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Coal mining practices reduce the microbial biomass, richness and diversity of soil

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ABSTRACT

Changes within the environment by mining processes include increased soil acidification, compaction, erosion, and air and water pollution. As it affects directly the soil microbial community, it may trigger severe impact on the biogeochemical cycles. The objectives of this study were to evaluate how the soil microbial community changes over time after mining and soil reconstruction at the largest coal mine in Latin America. To address this question, we compared soils from undisturbed native forest and grassland to five different sites at various stages of recovery post coal mining. We compared and correlated the soils' chemical features to the soil microbial biomass, soil enzyme activity, and prokaryotic phylogeny and diversity. 42 samples were collected from the seven sites and the V4 region of the 16S rRNA gene was sequenced on the Illumina HiSeq platform. Reads were classified to identify 525 genera from Bacteria and Archaea domains. The composition of the microbial community of post-mined sites was significantly different from the undisturbed sites. Soil from the reconstructed sites showed a significantly lower microbial diversity and biomass, and lower activity of the dehydrogenase and β-glucosidase enzymes when compared to undisturbed soils. The most abundant genera found in the reconstructed soils were *Thiobacillus, Sphingomonas, Novosphingobium, Acinetobacter*, and *Variovorax*. *Bradyrhizobium* was the most abundant genus in the undisturbed forest site, and *Bacillus* in the undisturbed grassland site. Microbial diversity increased as sites recuperated, but the microbial composition and abundance of post mined soils remained changed.

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1. Introduction

Candiota, located in south Brazil, is the biggest coal mine in Latin America. The coal extracted from Candiota, an opencast mine, is situated within two adjacent layers that are 200 centimeters thick each constituting a rate of 4 cubed meters of coal "in situ" by square meter of land mine, a rare occurrence among the coals. Each year, 1.7 million tons of coal is extracted from its 3 billion ton reserve supplying the Property Union CGTEE Thermoelectric Plant President Médici, which generates 440 megawatts of energy (CGTEE, 2014).

Coal mining contributes to both the social and economic development of the region (Koppe and Costa, 2002); however, mining large areas of land results in drastic structural and biological changes, triggering many environmental concerns (Potter et al.,1988; Kampf et al., 2000; Sydnor and Redente, 2002; Wong, 2003; Sheoran et al., 2008; Chaubey et al., 2012). After the coal is mined, the mining companies are obligated to restore the landscape in order to avoid land degradation in a process called soil reconstruction. This process includes reallocation of the original soil layers over the craters followed by the stabilization of the soil chemical properties and then reestablishment of vegetation. The final form is, called 'reconstructed soil' (Koppe and Costa, 2002; Gaivizzo et al., 2002). Typically, after mining, the soil surface layers become contaminated with pyrite (FeS_2) that later oxidizes to sulphuric acid when exposed to oxygen and water, which results in high levels of soil acidification (Fanning and Fanning, 1989). The loss of soil structure, decrease of organic matter, increase in bulk density (BD> 1.6 g cm^{-3}), and low rate of water infiltration into the soil ($R < 5$ cm h^{-1}), leads to runoff and soil erosion (Kampf et al., 1997; Guebert and Gardner, 2001). Minimizing environmental

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damages when forming a constructed soil is of utmost importance. However, without strict implementation of the landscape reconstruction procedure and soil management, soil and biome degradation is often irreversible with substantial loss of microbial and plant diversity.

Microbial activity is a key factor affecting the functioning of all terrestrial systems. The microbial functional stability increases with the availability of soil resources (McGuire and Treseder 2010; Shade et al., 2012; Griffiths and Philippot, 2013; Pagaling et al., 2013; Tardy et al., 2014). The soil organic matter (SOM) is a soil resource that nourishes the microbial community and its content is usually deficient in post-mined soils, due the high disturbance of the topsoil. It may decrease the microbial diversity, as well the soil functional stability. Predictive maps of global microbial diversity have being generated by integrating environmental data and molecular analyses of the bacterial community (Larsen et al., 2012; Fierer and Ladau, 2012), but the resilience of soil microbial diversity following disturbance remain unknown.

We hypothesized that the soil microbial community from post-mined soils may present different composition and diversity comparing to the original microbial community. Than, in this study we compared the soil microbial community of disturbed (after coal mining processes) and undisturbed (native forest and grassland) soils from the same edaphoclimatic area. We used multiple approaches, such as 16S rRNA next generation sequencing (NGS), quantification of microbial biomass and enzymatic activity, and soil chemical feature analysis to assess the overall processes occurring within the soils. Our goal was to detect the magnitude of these changes within the soil microbial community in Candiota's coal mine, Brazil.

2. Materials and methods

2.1. Environmental characterization

The studied area is used by the mining company Rio Grande do Sul (CRM), and it is located in Candiota at −31°33′55″S and −53°43′30″W with an altitude of 230 m. According to Köppen climate classification, the region has a humid subtropical climate. The average annual temperature is 17.2 °C, and the average annual rainfall is 1262 mm. The soil is classified as an Acrisol according to the FAO system (Driessen et al., 2001). After the coal extraction, the mining company restored the landscape, replacing the soil layers that were previously removed, included A horizon and applying limestone to raise the pH to 6.0. To complete the process, they seeded a blend of grasses on the site. In this study, forest and grassland areas were treated as controls, and sites where mining had occurred were treated as the experimental condition.

2.2. Sampling

The samples were collected in the Companhia Rio-Grandense de Mineração-CRM, Brazil that has a total area of 20,000 ha. The 42 individual soil samples (seven sites x six replicates each) represent five disturbed sites (after coal mining) with different ages of restoration: 3, 4, 5, 6 and 19 years of reconstruction, and two sites of undisturbed soils (controls, from the same geographic region): native grassland (with diverse native grasses and legumes composing the plant community), and native forest (subtropical vegetation with 2 to 5 m trees), both preserved sites located from less than 1000 m of the coal mine. Each of the 42 samples is a composite of ten subsamples (collected within 0.5 ha of each site) pulled from 1 to 10 centimeters deep after the superficial vegetation was removed.

2.3. Chemical and physical characterization

A neutral solution of KCl mol L^{-1} was used to extract Calcium (Ca), Magnesium (Mg), and Aluminum (Al). Levels of Ca and Mg were determined by atomic absorption spectrophotometry, and Al concentration was determined by neutralization with Sodium hydroxide (NaOH) titration (Tedesco et al., 1995). Potassium was extracted by using solution of Sulfuric acid 0.05 mol L^{-1} + HCl 0.05 mol L^{-1} and determined by flame photometry (Tedesco et al., 1995). Total acidity (H + Al) was extracted using calcium acetate solution (0.5 mol^{-1} L) buffered to pH 7.0 and quantified by titration with NaOH neutralization (Tedesco et al., 1995). The sum of bases (S), base saturation (V%), and cation exchange capacity (CEC) at pH 7.0, were than calculated. Organic carbon was analyzed according to Tedesco et al. (1995). Cadmium (Cd), Copper (Cu), Nickel (Ni), and Lead (Pb) were extracted using H_2SO_4 0.05 mol L^{-1} + HCl 0.05 mol L^{-1} solution, and their concentrations were determined by plasma spectrometry according to Tedesco et al. (1995). The pH levels were determined in water and KCl mol L^{-1} with potentiometer.

2.4. Microbial biomass C, respiration C, and enzymatic characterization of the soil

Carbon (C) from the microbial biomass was extracted using the chloroform fumigation and extraction method (Vance et al., 1987). For this, aliquots of 25 g of soil (dry weight based) were subjected to 48 h fumigation with chloroform in a glass desiccator. Triplicates of each sample from fumigated and non-fumigated soils were shaken for 1 h at 180 strokes per minute with 0.5 M of K_2SO_4 at a ratio of 5:1 (weight of extractant to dry soil weight) and filtered. Soluble carbon levels were determined for both fumigated and non-fumigated soils. Dissolved organic carbon (DOC) from the soil solution was extracted through potassium dichromate digestion, following the Jenkinson and Powlson method (1976). The amount of soluble C from the fumigated and non-fumigated soil extracts was calculated using the following equation:

$$\left[\text{Biomass}C = \frac{(C_f - C_{nf})}{K_{ec}}\right]$$

where C_f is C in fumigated extract, C_{nf} is C in non-fumigated extract, and K_{ec} is the proportion of the microbial C that is extracted from the soil.

The level of C from microbial respiration was estimated by measuring the amount of CO_2 released from the soil during 40 days of incubation in accordance to Stotzky and Pramer's methodology (1972). Three replicates of 200 g from each soil sample (dry weight based) were incubated for 40 days at 25 °C in closed, airtight 1L bottles with small beakers containing NaOH 0.5 N solution, which retains the CO_2 resulting from microbial biological activity, through forming Na_2CO_3 in the solution. Every 5 days (totaling eight measurements), CO_2 from the solution was measured. For this, 10 ml of 0.2 N $BaCl_2$ was added to the beakers containing NaOH in order to precipitate the carbonate and avoid its interference with the titration. Titration with HCl was performed using phenolphthalein as an indicator. Metabolic coefficient was determined according to Anderson and Domsch (1993). The concentrations of β-glucosidase and dehydrogenase enzymes were evaluated in the soil samples according to Tabatatai (1994).

2.5. DNA extraction and PCR

For each sample, DNA was extracted from 0.8 g of soil using the MoBio PowerSoil™ DNA Isolation Kit (Carlsbad, CA, USA) in accordance with the manufacturer's protocol. All genomic DNA

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