SOIL MOISTURE GOVERNS BACTERIAL AND ARCHAEOAL COMMUNITIES IN SUBTROPICAL GRASSLANDS

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Resumo:
Grasslands in regions with subtropical clime are exposed to a wide range of seasonal variations in temperature and rainfall that considerably influence moisture and temperature of the soil. These factors influence many biological processes mediated by microorganisms such as organic matter decomposition and the cycling of nutrients. The Brazilian Pampa (the area of study) is located in the South Temperate Zone and has subtropical climates with four well-characterized seasons. Grasslands, with sparse shrub and tree formations, are the dominant vegetation. Climate presents a annual range of temperature from 0°C to 35°C and a rainfall around 1,400 mm. In this work, we aimed to better understand how seasonal variations of moisture and temperature affect the microbial communities. A microcosm was designed to mimic natural climate variations. Twenty-seven soil cores were collected from natural grasslands and placed into pots with the same dimensions. The experimental design was completely randomized, with a 3x3 factorial arrangement with three replicates for all treatments. Three different moisture conditions were applied: permanent wilting point (equivalent to 8% moisture), 70% of field capacity (equivalent to 16% moisture) and field capacity (equivalent to 23% moisture), all of them kept constant for the whole treatment. Each treatment was incubated at three different temperatures: 10°C, 20°C and 30°C during 20 days. After incubation, soil samples were collected from microcosms systems and microbial DNA was extracted and sequenced by means of Ion PGM Platform. Data from sequencing was analyzed according the Brazilian Microbiome Project and community analyses were performed using the R environment. Results shown an increase in diversity indexes on treatments under higher moisture (p

Palavras-chave: 16S gene, Microbial Ecology, Next Generation Sequencing, Pampa Biome, Soil Temperature

Modalidade de Participação: Iniciação Científica
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1. INTRODUCTION

Grasslands in regions with subtropical clime are exposed to a wide range of seasonal variations in temperature and rainfall that considerably influence moisture and temperature of the soil (DENG et al., 2012). These conditions strongly affect important processes like plant growth, organic matter decomposition and the cycling of nutrients (KARHU et al., 2014). Microorganisms are the key drivers of these processes and their activity are intimately correlated and modulated by those factors once they influence soil parameters like gaseous diffusion processes and cell viability to biochemical reactions (PREVOST-BOURE et al., 2011).

The Brazilian Pampa (area of this study) is located between latitudes 28°00’ S and 34°00’ S and longitudes 49°30’ W and 58°00’ W, within the South Temperate Zone and has subtropical climates with four well-characterized seasons. Grasslands, with sparse shrub and tree formations, are the dominant vegetation. The annual range of temperature in the sapling site has a minimum of 0ºC and a maximum of 35ºC. The rainfall is well distributed during the year, with an annual rainfall around 1,200-1,600 mm (OVERBECK et al. 2007; ROESCH et al., 2009). Grasslands, with sparse shrub and tree formations, are the dominant vegetation. Because of this natural grasslands, livestock production is one of the main economic activities serving as source of forage for around 18 million animals, mainly cattle and sheep (IBGE 2017).

In this work, we aimed to obtain a better understanding of how seasonal variations of moisture and temperature affect the microbial communities and what is the particular influence of each one individually in the microbial ecology by means of Next Generation Sequencing and bioinformatic analysis.

2. METODOLOGY

An experimental microcosm was designed to mimic the natural climate variations (e.g. moisture and temperature variations) over the year in the Brazilian Pampa biome. Soil used in the experiment was sampled from a homogeneous area of native subtropical grasslands (29° 45’ S, 53° 45’ W) used for cattle grazing, with no input of fertilizers other than animal manure. The soil temperature and moisture applied in the microcosms were based on measurements over the year in the same grassland area where the soil cores were collected.

Twenty-seven cores (blocks of 15 x 20 x 25 cm) were collected carefully with a shovel from the upper soil layer (0-20 cm) in autumn with a minimum distance of 50 cm to ensure the homogeneity of soil characteristics and to avoid spatial autocorrelation of the microbial community. Each core was placed into a pot with the same dimensions and kept at the same sampling air temperature and soil moisture (28°C and 0.20 kg.kg⁻¹ (w/w), respectively) in laboratory.

The experimental design was completely randomized, with a 3x3 factorial arrangement with three replicates for all treatments. Based on the information on the permanent wilting point and field capacity, three levels of soil moisture were applied i.e., 0.08 kg.kg⁻¹ (permanent wilting point, or 8%), 0.16 kg.kg⁻¹ (70% of field capacity, or 16%) and 0.23 kg.kg⁻¹ (field capacity, or 23% w/w) and kept constant during the whole experiment by weighing the pots and by adding sterile distilled water. Within
the three above mentioned soil moistures, the microcosms were incubated at three different temperatures (10 °C, 20 °C and 30 °C) during 20 days.

After incubation, soil samples were collected from the microcosms systems. DNA extraction were carried out with 2g of soil by using the PowerSoil® DNA Isolation Kit (MoBio laboratories, Inc., Carlsbad, CA, USA). Microbial communities were determined by amplification of the V4 region from the 16S rRNA gene using the bacterial/archaeal primer 515F/806R (CAPORASO et al., 2012). Amplicons were further sequenced on the Ion PGM Platform™.

Data from sequencing was analyzed according the Brazilian Microbiome Project (PYLRO et al., 2014). Briefly, an OTU table was built using the UPARSE pipeline (EDGAR, 2013). The reads were truncated at 200 bp and quality filtered using a maximum expected error of 0.5 and further clustered into OTUs a 97% similarity cutoff, being removed the chimeras and unique sequences (singletons).

Community analyses were performed in R environment (R Core Team 2008) using the phylseq package (MCMURDIE & HOLMES, 2013). Alpha and beta diversity were analyzed and dissimilarities were tested using PERMANOVA analysis.

3. RESULTS and DISCUSSION

After quality filtering, a total of 402,614 sequences were obtained. Alpha and beta diversity measurements indicated moisture as the main factor responsible for changes in microbial community. Alpha diversity measures have shown a considerable difference between the three moisture assays (Figure 1; two-way ANOVA, p < 0.05), whereas temperature was not significantly important (Figure 1). Values of 23% of moisture (that means the field capacity) increased the diversity of the communities.

![Figure 1](image.png)

Figure 1. Alpha diversity measures. A) Chao1 diversity index; B) Shannon diversity index. Box plots having the same letter are not significantly different (p-value > 0.05) according to the pairwise Tukey's test between moisture regimes. Temperature regimes were not significantly different.

The ordination carried out by using Bray-Curtis dissimilarity also indicated soil moisture was the main driver for shaping the community structure (Figure 2). A clear group composed by microbial communities from soils under 23% of moisture can be observed. Same figure has shown that temperature do not affect the distribution of
microorganisms in tested soils, what can be seen by the dispersion of the replicates on the plane.

These results may suggest a capacity of adaptation of the bacterial and archaeal communities to the seasonal variations of temperature in subtropical climates. Besides, moisture appears as a variable with not only biochemical but also physical important effects, carrying microbial cells for different places and reducing oxygen levels.

Figure 2. Principal coordinates analysis (PCoA) ordination based on Bray-Curtis dissimilarity of microbial community structure based in rDNA partial sequencing. The variations explained by the first two axes are indicated on the graph. Different forms mean different temperature treatments whereas different colors mean different moisture levels.

Further multivariate analysis of variance confirmed the ordination results (Table 1). Neither temperature nor the interaction between moisture and temperature affected the microbial community. Analysis between moisture levels indicated that the microbial community also was influenced by the different moisture regimes while temperature variation did not affect the structure of the microbial community.
Table 1: Results of PERMANOVA analysis of the Bray Curtis dissimilarities for microbial community structure at a 97% similarity cutoff level for OTU clustering.

<table>
<thead>
<tr>
<th>Environmental Factors</th>
<th>R² *</th>
<th>Adjusted p-values**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>0.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.06</td>
<td>0.431</td>
</tr>
<tr>
<td>Moisture:Temperature</td>
<td>0.12</td>
<td>0.427</td>
</tr>
<tr>
<td>Moisture pairs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23% vs 16%</td>
<td>0.10</td>
<td>0.009</td>
</tr>
<tr>
<td>23% vs 8%</td>
<td>0.25</td>
<td>0.003</td>
</tr>
<tr>
<td>16% vs 8%</td>
<td>0.19</td>
<td>0.003</td>
</tr>
<tr>
<td>Temperature pairs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C vs 20°C</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>10°C vs 30°C</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>20°C vs 30°C</td>
<td>0.05</td>
<td>1</td>
</tr>
</tbody>
</table>

* R-square mean the influence of the tested variable in the communities structure modulation. ** significant p-values are highlighted in bold.

Data obtained suggests moisture is more important than temperature to modulate microbial communities on Pampa Biome. Although temperature has been recognized to be determinant for the composition and physiology of microorganisms in global environments (LIPSON, 2007), the results of this study have shown temperature with less importance at local scales, particularly in subtropical ecosystems where the community might contain a widely adaptive (such as dormancy) capacity to survive for large variations in temperature (JANGID et al. 2011).

4. FINAL CONSIDERATIONS

Based on this result, we can conclude that moisture have a greater importance in the shaping of the microbial community than temperature, especially in the field capacity level, when bacterial cells are able to disseminate themselves across the environment. Besides, temperature has caused no significant difference in the microbial communities.

5. REFERENCES


JANGID, K.; WILLIAMS, M. A.; FRANZLUEBBERS A. J.; SCHMIDT, T. M.; COLEMAN, D. C. et al. Land-use history has a stronger impact on soil microbial


