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Amazon basin pasture soils reveal susceptibility to phytopathogens and lower fungal community dissimilarity than forest

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ABSTRACT

The advance of livestock towards the hydrographic basin of Mutum-Paraná River, in the state of Rondônia contributes to the conversion of the amazon forest to pasture. This process can decrease the plant diversity, contributing to loss of endemic microorganisms and natural enemies leading to the replacement of native biota by non-native and generalist species. Thereby, we hypothesized that this conversion changes fungal composition, reduce fungal alpha diversity and community dissimilarity and increase the incidence of potential phytopathogenic genera at pasture soils compared to forest. Soil of 10 sampling points from forest and 10 from pasture were collected at the Mutum-Paraná River basin. Via ITS amplicon sequencing of the total soil DNA, differences in composition and in the taxonomic diversity between the two environments were addressed. The phylum Ascomycota predominated in both forest and pasture. Basidiomycota presented lower percent in both, but was higher in pastures. Zygomycota and Glomeromycota presented opposite tendencies, the first being predominately present in forest and the second only present in pasture with low incidence. The fungal diversity was higher in pasture soils, contrasting our hypothesis. There were also significant correlations between soil physicochemical properties and fungal community. The reduction of the dissimilarity in pasture was confirmed and this signalized for a biotic homogenization. In addition, higher incidence of potential phytopathogenic fungi was observed in pasture. These results contribute to a better understanding of how fungal communities are driven by forest-to-pasture conversion.

1. Introduction

The Amazonian forest regions have been subjected to high rates of land use conversion (Mendes et al., 2015a), since areas of native forest have been transformed into pasture areas and/or agricultural fields. In Rondônia, advancement of livestock on the areas of the Mutum-Paraná River basins has led to the destruction of the Open Ombrophylous Forest (de Aragão et al., 2014). These changes alter the vegetal composition and the soil physicochemical properties, influencing the biodiversity (Lauber et al., 2008) and functioning of terrestrial ecosystems, since forest-to-pasture conversion reduces the diversity and richness of functional genes leading to lower stability of microbial functions after environmental oscillations (Paula et al., 2014).

The increase in bacterial diversity by the conversion of Amazon forest to pasture and agriculture has been reported in several studies (Cenciani et al., 2009; Jesus et al., 2009; Mendes et al., 2015a; Mendes et al., 2015b; Rodrigues et al., 2013). Forest bacterial communities are related to a higher soil acidity and increases in concentration and saturation of aluminum than in pasture (Jesus et al., 2009; Rodrigues et al., 2013; Mendes et al., 2015a).

Fungi are less explored in this context and usually respond less to soil physicochemical characteristics, such as pH, as demonstrated by some studies (Orgiazzi et al., 2013; Rousk et al., 2010). On the other hand, a positive relationship between plant and fungal diversity has already been reported (Carney and Matson, 2006; Peay et al., 2013) and plant community is mentioned as a stronger driver of fungal composition than soil properties (Mueller et al., 2014). Thus, changes in plant composition may play a key role in soil fungal communities (Fracetto

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et al., 2013), which tend to be more sensitive to these changes, as in the case of mycorrhizal associations and decomposing fungi (Lauber et al., 2008).

The reduction of fungal alpha diversity by the conversion of natural to agricultural environments (De Castro et al., 2008) and the greater fungal richness in forest environments have already been observed (Mueller et al., 2016). In addition, altered environments lead to a biotic homogenization of fungi composition between different points, which become less dissimilar from one another (Alele et al., 2014). This process may be the result of the loss of endemic microorganisms from forests and the replacement of native biota by non-native organisms and/or generalist species (Alele et al., 2014; Rodrigues et al., 2013).

Pristine ecosystems harbors equilibrium and population balance due to the presence of natural enemies (Ellison and Evans, 1996) such as soil microorganisms capable to aid in the natural control of diseases and pests (Moreira et al., 2010). Environmental changes caused by anthropic activities can decline soil structure (Bailey and Lazarovits, 2003) and affect microbial composition and functions (Mendes et al., 2015a) possibly leading to the loss of this natural control and to the dominance of certain pathogenic microorganisms.

In this sense, we hypothesized that forest-to-pasture conversion would lead to changes in fungal composition, reduction of alpha diversity and fungal community dissimilarity and to the increase of phytopathogenic species incidence as a result of natural enemies loss. Therefore, this study aimed to verify, how forest-to-pasture conversion at the Mutum-Paraná River basin affects the fungal composition, dissimilarity and diversity.

2. Material and methods

2.1. Soil collection at the Mutum-Paraná River Basin – RO

Two expeditions throughout the Mutum-Paraná River Basin were carried out in September 2014 and September/October 2015. In the first, we collected at four forest and four pastures sites; in the second, at six forest and six pasture sites. The climate of the region is classified as Aw (Koppen's classification) ranging between 1600 and 1900 mm of annual precipitation and average temperature of 20 °C.

The soil sampling methodology was adapted from (Faoro et al., 2010). In each site, 1 m^2 of soil was cleaned to avoid the organic matter in decomposition. Five subsamples were taken from soil between 0 and 20 cm depth, pooled and homogenized in plastic bags forming a composite sample (Fig. A1). Each composite sample was kept in ice until the return to the laboratory. Collection tools were cleaned with water and surface sterilized with 70% alcohol. The composite samples were collected in duplicate, therefore, 20 composite soil samples were obtained from 10 forest sites and 20 composite soil samples from 10 pasture sites.

Each sampling site was georeferenced with GPS (Global Positioning System). The UTM (Universal Transverse Mercator) coordinates were used in the first expedition (Samples F1, F2, P3, F4, P5, P6, P7 and F8) and the geographical coordinates in the second (Samples F9, P10, F11, P12 P13, F14, P15, F16, P17, F18, P19, and F20) (Table A1). The Datum used was the SAD 69 (South American Datum – 69).

2.2. Soil physicochemical analysis

A portion of each soil sample was destined to soil physicochemical components analysis at the Brazilian Agricultural Research Corporation (EMBRAPA RONDÔNIA – CPAFRO) located in Porto Velho, Rondônia – Brazil. The macro and micronutrients Cu, Fe, Mn, Zn, P, K, Ca, Mg and Al, and the potential acidity (H + Al), cation exchange capacity (CEC), aluminum saturation (m), base saturation (V), pH, and organic matter percentage (OM) were analyzed.

2.3. Soil DNA extraction and ITS region sequencing

We extracted the total DNA from samples with 0.5 g of soil from each duplicate using the PowerSoil DNA Isolation Kit (MoBio Labs, USA) and following the manufacturer's recommendations. The DNA was sent to Argonne National Laboratory (USA) to perform a 250 bp paired-end sequencing by the Illumina MiSeq platform (Illumina Inc., US) using modified primers set ITS1F/ITS2 (Internal Transcribed Spacer) targeting a shorter region of the ITS (Smith and Peay, 2014). All the molecular methods such as PCR reaction, program and purification followed (Smith and Peay, 2014).

2.4. ITS amplicon sequencing analysis

The results were analyzed by the Quantitative Insights Into Microbial Ecology Software (QIIME v.1.8.0) (Caporaso et al., 2012), ITSx (Bengtsson-palme et al., 2013), USEARCH (Edgar, 2010) and UCHIME (Edgar et al., 2011) using the pipeline developed by the Brazilian Microbiome Project (Pylro et al., 2014). We used only de forward file (R1.fastq) since reads are variable in length. Quality filtering of the sequences were performed using USEARCH discarding reads with number of expected errors (E_max) higher than 0.5. The singletons were also removed using USEARCH. The ITSx software were used to extract only the ITS1 region from fungi. Thus, residual fragments of small subunit (SSU) and 5.8S ribosomal genes were removed and eventual ITS1 reads from other eukaryotes were not kept for further analysis. Afterwards, we used UCHIME to remove the chimeras. Sequences with similarity greater than 97% were grouped in the same Operational Taxonomy Unit (OTU) and the taxonomy was assigned to OTUs using UNITE (Kõljalg et al., 2013) as the reference database. The data were not rarefied for the subsequent analysis.

We used QIIME v.1.8.0 for calculating rarefaction curves and Good's coverage index. Alpha diversity indexes (such as Chao1, Shannon, equitability and dominance) were calculated at genera level. The online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) allowed verifying the amount of shared and exclusive genera from forest and pasture samples. The statistic analysis were done by the softwares R and Minitab.v.17.1.0. Student's *t*-test evaluated the differences between the averages of the four diversity indexes at 5% significance, considering the 20 pasture samples (10 points in duplicate) and the 20 forest samples as a repetition of each environment. Pearson correlation coefficient checked the correlation between diversity (Shannon index) and physicochemical characteristics of the soil. Since environmental samples are quite variable, a correlation with up to 10% of significance was considered significant.

The incidence percentages of the phyla Ascomycota, Basidiomycota, Glomeromycota and Zygomycota in forest and pasture were calculated considering only sequences classified into some phylum, i.e. identified sequences. In addition, we evaluated differences in the incidence of ascomycetes potentially phytopathogenic previously reported in the state of Rondônia (Fernandes et al., 2007; Ferreira et al., 2003) and *Trichoderma*, widely used for the biocontrol of phytopathogens (Machado and Silva, 2013; Mendoza et al., 2015; Walunj et al., 2015).

2.5. Multivariate and heatmap analysis

First, a Detrended Correspondence Analysis (DCA) evaluated the gradient size of genera distribution. A table containing the number of reads of each genera (species matrix) was used as input data, and a log transformation was performed. The DCA showed a linear distribution of the data (length of gradient < 3, precisely 2.187), thus suggesting a Redundance Analysis (RDA) as the most appropriated mathematical model. Thereby, a RDA was performed using as input the table containing the number of reads of each genera and a table of physicochemical data. Monte Carlo permutation test (1000 permutations) was made to select the environmental variables significantly correlated to