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Land-use system shapes soil bacterial communities in Southeastern Amazon region

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ABSTRACT

The expansion of the agriculture has become the main agent of disturbance in the Amazon region, and such alteration has consequences on soil microbial communities, which represent the majority of biodiversity in terrestrial ecosystems. In this study we assessed the effects of land-use changes on physicochemical soil properties and, consequently, on the bacterial communities in soils from Southeastern Amazon, Brazil. Soil samples were collected in four distinct land-use systems, *i.e.* native forest, deforested area, agricultural and pasture fields. The soil bacterial community abundance, structure and composition were addressed using qPCR, one molecular marker (T-RFLP) and high-throughput sequencing of the bacterial 16S rRNA gene, respectively. Obtained data were analyzed using multivariate techniques. We found that the type of land-use had a primary effect on the soil bacterial communities, whereas parameters such as pH, C, N, NO_3^- and K content significantly correlated to overall community structures. We observed that the abundance and taxonomic diversity of the bacterial 16S rRNA changed to a higher extent according to the land-use system, but they also showed significant temporal turnover within sites. From the total 27 bacterial phyla identified, 12 presented clearly differential distribution across the four land-use systems. Comparison among all sites revealed Acidobacteria and Chlamydiae to be higher abundant in forest soil, Actinobacteria in deforested site, Nitrospira and Deinococcus-Thermus in agriculture and Firmicutes in pasture. When data of specific phyla were correlated to specific soil properties, we demonstrated that parameters such as Al saturation index, Al, base saturation index, Mg and Ca presented correlation with the most number of bacterial groups detected. Thus, we suggest that several soil parameters besides pH should be taken into account when assessing the impacts of land-use change on the microbial communities.

1. Introduction

The exploration of natural areas for agricultural purposes has promoting large-scale land-use changes around the globe, particularly in tropical areas (Cerri et al., 2007). The Amazon rainforest is the largest reservoir of biodiversity of the world, however the expansion of cattle and agriculture in Brazil has led to deforestation of large areas of primary forest (Dirzo and Raven, 2003; Laurance and Peres, 2006; Soares-Filho et al., 2009). The conversion of natural forests into agricultural fields or pastures are anthropogenic activities commonly found in several landscapes, being considered key environmental impacts that affect the biodiversity and ecological functioning of terrestrial ecosystems (Sala et al., 2000). These changes cause progressive and cumulative soil disturbances that shift intrinsic soil characteristics, by promoting qualitative and quantitative changes in organic matter, carbon and nitrogen, shifting soil moisture, pH, as well as the dwelling microbial communities in this systems (Don et al., 2011; Doran and Zeiss, 2000; Kuramae et al., 2012; Lauber et al., 2008; Lin et al., 2011).

Microorganisms in the soil represent a significant portion of the living organisms, mainly due to their highest abundance, diversity and multiple metabolic activities (Kirk et al., 2004; Ovreas, 2000; Torsvik et al., 1990). It is well known that microbial communities play fundamental roles in biogeochemical processes, being considered key organisms for the metabolic functioning of nutrient cycling, thus having an undoubted role in maintaining the soil

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quality (van Elsas et al., 2006). In this sense, it is essential to understand the interrelations between soil organisms and the local environment through the assessment of microbial community structures and diversity in soils, to promote a better view on how these communities respond to anthropogenic disturbances (Ranjard et al., 2000).

Although there has been an increase number of works related to microbial communities in Amazon soils, still remains necessary gather new information about the effects of land-use change on the soil physicochemical properties and their relationship to specific microbial groups. In this study we examined changes in bacterial communities in soils under different land-use systems in Southeastern Amazonia, Brazil. Considering the growing expansion of agricultural areas in this Amazon region, this work depicts the consequences of land-use change on bacterial communities structure and composition and the correlation of specific bacterial phyla to soil physicochemical properties. Here, we raise data for a better understanding of the outcome of deforestation on microorganisms, and provide information that can be suitable for future development of sustainable approaches for ecosystem uses.

Molecular techniques such as quantitative PCR, T-RFLP, and high-throughput sequencing of the 16S rRNA gene were used to access the bacterial community abundance, structure and composition, respectively. Statistical analyses were conducted to access differences in microbial communities and to correlate the data to local soil physical and chemical properties at four land-use systems, *i.e.* native forest, deforested site, agriculture and pasture. Here, we hypothesize that the soil bacterial communities respond to land-use change, and these responses are related to soil physicochemical alterations caused by different management in the process of land-use conversion. In order to test this hypothesis, we addressed the following questions: (1) how are the bacterial abundances altered across different land-uses? (2) How are the bacterial community structure changed in each land-use system? (3) What are the specific bacterial groups select in each land-use type and what is their relation to the soil physicochemical properties?

2. Materials and methods

2.1. Soil sampling

Soil samples were collected in the Southeastern Brazilian Amazon, in the state of Mato Grosso, Brazil, in the Ipiranga do Norte municipality, in four land-use system, as follow: native forest, deforested site, agriculture and pasture (details in Table 1). The four treatments are located in the same soil type and are close to each other. Oxisol is the predominant soil order of the sampling sites (Secretaria de Estado de Planejamento e Coordenação Geral, 2001) and the climate is classified as Am (Koppen's classification) with annual average temperature of 28 °C and average precipitation of 2000 mm. Soil samples were collected in October 2009, April 2010 and November 2010, spanning the cycles of soybean cropping and intercropping. However, the sampling in the deforested site was performed only in two periods (October 2009 and April 2010), due to the fact that deforested sites do not remain unused for a long time. In the subsequent sampling (November 2010), this area had been used for other agricultural purposes. In the agricultural area, the soil was prepared through the application of fertilizers, pesticides and liming treatment to increase soil pH to 5–6. In this area, the annual crop rotation was: millet, soybean, and maize, under no-tillage.

For the sampling, PVC tubes (5 cm diameter by 25 cm long) previously sterilized were used to collect the 0–20 cm topsoil layer. In each area five sampling points were established, distancing 20 m among them. At each point, five samples were collected keeping a minimum distance of 50 cm between them, and the samples were pooled to obtain one composite sample per point. One part was kept at 4 °C and another at −20 °C. The samples kept at −20 °C were used for molecular analysis, while samples kept at 4 °C were used to characterize the physicochemical properties of the soil. The soil samples were processed within 72 h after sampling.

2.2. Soil physicochemical parameters

Soil chemical and physical properties were determined for each sample based on 400 g of soil, performed at the Laboratory of Soil Analysis at "Luiz de Queiroz" College of Agriculture (ESALQ/USP, Piracicaba, Brazil) according to the methodology described by van Raij et al. (2001). Briefly, soil pH was measured in a 1:2.5 soil/water suspension. Exchangeable Al, Ca, and Mg were extracted with KCl 1 M. Calcium and Mg were determined by atomic absorption spectrometry and Al by acid–base titration. Phosphorus and K were extracted by ion-exchange resin. Potential acidity (H + Al) was estimated by an equation based on the pH determined in SMP buffer solution (pH SMP). Available micronutrients (Fe, Mn, Zn, and Cu) were extracted by Mehlich 1 and determined by atomic absorption spectrometry. Boron was extracted with hot water and determined by spectrophotometry with azomethine-H at 420 nm. Some of the results allowed the calculation of other parameters such as exchangeable bases (SB), 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 SB and CEC; and Al saturation (m%), the percentage relation between exchangeable Al and CEC. Soil texture was determined using Bouyoucos densimeter after shaking the soil vigorously with NaOH 1 M as dispersant. Total nitrogen was determined by Kieldahl method; NH^{4+} and NO^{3-} by Raney/Kieldahl.

2.3. DNA extraction, PCR amplification, and T-RFLP

DNA extraction from 250 mg of soil was carried out for the five samples in each sampling point using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), according to the manufacturer's protocol. DNA quality and quantity were measured by 1% TSB (Brody and Kern, 2004) agarose gel electrophoresis and NanoDrop 1000 spectrophotometry (Thermo Scientific, Waltham, EUA). Terminal Restriction Fragment Length Polymorphism (T-RFLP) fingerprinting was used to characterize the bacterial community structure in all samples. The bacterial 16S rRNA gene was amplified with the primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-ACCTTGTTACGACTT-3′) (Amann et al., 1995). The forward primer was labeled with 6-FAM (6-carboxyfluorescein) at the 5′ end. The 25 μL reaction mixtures contained 2.5 μL of reaction buffer 10X (Invitrogen, Carslbad, CA, USA), 1.5 μL of $MgCl_2$ (50 mM), 1 μL of the primer set (5 pmol each), 0.2 μl (5 U) of *Platinum Taq* DNA Polymerase (Invitrogen), 0.5 μL of deoxyribonucleotide triphosphate mixture (2.5 mM each), 0.25 μL of Bovine Serum Albumin (1 ng ml^{-1}), 1 μL of DNA template (approximately 50 ng) and 18.05 μL of sterilized ultrapure water. PCR amplification was performed using a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following amplification cycles: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 59 °C for 45 s, and 72 °C for 1 min with a final extension step at 72 °C for 15 min. Negative controls without DNA were run in all amplifications. The PCR products were purified using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) after analysis by gel electrophoresis. After purification, 5 μL of amplification products (nearly 60 ng) were digested with the endonucleases *Msp*I (Invitrogen) in 15 μL reactions for 3 h at 37 °C. The digests were purified by sodium acetate/EDTA precipitation, and then mixed with 0.25 μL of the