MACRAE et al., 2013; PAJARES; BOHANNAN, 2016). In this manner, soil microorganisms (e.g. Thaumarchaeota and Crenarchaeota) help to mediate mineralization of organic material and contribute to large part of available nitrogen (CAMENZIND et al., 2017; CLEVELAND et al., 2013).

Pasture samples presented a high abundance of sequences related to metabolism of carbohydrates and stress response (cell division and cell cycle, dormancy and sporulation, and ‘motility and chemotaxis’). This high number of sequences assigned to metabolism of carbohydrates can be explained by previous studies that showed that land-use change followed by pasture establishment leads to great changes in the nutrients availability (FEIGL et al., 1995; KROEGER et al., 2018; RANJAN et al., 2015). Moreover, grasses (*Urochloa* genera) have the potential of adding great amounts of organic carbon into the soil, due to continuous root exudation (CENCIANI et al., 2009; CERRI et al., 2003). Lastly, the high number of sequences assigned to stress response in pasture is resulted of many environmental stresses that soil microorganisms are exposed in this area, including prolonged

sunlight exposure at the soil surface and variation of temperature throughout the day, which also explain the dominance of Firmicutes and Chloroflexi, as stated above (RODRIGUES et al., 2013).

Secondary forest samples presented a dominance of sequences related to respiration and sulfur-metabolism. Soil respiration is an important indicator of soil health because it indicates the level of microbial activity (ASHARDIONO; CASSIM, 2014). The amount of soil respiration is an indicator of nutrients contained in organic matter being converted to forms available to microorganisms and plants, e.g. nitrate-nitrogen as NO_3^- and sulfate as SO_4^{-2} (MAURYA et al., 2018). In this manner, over 95% of the sulfur (S) present in the soil is in the organic form (GAHAN; SCHMALENBERGER, 2014). However, this organic-S is not directly available to plants which rely upon microbial processes to increase its availability (KERTESZ et al., 2007). Many bacteria in soil are capable of mineralizing organic sulfur including *Comamonas, Enterobacter, Salmonella, Serratia,* and *Pseudomonas* (HUMMERJOHANN et al., 2000). Interestingly, all these genera belong to Proteobacteria, the most abundant phylum in our secondary forest samples (Figure 9).

Figure 9 - Boxplot showing the distribution in the proportion of the genera related to sulfur metabolism in samples from (PF) primary forest, (SF) secondary forest and (PA) pasture soil in Amazon region. Boxes indicate the IQR (75th to 25th of the data). The median value is shown as a line within the box and the mean value as a star. Whiskers extend to the most extreme value within 1.5*IQR



We then used network analysis in order to understand the microbial community dynamics and to compare the complexity of networks operating in the primary forest, pasture, and secondary forest. Our data revealed that pasture network was 2- and 3-fold more complex than primary and secondary forest, respectively. The higher complexity and connectivity in pasture area could be a response to many environmental stresses that soil microorganisms are exposed in this area (GOSS-SOUZA et al., 2017). It has been suggested that stress conditions lead to an increase in taxonomy and functional diversity and, consequently, reflect in a more complex ecological network (MENDES et al., 2017). Although pasture was more complex than the other sites, the modularity of the primary forest network was higher than pasture and secondary forest. The modularity is characterized by the presence of different groups of nodes with high numbers of interconnections within (NEWMAN, 2006). A modular network structure presents a more diversity in species roles and functionality, increasing niche overlap (POUDEL et al., 2016). This network property reinforces the effect of land-use change on the

microbial community dynamics, where taxonomic changes could lead to functional shifts in the ecosystem. The pasture area could also be distinguished from primary and secondary forests by the keystone species (depicted as nodes with larges sizes in the network) that dominated the correlations within the community, composed by genera belonging to Proteobacteria, Firmicutes and Archaea. Interestingly, the dominant groups in primary and secondary forest networks belong to Actinobacteria and Proteobacteria, revealing a return to 'forest-like' network dynamic condition after pasture abandonment.

Natural ecosystems around the world have been degraded by anthropic activities and the restoration of such areas is needed to protect the biodiversity (HOBBS; HARRIS, 2001). However, it has been demonstrated that restoration can take decades (EVINER and HAWKES, 2008). Given the genetic and ecological consequences of tropical biodiversity loss (ALLISON; MARTINY, 2008), we asked whether there are signs of resilience in the microbial community in our studied areas. Our study, although limited, indicates that restoration of community composition is under way after 15 years of re-establishment of a secondary forest. Our data indicated that pasture abandonment has led to a more similar structure between the forests sites (i.e. primary and second forests), with the functional profile being more similar than the taxonomy. More specifically, we observed a recovery of 18 out of 33 phyla, and 10 out of 28 functions indicating a progressive re-establishment of 'forest-like' taxon and functions when comparing the two forest sites. Considering that soil microbial communities are tightly interlinked with soil healthy and plants growth, the information obtained in our research could help to boost forest restoration in abandoned Amazon soils.

6 CONCLUSION

In this study we showed that land-use change in Amazon Rainforest soils has a primary effect in the microbial community composition and functioning. Despite the soil type is the same for all sites Oxisol (Typic Haplustox), different land-use systems were clearly distinct in soil physicochemical properties and, consequently, shaped the microbial community structure. Changes in Al, m% and pH, caused mainly by anthropic activities through soil management, were the main parameters that affect most of microbial groups. Taxonomic changes were followed by functional changes in the community; however, we observed that microbial community functionality presented signs of resilience and recovery a way faster than taxonomy after pasture abandonment and re-establishment of secondary forest. On the basis of our results, we suggest that a better understanding of soil microbial communities, its functions and correlations with environmental parameters is a powerful tool for the development of a more sustainable agriculture and better restoration programs.

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