UNIVERSIDADE FEDERAL DE VIÇOSA

ISABELLE GONÇALVES DE OLIVEIRA PRADO

DIVERSIDADE DE FUNGOS, COM ÊNFASE EM FMA, EM ÁREAS AFETADAS PELO REJEITO DE MINERAÇÃO DE FERRO AO LONGO DE TRÊS ANOS APÓS O ROMPIMENTO DA BARRAGEM DO FUNDÃO

VIÇOSA - MINAS GERAIS - BRASIL
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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Doctor Scientiae*.

Orientadora: Maria Catarina Megumi Kasuya

Coorientadoras: Cynthia Canêdo da Silva
Marliane de Cássia Soares da Silva

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RESUMO

PRADO, Isabelle Gonçalves de Oliveira. D.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Diversidade de fungos, com ênfase em FMA, em áreas afetadas pelo rejeito de mineração de ferro ao longo de três anos após o rompimento da barragem do Fundão.** Orientadora: Maria Catarina Megumi Kasuya. Coorientadoras: Marliane de Cássia Soares da Silva e Cynthia Canêdo da Silva.

Os impactos ambientais decorrentes de atividades de mineração são problemas recorrentes que o Brasil vem enfrentando. Em novembro de 2015, com o rompimento da Barragem do Fundão em Bento Rodrigues, Minas Gerais, uma onda de rejeitos da extração de minério de ferro foi lançada no ambiente causando a destruição do patrimônio público e religioso e gerando um impacto ambiental de grande proporção. O rejeito depositado sobre o solo do local passou a constituir o Tecnosolo, sendo suas propriedades e funções derivadas da atividade humana técnica. Para o restabelecimento das funções ecossistêmicas desenvolvidas pela biota do solo, a revegetação, com a introdução de plantas e a microbiota associada ao sistema radicular das plantas foi implementada. Entre esses microrganismos, os fungos micorrízicos arbusculares (FMA) exercem influência no crescimento, na aclimatação dos vegetais em condições de estresses bióticos e abióticos e possuem um potencial de atuarem no funcionamento e estabilidade dos ecossistemas, ciclagem de nutrientes, processos de recuperação, restauração ambiental e reflorestamento. Dessa forma, a análise da composição microbiana da área impactada torna-se uma estratégia importante a fim de utilizá-la na recuperação das áreas atingidas pelo rejeito de mineração. Este trabalho propôs estudar a diversidade de fungos presente nos solos sob sistemas de revegetação usando gramíneas e leguminosas ao longo de três anos, após o rompimento da barragem do fundão, fazendo uso de técnicas tradicionais e moleculares, a fim de monitorar a biodiversidade fúngica, em especial os FMA. As amostragens foram realizadas em março e setembro de 2016, 2017 e 2018. Foram coletadas amostras dos solos e sistema radicular de cinco áreas ao todo, sendo REC1, REC2 e REC3 áreas afetadas pelo rejeito em processo de recuperação, PASTrec área de pastagem afetada pelo rejeito, PAST e UND áreas de pastagem e de floresta respectivamente, adjacentes às áreas afetadas. Foram realizadas análises químicas dos solos e teor de matéria orgânica. A extração do DNA foi realizada a partir de amostras dos solos. Para análise de fungos totais foi realizado o sequenciamento ITS – Illumina MiSeq. A análise da composição da comunidade de FMA foi realizada por DGGE, porcentagem de colonização micorrízica e análise dos morfotipos pela extração de esporos do solo. Pelos resultados dos dados químicos das amostras de solo, observou-se a separação em dois grupos, aqueles afetados (REC e PASTrec) e não afetados (UND e PAST). Embora tenham se passado três anos, as características químicas dos Tecnosolos não sofreram grandes alterações. Entretanto, para os fungos, observou-se um aumento na diversidade, havendo prevalência de determinados grupos, com predominância de Ascomycota e Basidiomycota, com formação de três agrupamentos, sendo UND, PAST e áreas afetadas pelo rejeito. Houve variação na composição da microbiota em REC ao longo dos anos. Em 2016, REC2 apresentou distribuição distinta da comunidade fúngica e em 2017 e 2018 essa área se aproximou de outras áreas de REC. Avaliando a similaridade de OTUs total entre todas as áreas foi observada maior similaridade de REC com PAST indicando uma aproximação das áreas afetadas com as pastagens. Analisando dados de FMA de REC em relação à UND foi observado um aumento na similaridade em função do tempo. Conclui-se que o processo de revegetação vem produzindo um efeito positivo na diversidade de fungos, incluindo FMA, revelando-se mais sensíveis que os dados químicos. O manejo da área em processo de revegetação e a implementação de outras espécies vegetais, como arbóreas nativas, serão imprescindíveis para que haja progresso de recuperação. Análises periódicas da diversidade fúngica servirão de ferramenta para monitorar se o manejo está sendo adequado.

Palavras-chave: Fungos micorrízicos arbusculares. Monitoramento. Análise de cronosequência. Sequenciamento de Nova Geração. Práticas de manejo. Área de mineração.

ABSTRACT

PRADO, Isabelle Gonçalves de Oliveira. D.Sc., Universidade Federal de Viçosa, February, 2020. **Diversity of fungi, with emphasis on AMF, in areas affected by iron ore mining waste, three years after the Fundão dam collapse.** Advisor: Maria Catarina Megumi Kasuya. Co-advisors: Marliane de Cássia Soares da Silva and Cynthia Canêdo da Silva.

Environmental impacts of mining activities are recurring problems in Brazil. In November 2015, the collapse of Fundão Dam in Bento Rodrigues, Minas Gerais, launched a wave of iron ore mining waste (IOMW) in the environment, causing the destruction of public and religious heritage and an enormous environmental impact. The IOMW deposited on the local soil was then called Technosol and its properties and functions derive from technical human activity. The introduction of plants and microbiota associated with the plant root system was implemented for the restoration of ecosystem functions developed by soil biota and revegetation. Among these microorganisms, arbuscular mycorrhizal fungi (AMF) are able to affect growth, the acclimatization of plants under biotic and abiotic stress conditions and have potential to influence the functioning and stability of ecosystems, nutrient cycling, recovery processes, environmental restoration and reforestation. Thus, the analysis of microbial composition in impacted areas is an important strategy for its use in the recovery of the areas affected by IOMW. This work aimed to study the diversity of fungi present in soils under revegetation systems using grass and leguminous species over three years, after the rupture of Fundão dam, using traditional and molecular techniques, in order to monitor fungal biodiversity, especially AMF. Sampling was carried out in March and September 2016, 2017 and 2018. The soils and root systems were sampled from five areas: REC1, REC2 and REC3 IOMW-affected sites under recovery process, PASTrec an IOMW-affected pasture, PAST and UND a pasture and a forest sites respectively, adjacent to the IOMW-affected sites. Chemical analyses of the soils and organic matter were performed. DNA extraction was performed from soil samples. ITS - Illumina MiSeq sequencing was performed for total Fungi. AMF community analysis was carried out by DGGE; the percentage of mycorrhizal colonization and morphotype analysis, by soil spore extraction. The analysis of soil chemical data revealed two distinct groups, IOMW-affected (REC and PASTrec) and non-affected (UND and PAST). Although three years have passed, the technosols chemical characteristics did not change significantly. However, the analysis of fungi showed increased diversity, with prevalence of certain groups and predominance of Ascomycota and Basidiomycota. Three distinct groups were observed, UND, PAST and IOMW-affected sites. Variation was observed in microbiota composition in

REC, over the years. In 2016, REC2 presented distinct fungal distribution, and, in 2017 and 2018, this area was similar to other REC sites. Assessing the similarity of total OTUs across all areas, a greater similarity between REC and PAST was observed, indicating an approximation of the IOMW-affected sites with pasture. Analyzing AMF data from REC and UND, an increase in similarity over time was observed. It is concluded that the revegetation process has been producing a positive effect on fungal diversity, including AMF, which is more sensitive than chemical data. The management of the area in the process of revegetation and the implementation of other plant species, such as native trees, will be essential for the recovery progress. Periodic analysis of fungal diversity will be used as a tool to monitor management adequacy.

Keywords: Arbuscular mycorrhizal fungi. Monitoring. Chronosequence analysis. New Generation Sequencing. Management practices. Mining area.

SUMÁRIO

1. INTRODUÇÃO GERAL

Com o crescimento demográfico e a intensificação do desenvolvimento tecnológico nas últimas décadas, atividades exploratórias cresceram e junto delas os problemas ambientais associados à poluição gerando um grande desastre global. Entre essas atividades, a mineração de ferro se destaca como a atividade mineral de maior exportação nacional (> 60 %), com uma participação fundamental no PIB (Produto Interno Bruto - 1,4 % de todo o PIB do Brasil) e na geração de empregos (dados do relatório de 2017-2018 do IBRAM - Instituto Brasileiro de Mineração). O Quadrilátero Ferrífero, no centro sul de Minas Gerais, abriga algumas das atividades de mineração de ferro mais intensas do mundo e dessa forma, a importância da Indústria Extrativa Mineral é substancialmente representativa na economia de Minas Gerais e contribui na definição do perfil socioeconômico do Estado (IBRAM, 2015). As atividades de mineração resultam muitas vezes em impactos ao meio ambiente de forma localizada, mas os componentes de rejeito, quando solubilizados, podem atingir cursos de água e afetar áreas extensas a centenas de quilômetros do local de mineração (Hashemi et al., 2015; Salomons, 1995). Além disso, diversos problemas ambientais são recorrentes, como desmatamento, processos erosivos do solo, transporte dos elementos explorados para outras áreas, incluindo os mananciais hídricos. Assim, extensas áreas se tornam degradadas e contaminadas com elementos-traço, causando impactos na vegetação, nos organismos do solo, nas águas superficiais e subterrâneas (Leal et al., 2016; J. Li et al., 2014; Y. Li et al., 2014).

Somado a todos esses impactos já previstos da atividade mineradora, em novembro de 2015, com o rompimento da Barragem do Fundão em Mariana, Minas Gerais, 34 milhões de m³ de rejeito da mineração de ferro foram lançados no meio ambiente, cobrindo 650 km ao longo do percurso nos estados de Minas Gerais (MG) e Espírito Santo (ES), uma área ribeirinha de 1.176,6 ha e 457,6 ha da Mata Atlântica (Omachi et al., 2018). Este desastre foi classificado, de acordo com a Defesa Civil Brasileira, em uma escala de I a IV, como um Desastre Nível IV (desastres de muito grande porte ou intensidade) e caracterizado como o maior desastre na história da mineração brasileira (IBAMA, 2015). O colapso da barragem causou danos ao ecossistema, às atividades econômicas e à sociedade como um todo. De acordo com o laudo técnico do IBAMA, os rejeitos lançados causaram destruição de áreas de preservação ambiental, destruição e fragmentação de habitats, perda da vegetação nativa que compõe o *hotspot* Mata Atlântica, destruição de áreas agrícolas e pastos entre outros danos ambientais, sociais e econômicos. O solo resultante da deposição do rejeito de minério, influenciado pela atividade humana técnica é denominado Tecnosolo (Rossiter, 2007) devido à sua nova composição e estrutura. Dessa forma, a área impactada pelo rejeito de mineração passou a contar desde então com essa cobertura de Tecnosolo.

A fim de mitigar os impactos causados por esse desastre, várias medidas foram tomadas pelo poder público e por empresas privadas envolvidas (Samarco, 2016). Devido ao alto custo e ineficiência de tecnologias de limpeza e também pela urgência em evitar uma maior distribuição desses resíduos por processos de lixiviação e ação dos ventos, programas de revegetação foram empregados nessas áreas (Chaturvedi et al., 2015). Os programas de revegetação iniciados na área impactada pelo rejeito de mineração de ferro objetivaram a contenção e carreamento de sedimentos e contaram com combinações de distintas espécies vegetais, entre leguminosas, gramíneas e outros grupos vegetais, a exemplo de feijão guandu, feijão de porco, soja-perene, estilosante, crotalária, entre outras capazes de se estabelecerem nas condições adversas impostas (Samarco, 2016). As associações entre as plantas e os microrganismos tornam o processo de biorremediação mais eficiente. Uma vez que os distúrbios ambientais decorrentes desse impacto tendem a reduzir a biodiversidade local comprometendo as funções do ecossistema desenvolvidas por microrganismos (Zhou et al., 2014) é necessário levar em consideração estratégias de recomposição da microbiota do solo. Para isso, a comunidade microbiana associada deve apresentar uma grande diversidade funcional.

Caracterizados como grandes atores ecológicos, os fungos desenvolvem diversas atividades de ciclagem de matéria orgânica e direcionamento de nutrientes através dos níveis tróficos em ambientes terrestres e aquáticos (Nilsson et al., 2019). A comunidade fúngica do solo desempenha um papel importante nos ciclos biogeoquímicos nos ecossistemas e pode ser significativamente afetada por distúrbios ambientais (Rosales-Castillo et al., 2018; Zhou et al., 2014), sendo sensíveis às alterações do meio em que se encontram e também aos fatores climáticos entre as estações. Acessar a composição e diversidade desse grupo durante o ano, avaliando estação chuvosa e seca, bem como os parâmetros físico-químicos do solo é uma estratégia de monitoramento da área impactada. Análises de sequenciamento de fungo total a partir de amostras de solo oferecem imensas possibilidades na área da micologia e o sequenciamento de nova geração das amostras do solo vem sendo recomendada (Nilsson et al., 2019).

Entre os microrganismos requeridos no processo de reabilitação de solos afetados, encontram-se os fungos micorrízicos arbusculares (FMA) (Cabral et al., 2015; Leal et al., 2016; Mathur et al., 2007; Neagoe et al., 2014; Spruyt et al., 2014; Wang, 2017; Yang et al., 2016). Os FMA pertencem ao filo Glomeromycota, subfilo Glomeromycotina (Mucoromycota), um clado monofilético representado atualmente por cerca de 300 espécies distribuídas em três classes, cinco ordens, 15 famílias e 38 gêneros (Błaszkowski, 2012; Goto et al., 2012; Oehl et al., 2011; Spatafora et al., 2016). Os FMA são caracterizados como organismos mutualísticos simbióticos associados a aproximadamente 80 % das espécies de plantas terrestres (Smith & Read 2008). A taxonomia desse grupo é atualmente baseada em diferenças na morfologia dos esporos e nas sequências de genes conservados (Morton, J.B.; Benny, 1990; Redecker et al., 2013). Explorar os efeitos benéficos dos FMA para o desenvolvimento das plantas podem levar à implementação de estratégias de restauração em locais impactados por mineração, além de fornecer novos insights sobre o processo de recuperação dessas áreas. A diversidade funcional desses simbiontes do sistema radicular é importante na aquisição de diferentes nutrientes limitantes, pois auxiliam em funções críticas do ecossistema (van Der Heijden et al., 2008; van der Heijden and Hartmann, 2016) e promovem um melhor desenvolvimento das espécies vegetais em áreas impactadas pela mineração de minério de ferro (Leal et al., 2016; Prado et al., 2019; Silva et al., 2018; Teixeira et al., 2017; Vieira et al., 2017). As metodologias envolvidas no estudo de FMA vão desde estudos morfológicos como a extração de esporos e colonização micorrízica, bem como o uso de ferramentas moleculares. O uso de ambas metodologias são complementares no estudo desse grupo.

Com as atividades de mineração e a presença do rejeito de mineração, as características químicas do solo são alteradas, criando um ambiente com condições rigorosas para o crescimento das plantas e diversificação da microbiota. Com a implementação do processo de revegetação em 2016, análises do Tecnosolo e da microbiota nesse ambiente foi avaliada semestralmente. Para o bom desenvolvimento da planta é necessário considerar diversos fatores, entre eles a questão nutricional e física do solo. Para contornar problemas nutricionais, a fertilização acaba sendo uma alternativa eficiente a curto prazo, entretanto, a longo prazo é difícil ter o controle dos nutrientes no solo, uma vez que esses nutrientes podem ser dissipados no meio, processados, transformados. Além disso, um gargalo no desenvolvimento da planta está relacionado à estrutura do solo, que quando se encontra altamente compactado, dificulta o desenvolvimento radicular. Diante disso, o uso da microbiota, a exemplo de FMA é uma estratégia a ser adotada em áreas de recuperação ambiental para garantir o desenvolvimento e estabelecimento da vegetação (Kimura and Scotti, 2016; Mukhongo et al., 2016) uma vez que atuam tanto na nutrição como na estruturação do solo. Estudos indicam que os FMA são capazes de sobreviver e crescer em terras degradadas pelo processo de mineração e que as aplicações desses fungos durante o plantio reduzem as doses de fertilizantes químicos (Verma and Verma, 2016) trazendo benefícios em longo prazo na estruturação do solo (Kimura and Scotti, 2016). A integração de indicadores biológicos do solo como a análise de fungo total e dos FMA, atrelados aos indicadores químicos e físicos torna a avaliação da qualidade do solo robusta e contribui imensamente com o processo de recuperação (Silva et al., 2018).

Diante do desastre ocorrido em Mariana com a deposição do rejeito de mineração de ferro, análises químicas e biológicas do solo são necessárias para qualificar a condição do Tecnosolo e desenvolver estratégias para a recuperação da biodiversidade. Portanto, a hipótese do trabalho é que o processo de revegetação e os períodos de amostragem (chuvoso ou seco) afetam a diversidade de fungos na área atingida pelo rejeito e que o fator tempo interfere nessa recomposição da comunidade microbiana. O objetivo deste estudo foi caracterizar a comunidade fúngica – com destaque ao FMA - em áreas afetadas pelo rejeito de mineração nas estações secas e chuvosas e relacionar esses dados com as variáveis químicas do solo e do Tecnosolo em uma análise de cronosequência durante um período de três anos. Dessa forma, as amostragens na área impactada pelo rejeito de mineração em Paracatu de Baixo iniciaram em março de 2016, exatos 4 meses após o acidente. Desde então foram realizadas seis coletas (março e setembro – 2016, 2017, 2018). As amostragens realizadas em 2016 foram publicadas em 2019 (Prado et al., 2019) e os resultados obtidos das amostragens de 2017 e 2018 foram analisados de forma integrada fazendo uma comparação com o que foi relatado nas amostragens do ano anterior.

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2. ARTIGO CIENTÍFICO 1

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Revegetation process increases the diversity of total and arbuscular mycorrhizal fungi in areas affected by the Fundão dam failure in Mariana, Brazil

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ABSTRACT

Mining activities have environmental impacts. The rupture of the Fundão tailing dam ejected a significant volume of iron ore mining waste (IOMW) and was characterized as the greatest disaster in mining history. The use of microorganisms to monitor the affected soils is ideal due to their sensitivity to environmental changes and their capacity for soil remediation, as they can promote plant growth under stressed conditions. This study aimed to evaluate the total fungi diversity and arbuscular mycorrhizal fungi (AMF) community structure of the area affected by IOMW that is currently under rehabilitation. Soil and root samples were collected from an impacted site without rehabilitation (REC1), two revegetated areas with grass and leguminous species (REC2 and REC3), and one reference area in the undisturbed forest (UND) during two seasons (rainy and dry). The total fungal diversity was analyzed by next generation sequencing. The AMF was identified morphologically and the number of spores, colonization, and denaturing gradient gel electrophoresis (DGGE) were identified for this group of fungi. Total fungi diversity, AMF community structure, and soil chemical indicators differed among areas and between seasons. UND exhibited the highest organic matter content whereas rehabilitated areas were more affected by pH and presented higher values. The total fungal diversity and AMF community structure in REC areas showed that spore number and species richness increased along with the revegetation process. *Glomus* and *Rhizophagus* comprised the most abundant genera. In both climate seasons, AMF diversity in UND was higher than in REC areas but increased diversity in REC was observed in subsequent sampling. The fungal community diversity in affected areas was altered by the revegetation process—which increased the total fungal diversity—as well as AMF helping the recovery of this area.

Keywords: Disturbed area, Soil recovery, DGGE, Next generation sequencing

1. Introduction

The Quadrilátero Ferrífero in central southern Minas Gerais, Brazil is home to some of the most intense iron mining activity in the world. On November 5, 2015 a rupture of the Fundão tailing dam occurred in Mariana, Minas Gerais belonging to Samarco Mineração SA (the company in charge of the iron exploration in the area, formed by Vale and BHP Billiton) resulted in 34 million m^3 of iron ore mining waste (IOMW) being launched into the environment, covering 650 km along the states of Minas Gerais (MG) and Espírito Santo (ES), a riverside area of 1176.6 ha, and carrying away 457.6 ha of the Atlantic Forest (Omachi et al., 2018). The IOMW caused 19 casualties and destroyed the village of Bento Rodrigues and 207 other properties (IBAMA, 2015). This disaster was classified as a Level IV Disaster according to the Brazilian Civil Defense classification and characterized as the biggest disaster in Brazilian mining history (IBAMA, 2015). Mining activities have a localized impact but the tailing components, when solubilized, can easily reach watercourses and affect areas hundreds of kilometers removed from the mining site, which poses a threat to human health and a constitutes a potential environmental hazard (Hashemi et al., 2015; Salomons, 1995), besides cytotoxicity and cellular DNA damage (Segura et al., 2016). Soil analyses near the area affected by IOMW presented high concentrations of chemical compounds such as sodium hydroxide (do Carmo et al., 2017), which may also be due to different agricultural soil inputs, watercourses, and plantations in proximity to the affected sites (Segura et al., 2016). Soil that has been influenced by technical human activity, such areas where IOMW is added, has been called technosol (Rossiter, 2007). Given the aforementioned considerations, this disaster provides a unique scenario for understanding fungal ecology.

The fungal community of soil plays an important role in biogeochemical cycles in ecosystems and can be significantly affected by environmental disturbances (Rosales-Castillo et al., 2018). The recovery of the soil's microbial community is a fundamental step in its restoration for sustainable and beneficial use (Asmelash et al., 2016; Rana et al., 2007). Arbuscular mycorrhizal fungi (AMF) are among the useful microorganisms in this process since they can increase plant survival, growth and nutrition, tolerance to stress conditions, and soil structure and quality (Cabral et al., 2015; Mathur et al., 2007; Wang, 2017; Yang et al., 2016), which can influence and optimize terrestrial ecosystem processes directly and indirectly (Rillig, 2004; Wang, 2017). Environmental stresses, like those present in areas following iron mining, reduce the biodiversity and result in compromised ecosystem functions. Monitoring programs of biodiversity in mining projects need to detect significant changes in biodiversity over time to be more effective (Dias et al., 2017). As consequence, exploring the effects of AMF may lead to restoration strategies for mining-impacted sites and may provide new insights into the recovery process, as no information can yet be found on this topic. The functional diversity of the root microbiome's symbiont members is important as they can complement one another by acquiring different limiting nutrients and thereby drive critical ecosystem functions (van Der Heijden et al., 2008; van der Heijden and Hartmann, 2016) and promote the recovery of areas impacted by iron ore mining (Leal et al., 2016; Silva et al., 2018; Teixeira et al., 2017; Vieira et al., 2017).

The limitations in dispersion and environmental filtering can affect the subsets of regional species in a given community and result in variations in the relative abundance of species that could colonize the area. This suggests that fungal dispersion may constitute an important consideration in this successional process (Nielsen et al., 2016). The richness and communal composition of soil fungal groups result from both direct and indirect effects of climatic variables, e.g., edaphic and spatial patterning (Tedersoo et al., 2014) and it is noteworthy that human activities, such as agricultural practices, also participate in the dispersal of these propagules (Mangan and Adler, 2002; Rosendahl et al., 2009).

The integration of soil biological indicators with chemical and physical indicators is an important factor in the evaluation of soil quality and the recovery process (Silva et al., 2018). In the face of the disaster that occurred in Mariana with IOMW, chemical and biological analyses of the soil are necessary to qualify the technosol condition and develop strategies for biodiversity recovery. Therefore, we hypothesized that the revegetation process and the period of sampling (rainy or dry) will affect detection of fungal diversity in the area affected by IOMW. The aim of this study was to evaluate the fungal community—mainly AMF—in areas affected by this residue in both periods and to correlate them with aspects of the recovery process.

2. Material and methods

2.1. Study areas and sampling of soil and plant

Soil samples were collected in the rainy summer (March 2016) and dry winter (September 2016) in Paracatu de Baixo (20°18′15″S 43°13′51″W), one of the impacted areas devoid of revegetation, and in other areas with revegetation systems (Table 1). During the samplings, each area was characterized for its vegetation coverage (Table 1; Fig. 1). The regional climate corresponds to type Cwa of the Köppen classification system, where temperatures ranged between 16.1 and 22.4 °C with an average of 19.7 °C. The total annual rainfall is 1375 mm, ranging from 13 mm in June to 275 mm in December (https://pt.climate-data.org/location/316464/). All areas are within the limits of the Atlantic Forest, a tropical forest and one of the most representative Brazilian biome with a global diversity hotspot [Law of the Atlantic Forest (11.428/2006)].

Table 1

Characterization of the area during the two samplings (Mar/2016 and Sep/2016).

Sampling	Area	ID	Vegetation cover during the samplings
Mar/2016	Forest	UND	Natural forest with typical species of the region
	Recovery 1	REC1	Contamination of mining tailings, absence of vegetal cover
	Recovery 2	REC2	Initial revegetation process near the forest
	Recovery 3	REC3	Initial revegetation process near the course of the river
Sep/2016	Forest	UND	Natural forest with typical species of the region
	Recovery 1	REC1	Initial natural revegetation process. Sparse vegetation
	Recovery 2	REC2	Intermediate revegetation process near the forest
	Recovery 3	REC3	Intermediate process of revegetation near the river

Soils were collected from REC1 (IOMW without revegetation process in the first sampling), REC2 and REC3 (recovering areas), and UND (undisturbed forest) (Fig. 1). Three composite soil samples (each composed of five sub-samples in 1 m2) were collected at a depth of 0–15 cm (Faoro et al., 2010) in each area. This sampling was supported by AGROFLOR Engenharia e Meio Ambiente (Viçosa, Brazil), the company in charge of the elaboration and execution of environmental projects in those areas.

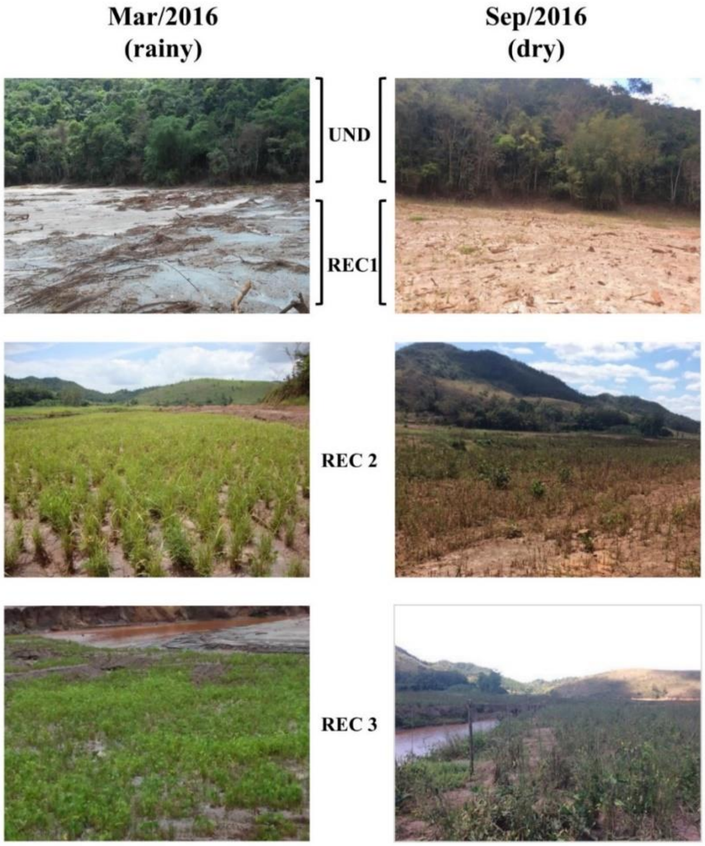

Fig. 1. Partial view and representation of the soil sampling sites corresponding to undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in the two periods of time (Mar/2016 and Sep/2016).

In the first sampling, the REC1 had no vegetation cover (Fig. 1). However, although some natural vegetation growth was evident in the second sampling, it was not possible to collect roots for the AMF colonization analysis. The reference area in the adjacency (unaffected by the IOMW) was taken from an UND (Table 1). A reforestation program in the IOMW-affected area was initiated in January 2016 by Samarco, a company in charge of the iron exploration in the area. The rehabilitation strategy included excavation of the IOMW and use of organic compost. > 250 ha have begun the revegetation process (Samarco, 2016). According to AGROFLOR, one of the revegetation enterprises, after the technosol had been revolved, NPK 8-28-16 (100 kg/ha) was applied at the time of seeding of a mixture of legumes and grasses (400 kg/ha) (Supplementary Table 1). NPK 20-05-20 (100–200 kg/ha) was applied after 10–30 days (Samarco, 2016).

The residue (IOMW) was classified as non-hazardous and non-inert to iron and manganese (IBAMA 2015). The routine chemical analyses of soil samples and organic matter were carried out in the Laboratory of Analysis of Soil Viçosa (Table 2) in Viçosa, MG, Brazil (Defilipo and Ribeiro, 1997).

Table 2

Statistical analysis of the average from three replicates of chemical characteristics of the soil in areas contaminated with mining tailings under recovery process (REC1, REC2, REC3) and in undisturbed forest (UND) in the two periods of time (Mar/2016 and Sep/2016).

	Mar/2016				**Sep/2016**			
Characteristics	UND	REC1	REC2	REC3	UND	REC1	REC2	REC3
pH (H_2O)	6.1 bc	7.6 a	7.2 ab	7.7 a	5.4 c	8.0 a	7.2 ab	8.0 a
P (mg dm^{-3})	10.2 a	5.6 a	26.8 a	22.7 a	4.8 a	7.8 a	8.4 a	8.7 a
K (mg dm^{-3})	127.7 a	8.7 c	19.7 bc	25.0 bc	88.3 ab	62.7 abc	50.7 abc	48.7 bc
Ca^{2+} (cmol$_c$ dm^{-3})	6.6 a	0.7 b	1.0 b	1.0 b	2.4 b	1.4 b	0.9 b	1.1 b
Mg^{2+} (cmol$_c$ dm^{-3})	1.7 a	0.0 b	0.1 b	0.1 b	1.2 a	0.0 b	0.1 b	0.0 b
Al^{3+} (cmol$_c$ dm^{-3})	0.0 b	0.0 b	0.0 b	0.0 b	0.4 a	0.0 b	0.0 b	0.0 b
H+Al (cmol$_c$ dm^{-3})	3.1 b	0.2 c	0.2 c	0.2 c	5.6 a	0.2 c	0.2 c	0.2 c
SB (cmol$_c$ dm^{-3})	8.6 a	0.7 b	1.1 b	1.1 b	3.8 b	1.6 b	1.1 b	1.3 b
CEC(t) (cmol$_c$ dm^{-3})	8.6 a	0.7 b	1.1 b	1.1 b	4.1 b	1.6 b	1.1 b	1.3 b
CEC(T) (cmol$_c$ dm^{-3})	11.7 a	0.9 b	1.2 b	1.3 b	9.3 a	1.8 b	1.1 b	1.3 b
V %	70.0 a	79.3 a	86.3 a	83.0 a	39.3 b	90.0 a	86.7 a	88.7 a
OM (dag kg^{-1})	7.2 a	0.6 b	1.2 b	1.0 b	4.8 a	1.0 b	1.3 b	1.1 b

Extractors used: P, K, = Mehlich1; Ca^{2+}, Mg^{2+} e Al^{3+} = KCl 1 mol.L^{-1}; H + Al = Calcium acetate 0.5 mol.L^{-1}; SB = Sum of exchangeable bases; CEC (t) = Effective cation exchange capacity; CEC (T) = Cation exchange capacity at pH 7.0; V = base saturation index; OM = organic matter. The data followed by the same lowercase letters in the same rows do not differ by Tukey test ($p < 0.05$).

2.2. *DNA extraction and fungal DNA sequencing*

Soil samples were stored at −4 °C of which 0.4 g were used for extraction of the total DNA using a Nucleo Spin Soil Kit (Macherey- Nagel, GmbH & Co. KG, Germany) according to the manufacturer's instructions.

The fungal diversity was evaluated by sequencing the ITS1 region of total DNA using the Illumina MiSeq platform. Sequencing was performed for all extracted DNA samples. The ITS1 region was amplified with the pair of primers ITS1F (Gardes and Bruns, 1993) and ITS2 (White et al., 1990). This primer set targets a shorter section of the fungal ITS region due to the shorter reading lengths provided by MiSeq.

The database and bioinformatics scripts to prepare, clean, and analyze the data were performed using the Quantitative Insights Into Microbial Ecology (QIIME 1.8.0 software) software system (Bokulich et al., 2013; Caporaso et al., 2010) according to the protocol established by the Brazilian Microbiome Project for ITS sequences (Pylro et al., 2014). In sequence cleaning procedures, readings that were < 140 bp in length, had a quality < 25 on the Phred scale, and displayed ≥1 base ambiguities were discarded. The sequences were assembled and the contigs were grouped as Operational Taxonomic Units (OTUs) with 97 % identity using the Uparse method (Edgar, 2013). Each OTU was assigned to a taxon through the tool available in QIIME using the UNITE database (Koljalg et al., 2014). The filtration of the fungal sequences and rarefaction of the samples were also performed. The Chao index was used to estimate the number of OTUs. Venn diagrams were performed in the JVENN software system to show unique and shared number of species among different areas (Bardou et al., 2014).

2.3. Arbuscular mycorrhizal fungi (AMF)

The occurrence of AMF was assessed by direct extraction of spores in 50 mL of soil using the wet sieving technique (Gerdemann and Nicolson, 1963) with water centrifugation, followed by centrifugation in a 50 % sucrose solution. Spores were considered viable if they displayed clear contents and intact spore walls under light microscopy. The spores were counted and mounted on slides with polyvinyl lactoglycerol (PVLG) and Melzer's reagent (v:v). Phenotypic characteristics were then observed under light microscopy (Olympus BX-50) for taxonomic classification. The identification was performed using the original species descriptions from Błaszkowski (2012) and the International Collection of Arbuscular and Vesicular-arbuscular Mycorrhizal Fungi (INVAM, 2016). The matrix for presence/absence of AMF was used to generate a dendrogram using the Euclidian's similarity index and cluster analysis with Ward's minimum variance method.

The roots were kept in FAA (formalin: alcohol-ethanol: acetic acid, 0.5:9:0.5) (v:v:v) and stored for later analysis to assess mycorrhizal colonization. The roots were subjected to bleaching in a solution of KOH 10 % (w:v) for 24 h, washed in water, and subsequently immersed in HCl 2 % (w:v) for 5 min followed by staining in 0.05 % trypan blue in lactoglycerol (w:v) at 70 °C for

40–60 min. Root colonization was quantified by using the gridline-intersect method (Giovannetti and Mosse, 1980). The presence of fungal structures, such as arbuscules, hyphae, vesicles, and spores were investigated.

2.4. DGGE of AMF communities

DNA fragments corresponding to the 18S rDNA genes from AMF were amplified by PCR and Nested-PCR reactions according to Liu et al. (2015). The reactions were performed in a micro centrifuge 0.2 mL tube using GoTaq® Flexi DNA Polymerase (Promega, Madison, USA), according to the instructions of the manufacturer, by adding elution buffer (20 mM Tris–HCl, 50 mM KCl, pH 8.4). All material used in the preparation of the reactions was previously sterilized. The first reaction for amplification of the 18S rDNA consists of 1 µL (20 ng of total DNA) from each sample, 2 µL of each primer (0.2 µM), being AML1 (5′-ATCAACTTTCGATGGTAGGAT AGA-3′) and AML2 (5′-GAACCCAAACACTTTGGTTTCC-3′) (Lee et al., 2008), 10 µL of buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.4), 4 µL de MgCl2 (2 mM), 2 µL dNTPs (200 µM of dNTP), 0,8 µL of BSA (0.5 mg mL−1) (Promega), 0.25 µL (1.25u) GoTaq DNA polymerase Flex® (Promega, Madison, USA) and 27.95 µL of nuclease-free water for a final volume reaction 50 µL. The cycling was performed on a Mastercycler Ep Gradient (Eppendorf) thermal cycler. The following regime was used: initial denaturalization step at 94 °C for 3 min, followed by 30 cycles of a denaturalization at 94 °C (1 min), 50 °C (1 min), 72 °C (1 min) and a final elongation at 72 °C, for 10 min. To confirm the PCR amplification and quality, the products were examined by agarose gel electrophoresis (0.8 %), stained with ethidium bromide (0.5 µg mL−1) and visualized under UV light and UV light photo documentation imaging system (Loccus Biotecnologic L-Pix Chemi).

The PCR products resulted in DNA fragments of approximately 560 bp. To obtain a smaller DNA fragment for carrying out the DGGE technique, a second reaction of PCR reactions was performed (Nested- PCR) using a set of primers NS31-GC (5′-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGTTGGAGGGCAAGT CTGGTGCC-3′) (Kowalchuk et al., 2002) and Glo1 (5′-GCCTGCTTTAAACACTCTA-3′) (Cornejo et al., 2004) with the same reaction mixture already detailed. The nested-PCR was conducted under the following conditions: an initial denaturalization step at 94 °C (5 min), followed by 35 cycles of a denaturalization at 94 °C (45 s), then 52 °C (45 s), 72 °C (1 min) and a final elongation at 72 °C, for 30 min. The products were examined by agarose gel electrophoresis (1.5 %), stained with ethidium bromide and visualized under UV light to confirm the PCR amplification and quality. The PCR products were stored at −20 °C until the time for the DGGE analyses. The analysis by denaturing gradient gel electrophoresis (DGGE) was performed to characterize the soil AMF community structure or profile. A mixture of Glomeromycota reference markers was performed using approximately 300 ng of them.

The following reference markers were used according to da Silva et al. (2014): a strain of *Rhizophagus clarus*, *Acaulospora koskei* SCT406A, *Acaulospora tuberculata* SCT250B, *Gigaspora albida* PRN201A, *Gigaspora decipiens* SCT304A and *Dentiscutata heterogama* PNB102A. The *Rhizophagus clarus* strain was obtained from the in vitro collection of the Laboratory of Mycorrhizal Associations, Universidade Federal de Viçosa -Viçosa, Brazil. The other isolates were obtained from the International Culture Collection of Glomeromycota, CICG - www.furb.br/cicg, at the Universidade Regional de Blumenau, Blumenau, Brazil. Approximately 250 ng of DNA from each sample of the products obtained by the nested-PCR technique were analyzed by DGGE (Dcode™ System – BIO-Rad California, USA).

DGGE was performed in a DCode apparatus (Bio-Rad) using polyacrylamide gels containing 8 % acrylamide:bisacrylamide (w:v) in (37.5:1) Tris-acetate-EDTA (TAE) buffer 1× (Tris/acetic acid/EDTA, pH 8.0) with denaturing ranging from 35 to 55 %. Electrophoresis ran in TAE 1× buffer at a constant temperature of 60 °C, at 80 V (10 min), followed by 60 V (12h). The 16 × 16 cm gels were 0.75 mm thick. They were stained, after completion of electrophoresis, for 30–40 min in a 1× SYBR GOLD (Sigma–Aldrich) solution, visualized under UV light, captured and digitized, using a photo documentation imaging system (Loccus Biotecnologic L-Pix Chemi). The dendrograms were constructed with the Bionumeric software version 6.0 (Applied Maths, Inc., Austin, Texas, USA) using the Dice's similarity index and cluster analysis with the Ward's minimum variance method.

2.5. Data analysis and statistics

All statistical analyses were performed using the statistical program R (Index, R, and Team, T.R.C, 2014). The data of chemical characteristics of soil were subjected to ANOVA and means were compared by Tukey's test ($p < 0.05$). The Constrained Analysis of Principal Coordinates (CAP) was performed to analyze the effect of chemical soil properties on soil fungi community composition. Diversity indices were calculated in PAST (Paleontological Statistics) version 3.22 software (Hammer et al., 2001). The AMF species richness was determined per sample and the abundance of viable spores of each AMF morphotype was used to calculate the Shannon diversity index and equitability index.

3. Results

3.1. Soil chemical properties

The pH values of UND and REC areas were quite different (Table 2). The REC areas invariably present higher pH values. The phosphorus content varied among the areas in both sampling periods (Table 2) but was elevated in areas undergoing revegetation (REC2 and REC3) in the first sampling. Potassium (K), Calcium (Ca), Magnesium (Mg), Al3+, Calcium acetate extractor (H + Al), sum of bases (SB), effective cation exchange capacity CEC (t), and cation exchange capacity CEC (T) presented higher values for UND in both samplings. The saturation index of bases (V) presented lower values in UND relative to REC (Table 2).

3.2. Total fungal diversity

After discarding low-quality sequences, 652,112 sequences containing only the ITS1 region (using the primer ITS1F) were retained. Sequence filtering was performed to eliminate non-fungal sequences, resulting in 383,438 retained sequences.

The majority sequences were classified into Ascomycota (56.2 %), followed by Basidiomycota (17.9 %), other fungi group (7.5 %) (Mucoromycota, Mortierellomycota, Rozellomycota, Glomeromycota, and others), and unidentified fungi phylum groups (18.4%) (Fig. 2). In the first sampling, Basidiomycota and Ascomycota prevailed in UND whereas Ascomycota predominated in the REC areas. In the second sampling, Ascomycota prevailed in all areas but Basidiomycota was increased in REC2 and REC3. The Mortierellomycota was present in UND, with 10.4 % in the first and 11.8 % in the second sampling. Likewise, the Rozellomycota was only present in UND, with 0.6 % in the first and 0.1 % in the second sampling. In the sequencing analysis, a low number of Glomeromycota was detected in UND alone in the first sampling (0.1 %).

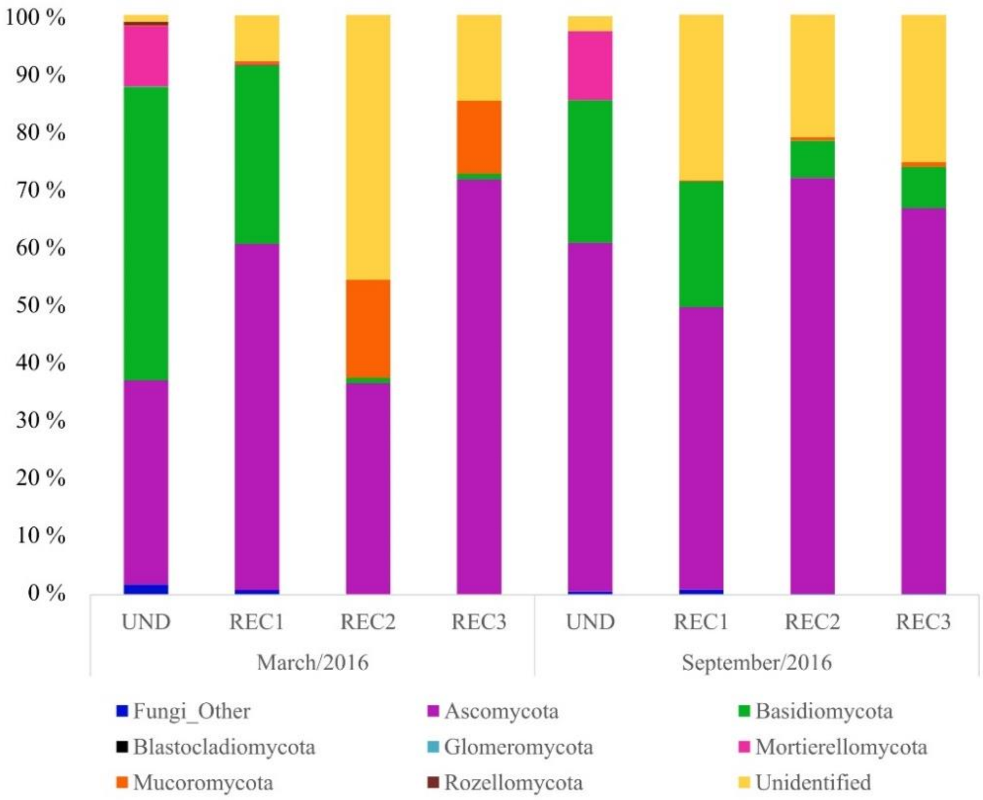

Fig. 2. Relative abundance of each phylum for fungi community composition corresponding to undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in the two periods of time (Mar/2016 and Sep/2016).

The species richness varied among the areas and sampling periods (Table 3). In the first sampling, the OTU richness was higher in UND and REC2, followed by REC3 and REC1 (Table 3). In the second sampling, no difference was observed among the areas. REC1 and REC3 presented increased OTU richness in the second sampling (Table 3). The comparison of the OTUs between areas in both sampling periods demonstrated that the number of fungal species in the core portion, accounting 56 OTUs, remained constant (Fig. 3). In UND, a decrease in OTUs was observed in the second sampling (dry season). In the areas undergoing recovery, an increased number of OTUs in REC1 and REC3 was observed, where vegetation covering had been in progress for six months (Fig. 3). REC2 did not change between the samplings.

Table 3

OTU richness from the sequencing analysis from the undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in the two periods of time (Mar/2016 and Sep/2016).

Area	OTU Richness	
	Mar/2016	Sep/2016
UND	275 Aa	241 Aa
REC1	94 Cb	184 Aa
REC2	242 Aa	245 Aa
REC3	162 Bb	243 Aa

*Means followed by the same capital letters in a column and lowercase letters on the lines do not differ significantly by the Tukey test ($p < 0.1$).

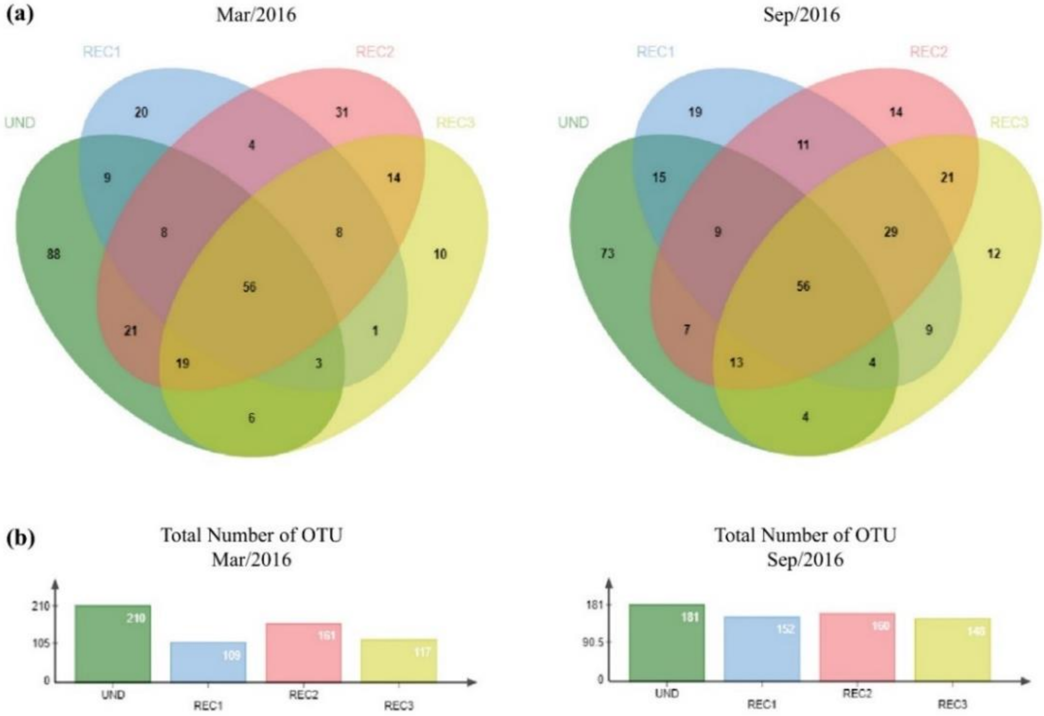

Fig. 3. a) Venn diagram depicting OTUs (similarity 97 %) that are shared or unique to soil sample and, b) the total number of OTUs found in undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in the two periods of time (Mar/2016 and Sep/2016).

The most abundant genera were the same in all areas during both samplings (Supplementary Table 2). UND was home to the greatest genera variance, namely *Auricularia*, *Castanediella*, *Clavulinopsis*, and *Myxocephala*, all of which are exclusive to this area. in the first sampling, *Fusarium*, *Bipolaris*, and *Aspergillus* were the most dispersed—- being found in all areas—and among the most abundant in REC areas, except *Aspergillus* in REC1 (Supplementary Table 2).

Chemical soil properties affected the distribution of OTUs of fungal communities (Fig. 4). The CAP showed a difference between the UND and REC areas in both samplings. The samples from the same site clustered together and showed a slight variation in REC1 in the second sampling (Fig. 4).

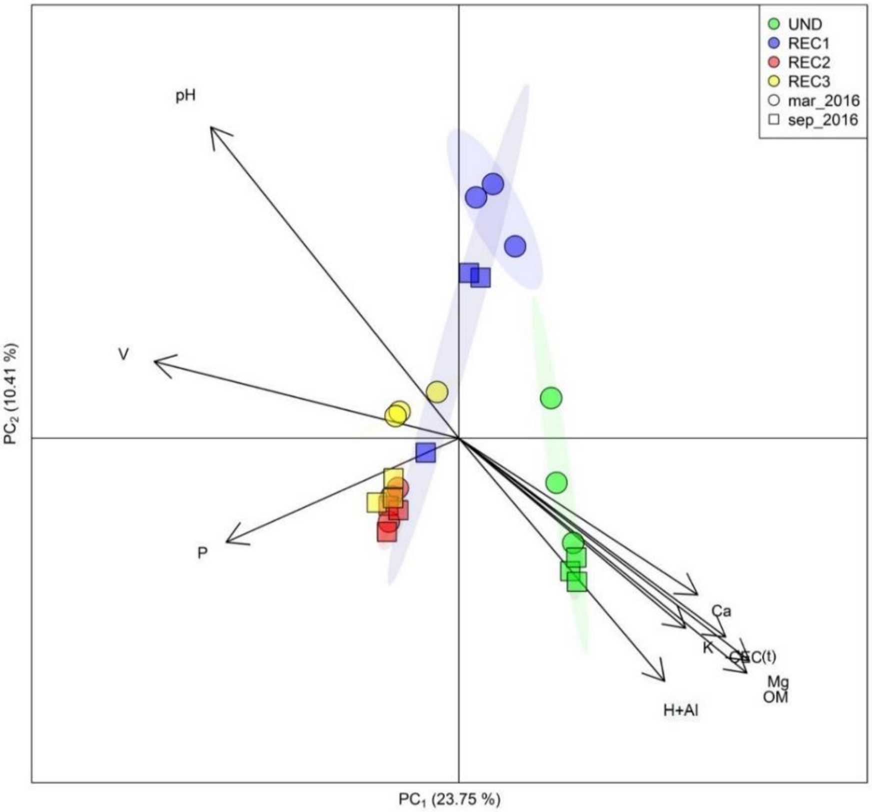

Fig. 4. Constrained Analysis of Principal Coordinates (CAP) of the relationship between soil fungal community structure and soil chemical properties. The soil properties are indicated with arrows, including soil pH, phosphorous content (P), base saturation index (V), potassium (K), soil organic matter (MO), effective cation exchange capacity (CEC (t)), magnesium (Mg), calcium (Ca), (H + Al).

In the first sampling, according to physicochemical properties, the distribution of UND samples were more dispersed than in the second sampling, while the REC areas presented varied distributions between the samplings. Overall, REC1 was more dispersed than other REC areas. In the second sampling, REC2 and REC3 presented a similar distribution. The second samples of REC1 were similar to the REC3 distribution of the first samples (Fig. 4). UND was strongly related by the organic matter, K, H + Al, K, Mg, CEC (t), and Ca, while the variation of REC areas was determined by the pH, base saturation, V, and P (Fig. 4).

3.3. AMF communities

A total of nine AMF morphospecies were observed by morphological analysis (Fig. 5). These morphotypes corresponded to three families. Glomeraceae presented higher specific richness, and Paraglomeraceae and Acaulosporaceae were represented by one species each. Glomeraceae was the only family with representatives in all areas sampled.

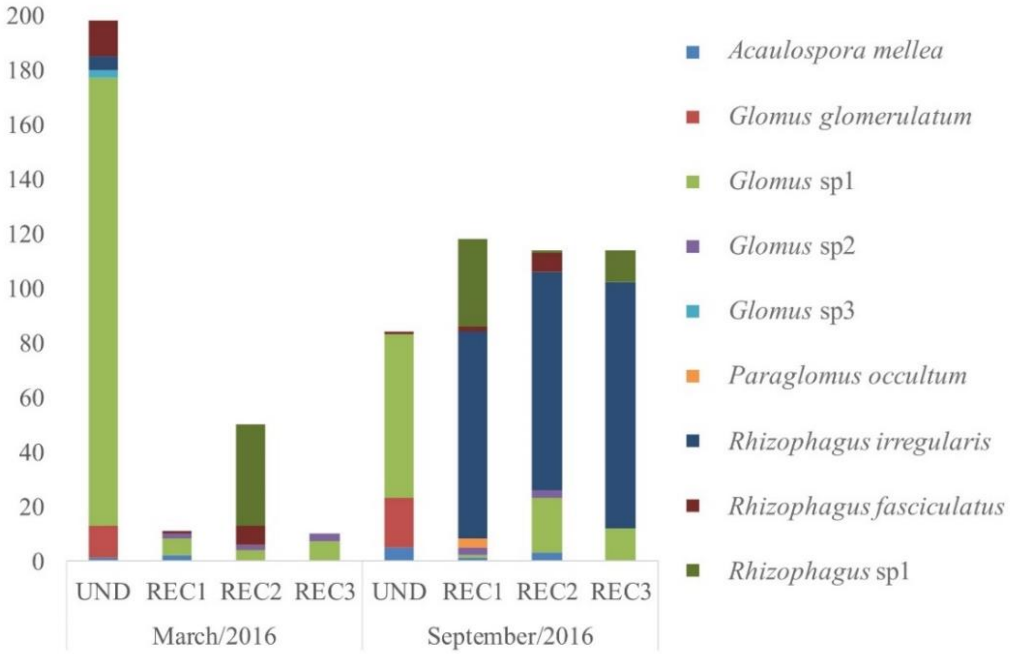

Fig. 5. Number of viable spores of each AMF species sampled in undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in two sampling periods (Mar/ 2016 and Sep/2016).

Glomus sp1, *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot), C. Walker & A. Schüßler, and *Rhizophagus* sp1 were the only species that accounted for > 50 % of the relative abundance. The presence of *R. irregularis* in all areas affected by IOMW in the second sampling was detected, while in UND it was detected only in the first sampling and displayed a low spore density (Fig. 5). *Glomus* sp1 was present in all areas in both samplings, with high sporulation in UND (Fig. 5). Shannon's diversity index and equitability index were higher in the second sampling (dry seasons) for all areas except REC1 (Table 4).

In both samplings, a higher number of spores was observed in UND compared to REC areas. A higher density of spores was observed in UND in March 2016. An increased number of spores was observed in REC in September 2016 (Table 4).

Table 4

Descriptors of AMF community. Percentage of root colonization (RC), spore density (SD), species richness (S), Shannon's index (H), Equitability (J), corresponding to undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in the two periods of time (Mar/2016 and Sep/2016).

	Mar/2016				**Sep/2016**			
	UND	**REC1**	**REC2**	**REC3**	**UND**	**REC1**	**REC2**	**REC3**
RC	53 Aa	N/A	44 Aa	16 Bb	34 Bb	N/A	35 Ba	80 Aa
SD	269Aa	11 Ba	29 Ba	11 Bb	180Ab	19 Ba	20 Ba	38 Ba
S	6	4	4	2	4	7	6	3
H	0.6878	1.169	0.8289	0.6109	0.7911	0.9739	0.9582	0.6606
J	0.5005	0.8429	03839	0.5707	0.5348	0.5979	0.8813	0.6013

*Means followed by the same capital letters on the lines in each periods of time do not differ significantly by the Tukey test ($p < 0.1$) and lowercase letters on the lines for the same area, between the two periods of time do not differ significantly by the Tukey test ($p < 0.1$); N/A, data not available.

The mycorrhizal colonization of REC1 was not evaluated due to its absence and the scarce vegetation cover in this area. In the first sampling, UND and REC2 presented higher colonization relative to REC3 (Table 4). In the second, REC3 presented higher colonization. A significant difference in the areas between the two samplings was observed. In UND, decreased colonization was seen whereas the converse was shown in REC3 (Table 4).

The banding patterns generated by DGGE revealed different OTUs as well as different profiles of AMF communities. The dendrogram was generated to estimate the similarity between the areas in both samplings (Fig. 6). No DNA from REC1 was amplified in the first sampling; however, there were some plants and some OTUs in the second sampling (Fig. 1). AMF were present in all disturbed areas. The dendrogram results showed that there were two clusters in the first sampling—REC2 grouped next to UND—with similarity nearing 50 % whereas REC3 comprised another group. In the second sampling, three main groups were observed; one represented by UND with similarity > 70 %; an- other by REC2 and REC3 with ~ 40 % similarity; and a third by REC1 with 50 % similarity (Fig. 6). In the first sampling, OTU richness was highest in UND, followed by REC2 then REC3 (Table 5). In the second sampling, no difference was observed among these areas. Between samplings, UND presented decreased OTU richness and REC3 presented increased OTU richness in the second sampling relative to the first (Table 5).

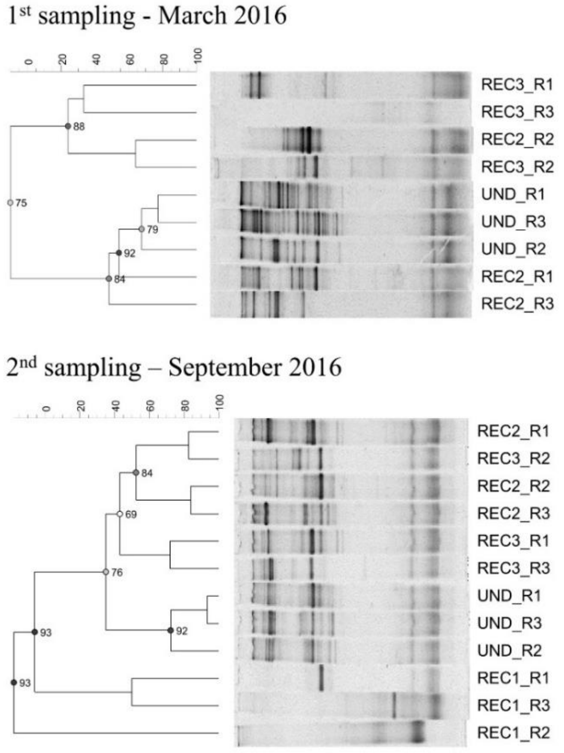

Fig. 6. Dendrogram following the DICE WARD analysis obtained from the DGGE band patterns of the AMF population, which shows the groupings of the areas in the first and second samplings. Undisturbed forest (UND); recovering areas (REC1, REC2, REC3); R1, R2, R3: sampling replicates.

Table 5

OTU richness from the DGGE analysis from the undisturbed forest (UND) and recovering areas (REC1, REC2, REC3) in the two periods of time (Mar/2016 and Sep/2016).

Area	OTU Richness	
	Mar/2016	Sep/2016
UND	20.666 Aa	15.333 Ab
REC1**	N/A	4.666
REC2	13.333 Ba	16.000 Aa
REC3	8.000 Cb	12.333 Aa

*Means followed by the same capital letters in a column and lowercase letters on the lines do not differ significantly by the Tukey test ($p < 0.1$).
** Due to absence of DNA in REC1, on March/2016, this area was not included in the statistical analysis; N/A, data not available.

4. Discussion

The areas affected by IOMW exhibit varying degrees of degradation which generates differences in chemical and biological characteristics (Fig. 1; Tables 1 and 2), and soil fungal diversity reveals that the revegetation process plays an important role in the development of the community's structure (Fig. 1; Table 1). The presence of fungi in all examined areas (Fig. 2) and species richness indices for the varying areas revealed an increase in OTUs following revegetation in REC1 and REC3 (Table 3). These results confirmed that different components of the root microbiome can be complementary in the acquisition of essential and limiting nutrients in the ecosystem (de Quadros et al., 2016; Casazza et al., 2017). Differences in soil microbiome among areas were mainly caused by the plant community, as observed in other mining site under a revegetation program (Vieira et al., 2018).

The predominance of the Ascomycota phylum, followed by Basidiomycota, is common in forests (Rosales-Castillo et al., 2018; Vieira et al., 2018). This information shows that REC areas are recovering their ecosystem functions. Another difference among areas and samplings was the presence of the Mucoromycota phylum in REC2 and REC3 in the first sampling; and of the Mortierellomycota phylum in UND in both samplings. Some fungi genera occur abundantly or exclusively in some areas, including *Auricularia*, *Castanediella*, *Clavulinopsis*, and *Myxocephala*, which were found only in UND (Supplementary Table 2). Auriculariales, are likely found in preserved forests and may be used as indicators of forest formation (Rosales-Castillo et al., 2018). The predominance of certain fungi genera in the soil interacting with certain plant species can ensure functional redundancy in different ecological contexts (Louca et al., 2018). Thus, if the fungal community composition changes under stressful conditions, different species can be recruited to produce the necessary compounds (Pradhan et al., 2014). Studies indicate the potential use of fungi as a clean and alternative technology for removing residues generated by mining and iron ore processing (Pradhan et al., 2014). Fungi and fungal products (e.g., enzymes) present in IOMW may be utilized as an efficient tool for the bioremediation of contaminated soil (Williams and Cloete, 2008; Moustafa, 2016; Verma et al., 2016).

The revegetation process showed the positive effect because although the fungal richness in the core portion (i.e., the presence of OTUs in all areas) remained constant among the samplings, the shared species increased in the second sampling due to the better vegetation covering after six months (Fig. 3). Soil microbes should be considered drivers of productivity diversity in terrestrial ecosystems (van Der Heijden et al., 2008). Microbial succession occurs and revegetation promotes the restoration of mine tailings (Li et al., 2016). The microbiota is sensitive enough to differentiate soil quality, therefore, chronosequence studies are important to optimize restoration processes (Li et al., 2014, 2016; van der Heijden and Hartmann, 2016). Organic matter, which determines the organic carbon content in the soil, was higher in UND than in REC areas (Table 2). The differences in fungi composition found between samplings (Fig. 3; Supplementary Table 2) could be attributed to climate, as observed in UND, and to the revegetation process.

The total diversity of fungi in REC areas and UND differed according to the chemical attributes of the soil (Figs. 3 and 4), as observed in mycorrhizal fungi in the Quadrilátero Ferrífero in both rainy and dry seasons (Silva et al., 2018). Microsites can be found in the soil and soil properties can also vary according to the seasons (Asmelash et al., 2016). The revegetation of IOMW areas has an impact on the physicochemical properties of the soil and microbial communities (Leal et al., 2016). AMF were dispersed in all areas (Figs. 5 and 6) and the increase in plant cover favors the establishment of obligatory symbiosis with plants, such as arbuscular mycorrhizal. Microbial composition, including AMF, can be affected by seasonal changes in soil and temperature variations (Cao et al., 2010; da Silva et al. 2014). The pH was highest in the REC areas (Table 2), which can affect AMF communities in agroecosystems and crops (Hazard et al., 2013), directly changing the physiological status of indigenous AMF by affecting spore germination (Giovannetti, 1983; Hepper, 1984), the regulation of AMF-specific phosphate transporters (Carrino-Kyker et al., 2017), the mobility and sorption of metals (Xu et al., 2017), and—indirectly—the AMF community by regulating soil nutrient bioavailability. As such, soil pH is the key environmental factor that drives microbial communities (Jansa et al., 2014; Stürmer et al., 2018).

The most common family found in the studied areas was Glomeraceae (Fig. 5). As previously described, pH is a significant factor affecting AMF communities (Table 2) and the high richness of Glomeraceae has been attributed to a pH closest to neutrality (Siqueira et al., 1989). *Glomus* and *Rhizophagus* were the most abundant genera (Fig. 5) and these genera can be the most common in disturbed areas (Horn et al., 2017). The morphospecies *Glomus* sp1 found in this study corresponds to *Glomus* sp2 and *Glomus* sp1 of the surveys conducted by Teixeira et al. (2017) and Vieira et al. (2017), respectively (Kemmelmeier, K; personal observation on reference slides). This morphospecies presents high frequency of occurrence in ecosystems impacted by iron- mining activity and surrounding ecosystems in the Quadrilátero Ferrífero (Teixeira et al., 2017; Vieira et al., 2017). Although present in all areas in this study, the number of spores of *Glomus* sp1 recovered in REC in September was lower than that of *R. irregularis* (Fig. 5). Glomeraceae species that sporulate rapidly may be favorably selected in impacted environments, with some genera or species showing the ability to survive and propagate using intraradical vesicles (de Souza et al., 2005) or strong sporulation in the roots of vascular plants, as observed in *Rhizophagus* spp. (Schüßler and Walker, 2010). Different isolates of *R. irregularis* from the same area may exhibit substantial divergences in functionality, which indicates adaptation to biotic and abiotic changes (Chen et al., 2018). *Rhizophagus fasciculatus* (Thaxter) C. Walker & A. Schüßler, found in UND, REC1, and REC2, can produce sporocarps (Gerdemann and Trappe, 1974) and have been reported as frequent food sources for rodents (Mangan and Adler, 2000). This movement of spores has been facilitated by small mammals and is, therefore, critical in the reintroduction of AMF following certain disturbances (Allen, 1987). *Acaulospora mellea* Spain & N.C. Schenck, the only Acaulosporaceae found in this study, showed high frequency of occurrence in other studies in the Quadrilátero Ferrífero (Teixeira et al., 2017; Vieira et al., 2017). *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker was already sampled in ecosystems impacted by mining activities (Leal et al., 2016; Melloni et al., 2003) and displayed tolerance to the adverse conditions inherent in these environments. *Glomus glomerulatum* Sieverd. and *Glomus* sp3 were exclusively sampled in UND and these species may not present the plasticity necessary to tolerate the conditions imposed by IOMW.

Root plants were colonized by AMF (Table 4), which reveals the presence of this association and its participation in the functioning of the ecosystem, since > 80 % of terrestrial plants form mycorrhizal associations (Brundrett, 2002). In the Atlantic Forest, the presence and diversity of AMF is high and even influenced by phytophysiognomies type (Duarte et al., 2018). In disturbed areas, such as REC1, the initial incidence of plants was quite low, which led to low levels of spore densities and fungal AMF richness (Figs. 1 and 6; Table 4). The Shannon's diversity index and equitability index were higher in the second sampling for all REC areas except for REC1 (Table 4), indicating that a successional process is underway in these areas, with establishment of AMF species that interact with one another and with both biotic and abiotic components. The decreased Shannon diversity index and equitability index in the second sampling of REC1 may have resulted from the establishment of highly sporulating AMF species, including *R. irregularis*, which accounts for ~ 64 % of the relative abundance (Fig. 5).

The composition and variability of AMF species richness observed in each area (Table 5) may be due to the different species of vegetation and to the degree of contamination by IOMW. There is a relation between plant cover and AMF communities (Tables 1 and 5) since no AMF DNA was found in the REC1 (Fig. 6) in the first sampling, where vegetation was absent. The similarity higher than 70 % presented by UND in both samplings shows the occurrence of many species of common AMF among sampled points. It also demonstrates that the community structure in forest areas is highly dynamic (Rosales-Castillo et al., 2018). The similarity between REC2 and UND of only 50 % in the first sampling shows the difference in the AMF community profile and also shows the presence of a common community, indicating the beginning of the settlement by AMF in this REC site. AMF are important for the revegetation process; they are considered pioneers and a potential mechanism to improve the restoration of degraded land (Asmelash et al., 2016; Wang, 2017). The DGGE revealed the presence of some bands (OTUs) in REC1 in the second sampling and, even in small quantities, may be due to the late process of natural revegetation that occurred in this area and by the proximity to the UND, which could be receiving leached material. In the first sampling, UND presented the highest OTU richness since it was undisturbed. The REC areas were in the initial stages of the revegetation process. As mentioned earlier, different forms of spore dispersion may contribute to the similarity among REC2 and REC3 with UND (~ 40 %). Spores formed by AMF can be dispersed by wind, predominantly in some arid ecosystems (Warner et al., 1987), or by animals (Mangan and Adler, 2000). It is noteworthy that human activities, such as agricultural practices, also participate in the dispersal of these propagules (Mangan and Adler, 2002; Rosendahl et al., 2009). Increases in AMF diversity was observed in the recovery areas throughout the revegetation progress (Figs. 5 and 6; Table 4). AMF in the rhizosphere represents a pool of species recruited by plants (Xu et al., 2017).

The combination of technosol and plant species that were established in the revegetation system can stimulate the selection of certain species of AMF at the expense of others (Supplementary Table 1) due to their capacity to adapt to soil disturbances (Purin et al., 2006). Revegetation increases plant litter and root exudates, which provide substrates to microorganisms, increase the microbial abundance (dos Santos et al., 2016; Leal et al., 2016; Silva et al., 2018), and select specific microbial populations (Dong et al., 2017). The revegetation process can accelerate environmental recovery and ecosystem functions in areas contaminated by IOMW. The understanding of AMF community structure is necessary for the management of IOMW-affected areas.

5. Conclusions

The fungal community diversity in IOMW-affected areas was altered by the revegetation process, which increased the total fungal diversity as well as AMF. Therefore, the diversity of these groups can be used as a bioindicator of the rehabilitation of an IOMW-affected area.

Supplementary data to this article can be found online at https:// doi.org/10.1016/j.apsoil.2019.05.008 (attached at the end of this paper).

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SUPPLEMENTARY MATERIAL

Supplementary Table 1

Seeds used in the revegetation process.

Scientific Name	**Common Name**	**Habit**
Avena spp.	Aveia-amarela, aveia-preta	Herb
Cajanus cajan (L.) Millsp.	Guandu	Shrub
Calopogonium mucunoides Desv.	Calopogonio	Herbaceous creeper
Canavalia ensiformis (L.) DC.	Feijão de porco	Herb
Crotalaria spp.	Chocalho de cascavel	Shrub
Cynodon dactylon (L.) Pers.	Capim-vaqueiro	Herb
Glycine wightii (Wight & Arn.) Verdc.	Soja-perene	Shrub
Lolium multiflorum Lam.	Azevém	Herb
Mucuna pruriens (L.) DC.	Mucuna-cinza, mucuna-preta	Liana
Paspalum notatum Flüggé	Batatais	Herb
Cenchrus americanus (L.) Morrone	Milheto	Herb
Raphanus sativus L.	Nabo-forrageiro	Herb
Sorghum bicolor (L.) Moench	Sorgo-forrageiro	Herb
Stylosanthes spp.	Estilosante	Herb
Vicia sativa L.	Ervilhaca	Shrub

Supplementary Table 2

Ten most abundant genera in each area / sampling from the sequencing analysis. The genera are ordered according to their abundance (number of OTUs reads). Undisturbed forest (UND); recovering areas (REC1, REC2, REC3); the two periods of time (Mar/2016 and Sep/2016).

Sampling	UND	REC1	REC2	REC3
Mar/2016	*Geastrum*	*Geastrum*	*Rhizopus*	*Aspergillus*
	Mortierella	*Pestalotiopsis*	*Aspergillus*	*Rhizopus*
	Trichoderma	*Bipolaris*	*Fusarium*	*Fusarium*
	Cladophialophora	*Fusarium*	*Bipolaris*	*Pseudallescheria*
	Preussia	*Preussia*	*Edenia*	*Bipolaris*
	Penicillium	*Rhodosporidiobolus*	*Alternaria*	*Rhodotorula*
	Mycena	*Pseudallescheria*	*Curvularia*	*Exserohilum*
	Myxocephala	*Peniophora*	*Westerdykella*	*Alternaria*
	Castanediella	*Penicillium*	*Exserohilum*	*Curvularia*
	Robillarda	*Westerdykella*	*Pseudocercospora*	*Paraconiothyrium*
Sep/2016	*Trichoderma*	*Ceratobasidium*	*Fusarium*	*Fusarium*
	Geastrum	*Fusarium*	*Edenia*	*Alternaria*
	Auricularia	*Aspergillus*	*Alternaria*	*Bipolaris*
	Clavulinopsis	*Dactylella*	*Bipolaris*	*Aspergillus*
	Mortierella	*Pseudallescheria*	*Aspergillus*	*Rhizopus*
	Penicillium	*Bipolaris*	*Poaceascoma*	*Cryptococcus*
	Clonostachys	*Veronaea*	*Rhizopus*	*Exserohilum*
	Cladophialophora	*Trichoderma*	*Lectera*	*Edenia*
	Tomentella	*Podospora*	*Rhizoctonia*	*Periconia*
	Pestalotiopsis	*Roussoella*	*Cryptococcus*	*Ceratobasidium*

3. ARTIGO CIENTÍFICO 2

Title: Mycobiome and AMF community in areas affected by iron ore mining waste, three years after the collapse of the Fundão Dam

Article to be submitted.
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Mycobiome and AMF community in areas affected by iron ore mining waste, three years after the collapse of the Fundão Dam

ABSTRACT

After the collapse of Fundão dam in Mariana, Brazil, the deposition of iron ore mining waste (IOMW) disrupted the aesthetics of the landscape, leading to nutritionally deprived habitats, altered ecosystem functions and compacted soils. This condition hinders the establishment of plants. The monitoring and insights of the composition, diversity and structuring of microbial soil communities, as arbuscular mycorrhizal fungi (AMF), are useful to evaluate and improve the rehabilitation process. This study aimed to compare the soil chemical properties, the total fungi species and AMF richness in IOMW with non-IOMW affected ecosystems, three years after the disaster. Soil and root samples were collected in dry and rainy seasons, in 2017 and 2018, in Paracatu de Baixo, Minas Gerais State, Brazil and were analyzed over 3 years. Five areas were sampled: IOMW-affected (REC1, REC2, PASTrec) and non-IOMW adjacent forest (UND) and pasture (PAST). Soil chemical analysis was performed to be analyzed together with biological data. Total fungal composition was evaluated by the sequencing of the ITS region, by Illumina MiSeq. The AMF community was evaluated by spore density and root colonization and DGGE. Higher values of pH were found in IOMW-affected and higher values of organic matter (OM) were observed in non-IOMW affected sites. The revegetation process produced a positive effect on soil fungal community, but this progress requires monitoring and intervention to promote suitable rehabilitation. AMF sporulation was positively correlated with high OM and lower pH. In the rainy season, it was observed high diversity in the fungal community and AMF colonization. Increased similarity in AMF features was observed among REC and UND sites over the samplings. Greater similarity of total fungi was observed between IOMW-affected and PAST, suggesting that the revegetation process may be heading towards a pasture condition. Periodic analysis is important, and soil microbiota, mainly AMF features, should be considered as an indicator of the recovery process evolution.

Keywords: Arbuscular Mycorrhizal Fungi, Chronosequence analysis, IOMW, ITS Sequencing, Management practices, Mining area, Rehabilitation

Highlights

- Mycobiomes are aligned with the recovery process.
- Similarity of the AMF community increases among REC and UND areas due to the recovery process.
- AMF in areas affected by IOMW contribute for the progress of revegetation process.
- The fungal diversity of REC areas is more similar to PAST.

1. Introduction

Tailings dam is the conventional solution for iron ore mining waste (IOMW) disposal in most Brazilian iron ore reserves. In “Quadrilátero Ferrífero”, a region in Minas Gerais rich in iron source, tailings dams are common, even with the high risk of collapse due to erroneous design, operation, monitoring and maintenance (Gomes et al., 2016). Alternatives for dams have been proposed, but their technical and economic viability must be analyzed before implementation (Gomes et al., 2016).

Four years have passed since the collapse of Fundão dam in Mariana, Minas Gerais state, Brazil, which culminated in the contamination of soils, rivers and destruction of villages and vegetation along the way (IBAMA, 2015). After the disaster, a revegetation program was started and carried out in the affected sites. In Paracatu de Baixo, a district of Mariana affected by the wave of mud with IOMW, monitoring started shortly after the implementation of the revegetation program, still in early 2016 (Prado et al., 2019). Since then, semiannual samplings have been carried out.

The recovery strategies involve soil restructuring, soil fertility, microbial populations, soil management, nutrient cycling and searching to restore the land so that it is as close as possible to its original condition (Sheoran, 2010). Although the previous condition is not the ideal in terms of preservation (i.e. degraded pasture), the use of different references, considering the degree of conservation (i.e. forest, pasture), can be even more informative. The revegetation of mining areas is often limited by soil physical and chemical properties, low levels of nutrients and poor soil physical structure (Mensah, 2016). Biological analysis and physiological characteristics of these areas can be used to direct the rehabilitation and reforestation activities (Mensah, 2016).

The analysis of this area, carried out in 2016, revealed a positive relation between the advance of the vegetation and increased soil fungal communities and arbuscular mycorrhizal fungi

(AMF), in the first year of revegetation (Prado et al., 2019). Comparison of microbial community composition between rehabilitating and non-IOMW affected sites contributed to the evaluation of rehabilitation success in mine lands (Sheoran, 2010).

Fungi are among the main ecological agents in the environment. Total fungi sequencing and bioinformatics analysis offer possibilities in the area of mycology (Nilsson et al., 2019 b). Insights related to soil chemical factors, composition, diversity and structuring of microbial communities are useful for evaluating and measuring the success of rehabilitation programs. This strategy can also provide a valuable feedback for the improvement of the rehabilitation practices (Gastauer et al., 2019).

Therefore, we hypothesized that the monitoring and chronosequence analysis of chemical and biological variables of the soil is fundamental to evaluate the direction of the revegetation process and the area under the revegetation program resembles the pasture area. So, this study aimed to analyses if the chemical and biological is indicating that vegetation cover is recovering the area. For this we characterize soil chemical properties, the total fungi and AMF communities in sampled area. It was used traditional and NGS techniques and conduced a comparation among affected-IOMW (REC) and non-IOMW affected sites covered by native Atlantic Forest vegetation (UND) and a family farm pasture (PAST). The samplings were conducted in rainy and dry seasons, in 2017 and 2018 in a chronosequence strategy for three years.

2. Materials and methods

The monitoring of the analyses started in 2016 (Prado et al., 2019). In 2017, (March and September) samplings were carried in REC1, REC2 and UND, the same areas of 2016, except for REC3, which was not possible to access due to physical impediment. In 2018 (March and September), samplings were conducted in the same areas as in 2017, and in two new areas, an adjacent family farm pasture (PAST) and an IOMW-affected family farm pasture (PASTrec).

2.1. Site description and sampling of soil and plant

The comparative analyses were conducted in these areas over two years (2017 and 2018) in two different period of times, rainy (March) and dry seasons (September). It was possible to compare with the initial condition right after the IOMW deposition (2016), as described by Prado et al. (2019). In order to analyze the direction and conditions of the ongoing revegetation process, a search for satellite images was carried out before the dam collapse (Supplementary Fig. 1). Analyzing images from 2015 to 2018 it is possible to verify that, in general, the REC areas had undergrowth cover before the accident, a similar condition to PAST, the non-IOMW affected family farm pasture that was sampled. The sampled areas were characterized by its vegetation cover and some observations of each area (Table 1; Fig. 1). Triplicate sample for soil and mix of roots were carried out for each site. Each soil sample was composed of five soil subsamples (depth 0–15 cm) and used for chemical analysis, AMF spore extraction and DNA extraction. These samples were stored in thermal boxes for transportation to the laboratory.

2.2. Soil chemical properties and DNA extraction and sequencing

The methodology described in Prado et al. (2019) was used for soil chemical analysis, soil DNA extraction and sequencing of the ITS region. The trim of the reads, pre-processing, quality and chimera filtering, and further analysis were performed for the analysis of the sequencing data, using the bioinformatics software packages. The overall analysis was conducted using Quantitative Insights Into Microbial Ecology Software (QIIME 1.9.1 version) (Caporaso et al., 2010), following the Brazilian Microbiome Project protocol for ITS sequences (Pylro et al., 2014). Quality ITS reads were aligned and clustered into OTU (Operational taxonomic unit) (97 % similarity). Consensus sequences were generated by USEARCH (version 8.1.1861), and assigned against UNITE database (https://unite.ut.ee/ - 18.11.2018 version) (Nilsson et al., 2019 a). This database was analyzed and some “unclassified” fungal sequences from this study were manually investigated by BLASTn in GenBank (blast.ncbi.nlm.nih.gov). Good’s coverage index was calculated to assess the coverage reached at the selected rarefaction level. Based on the *biom* file, the taxonomic profile, cluster, abundance and diversity index were estimated and calculated using the R software (Index and Team, 2014) and Microbiome Analyst web framework (microbiomeanalyst.ca/faces/home.xhtml) (Dhariwal et al., 2017).

2.3. Arbuscular mycorrhizal fungi (AMF)

The density of spores, root colonization and DGGE were analyzed for AMF, as described by Prado et al. (2019).

2.4. Chronosequence analyses

In order to evaluate and monitor the changes in the area of study over 3 years, all sequencing and chemical soil data were plotted together. Data from 2016 (Prado et al., 2019) for the same area were incorporated in new sequence analysis, using the methodology previously described.

Table 1

Characterization of sampled areas corresponding to undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018.

Period of sampling	Area	IOMW	Vegetation cover during the samplings
Mar/2017	UND	No	Natural forest with typical species of the region.
	REC1	Yes	Initial natural revegetation process. Very low plant cover and spaced vegetation with grasses. Presence of exposed IOMW.
	REC2	Yes	Presence of leguminous and grasses of low and medium size (intermediate).
Sep/2017	UND	No	Natural forest with typical species of the region.
	REC1	Yes	Sugar cane plantation (approximately one m in height).
	REC2	Yes	Presence of leguminous and grasses more advanced than last sampling, but under drought conditions.
Mar/2018	UND	No	Natural forest with typical species of the region.
	REC1	Yes	Sugar cane plantation in intermediate stage (approximately two m in height) and more vigorous than in the dry season.
	REC2	Yes	Presence of leguminous and grasses more advanced than last sampling and more vigorous than in the dry season.
	PAST	No	Pasture area with low vegetation cover and few specimens, but totally vegetated. Presence of grazing animals.
	PASTrec	Yes	Covered revegetation pasture with grasses and low density of plants.
Sep/2018	UND	No	Natural forest with typical species of the region.
	REC1	Yes	Sugar cane plantation (approximately two m in height), under drought conditions. Part of the sugar cane plantation in the sampled area already removed.
	REC2	Yes	Presence of leguminous and grasses more advanced than last sampling, under drought conditions and presence of grazing animals.
	PAST	No	Pasture area with low vegetation cover and few specimens, not totally vegetated, under drought conditions. Presence of grazing animals.
	PASTrec	Yes	Low vegetation cover with grasses and low density of plants, under drought conditions. Indicative of intense anthropogenic interference. Presence of grazing animals.

Fig. 1. Representation of sampled areas corresponding to undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018.

2.5. Data analysis and statistics

All statistical analyses were carried out using the R statistical program (Index and Team, 2014). The Scott Knott test was used for the multiple comparison of media.

The PCoA was performed to analyze chemical and biological soil data from different areas over the samplings. The correlation analysis was implemented with AMF data and soil chemical features. The following environmental variables were used in the analysis: soil pH, P, K, Ca, Mg, H+Al, CEC (t), V, OM.

3. Results

3.1.Soil description and chemical characterization

REC1 was naturally regenerated after the IOMW were deposited. Although no revegetation program was installed, this area was directly affected by forest site (UND) and it was possible to observe some plants in 2016 and March/2017 (Fig. 1). In September/2017, sugar cane was planted in this area. The same vegetation cover reported in 2016 (Prado et al., 2019) was observed in REC2, despite the changes detected throughout the rainy and dry seasons and caused by cattle grazing. In general, the vegetation was greener and more vigorous in March (rainy) than in September (dry) (Fig. 1). This observation was not so clear for UND.

PAST presented grass coverture, but the density was higher in rainy seasons than in dry seasons, although cattle were grazing all the time. The revegetation process applied in PASTrec was similar to that used in REC2, with grass and legumes, and the fertilization process was expressive (Table 1). There was observed a loss in the diversity of vegetation cover in PASTrec between the samplings in 2018 and a predominance of *Urochloa* sp.

The difference in soil chemical proprieties between IOMW and non-IOMW affected sites is still considerable, after 3 years of revegetation. Higher values of pH, P and V are observed in IOMW-affected sites (Table 2). Soil pH was 4.5 - 5.4 in non-IOMW affected (UND and PAST) and 6.6 – 8.0 in IOMW affected sites (REC and PASTrec) (Table 2). On the other hand, higher Mg, Al, H+Al, CEC and OM rates were observed in non-IOMW affected sites (Table 2). Although used as control, difference between UND and PAST was also found, such as higher OM content in UND (Table 2).

Table 2

Soil chemical characteristics. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018.

Soil characteristics	Mar/17			Sep/2017			Mar/18					Sep/2018				
	UND	REC1	REC2	UND	REC1	REC2	UND	REC1	REC2	PAST	PASTrec	UND	REC1	REC2	PAST	PASTrec
pH (H_2O)	5.3	7.7	7.3	5.4	8.0	7.0	4.8	7.5	6.9	5.3	6.6	4.5	7.3	6.6	4.9	7.0
P (mg dm^{-3})	3.4	8.1	6.8	2.7	11.9	7.5	1.6	6.2	6.2	1,4	5.4	1,7	7.5	7.2	1.4	6.7
K (mg dm^{-3})	71	48.7	33.7	76.7	169	46.3	52.6	23.0	13.3	78.0	22.0	60.3	27.0	24.0	55.3	41.3
Ca^{2+} ($cmol_c$ dm^{-3})	0.9	1.0	1.0	1.7	1.2	0.9	0.6	0.7	0.6	0.9	0.8	0.8	0.8	0.8	0.8	1.2
Mg^{2+} ($cmol_c$ dm^{-3})	0.7	0.0	0.1	0.8	0.1	0.1	0.4	0	0.1	0.3	0.1	0.4	0.0	0.1	0.2	0.1
Al^{3+} ($cmol_c$ dm^{-3})	0.9	0.0	0.0	0.5	0.0	0.0	0.8	0	0	0.3	0	0.9	0	0	0.4	0
H+Al ($cmol_c$ dm^{-3})	4.9	0.2	0.2	4.4	0	0	6.1	0.3	0.4	3.4	0.7	6.8	0.7	1.0	4.1	1.2
SB ($cmol_c$ dm^{-3})	3.3	1.2	1.2	2.6	2.1	1.1	1.2	0.8	0.7	1.4	1.0	1.4	0.9	1.0	1.1	1.4
CEC(t) ($cmol_c$ dm^{-3})	3.9	1.2	1.2	3.0	2.1	1.1	2.0	0.8	0.7	1.7	1.0	2.3	0.9	1.0	1.5	1.4
CEC(T) ($cmol_c$ dm^{-3})	7.6	1.3	1.4	7.0	2.1	1.1	7.3	1.0	1.2	4.9	1.7	8.2	1.6	2.0	5.3	2.6
V %	25.0	92.7	87.7	35.7	100	100	16.3	81.7	63.3	28.7	59	16.6	60.0	50.3	21.9	53.6
OM (dag kg^{-1})	4.1	0.9	1.2	3.5	1.1	1.4	3.1	1.1	1.2	2.2	1.2	3.2	0.5	0.6	1.75	0.8

Extractors used: P, K, = Mehlich1; Ca^{2+}, Mg^{2+} e Al^{3+} = KCl 1 mol.L^{-1}; H + Al = Calcium acetate 0.5 mol.L^{-1}; SB = Sum of exchangeable bases; CEC (t) = Effective cation exchange capacity; CEC (T) = Cation exchange capacity at pH 7.0; V = base saturation index; OM = organic matter.

3.2.Total fungal community composition

3.2.1. Taxonomic composition

After the discard of the low-quality sequences, the sequences were aligned and clustered into OTU. Following the raw data processing and filtering steps, a total of 412.079 fungal ITS2 sequences remained for community analysis. The rarefaction analysis indicated that the libraries provided an adequate sampling of fungal diversity of all the samplings under study and high Good's coverage rate, ranging from 0.961 - 0.999 (Table 3). The data correspond to 1.593 observations. Only filtered Fungi, which account for 92 %, were analyzed. 1.534 OTU were identified at phylum level. Among these total Fungi, Ascomycota was the most abundant, with 75.8 % (1252 OTU), followed by Basidiomycota, 10.6 % (225 OTU), while Mortierellomycota, Blastocladiomycota, Glomeromycota, Mucoromycota and others accounted for 5.5 % (57 OTU). Below the phylum level, the success for taxonomic assignment decrease at lower taxonomic levels and 804 OTU was identified at the genus level, and 350, at species level.

Table 3

Information from data sequencing: samples Good's coverage, Chao1 Index and Observed OTU. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018.

Year	Period	Area	Good's Coverage	Chao 1	Observed OTU
2017	Mar	UND	0.965 - 0.978	428.5	346
		REC1	0.977 - 0.983	354.3	291
		REC2	0.964 - 0.982	467.2	334
	Sep	UND	0.965 - 0.971	412.1	305
		REC1	0.976 - 0.977	349.3	232
		REC2	0.973 - 0.978	400.7	298
2018	Mar	UND	0.961 - 0.974	463.0	333
		REC1	0.980 - 0.990	328.1	270
		REC2 †	0.995 - 0.997	102.1	93
		PAST	0.976 - 0.977	379.7	310
		PASTrec	0.984 - 0.999	225.9	203
	Sep	UND	0.988 - 0.996	246.4	229
		REC1	0.978 - 0.979	276.6	197
		REC2	0.970 - 0.976	423.4	306
		PAST	0.982 - 0.986	267.4	222
		PASTrec	0.963 - 0.986	504.9	350

†One repetition dropped out of sequencing analyses.

3.2.2. Fungal richness and diversity

OTU distribution in each sampling-area showed a higher diversity in rainy samplings in 2017 (Table 3). The same behavior was observed in 2018, except in REC2 and PASTrec, in accordance with Chao 1 estimator (Table 3). The abundance of fungal groups at lower levels, such as Family / Genus / Species, was analyzed in each area. The most represented families and their respective most abundant genus in all studied area were: Nectriaceae (12 %, g. Fusarium), Hypocreaceae (11 %, g. *Trichoderma*, *Myxocephala*), Mortierellaceae (8 %, g. *Mortierella*). The two most abundant species were *Fusarium oxysporum*, with high abundance in all areas, except in UND, and *Fusarium incarnatum*, which was abundant in REC areas. The genus *Trechispora* and the species *Mortierella horticola* are found in non-IOMW. *Poaceascoma helicoides* are found only in IOMW.

Comparison of the levels of diversity over the years between IOMW-affected and non-IOMW affected sites showed greater similarity between IOMW-affected and PAST. Regarding the UND area, REC1 presented similar decrease from March to Sep/2017. REC 2 showed an increase in Sep/2018 over the previous samplings. Although analyzed for one year, PASTrec showed a significant increase in similarity, compared to UND (16 - 27.6 %) and PAST (28.3 - 49.4 %) (Fig. 2). The set of primers used on sequencing analysis were not ideal for covering the Glomeromycota phylum. However, 27 OTU were distributed in all areas, in March/2018. These OTU were formed by three classes of Glomeromycota: Archaeosporomycetes (1), Glomeromycetes (7), Paraglomeromycetes (5), and others, classified as unidentified Glomeromycota groups (14).

3.2.3. Effect of soil chemical properties and fungal OTU on areas and samplings

According to the analysis (Fig. 3a and 3b), chemical characteristics displayed distinct trends at non-IOMW and IOMW-affected sites, in 2017 and 2018 (Fig. 3a). In September/2017, REC1 differed from other areas, but still showed greater similarity with REC areas, compared to non-IOMW. UND and PAST were similar in chemical factors (Fig. 3a) but differed in their fungal community (Fig. 3b). IOMW-affected sites showed similar distribution in fungal composition over samplings (Fig. 3b) and differed from non-IOMW affected sites.

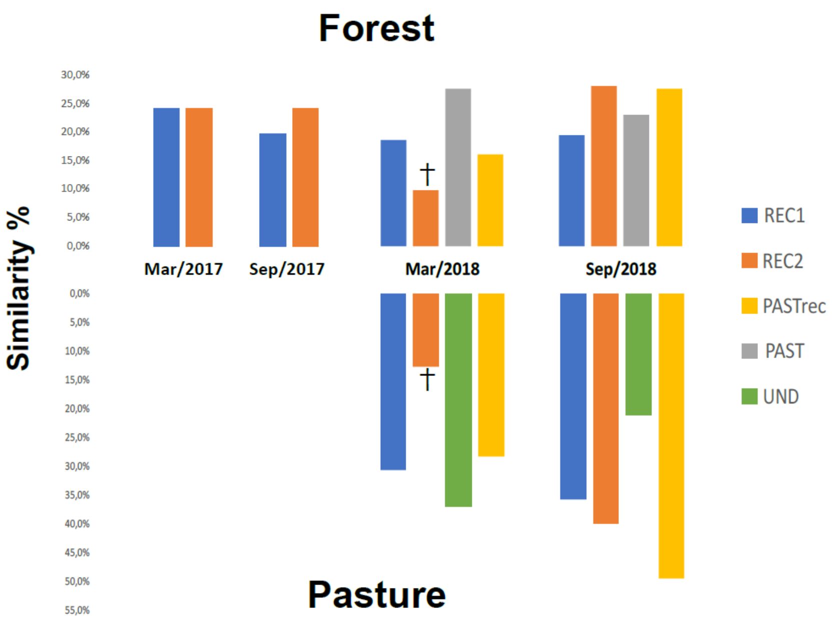

Fig. 2. Similarity (%) among OTU diversity in IOMW sites against non-IOMW. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018.

†One repetition dropped out of sequencing analyses.

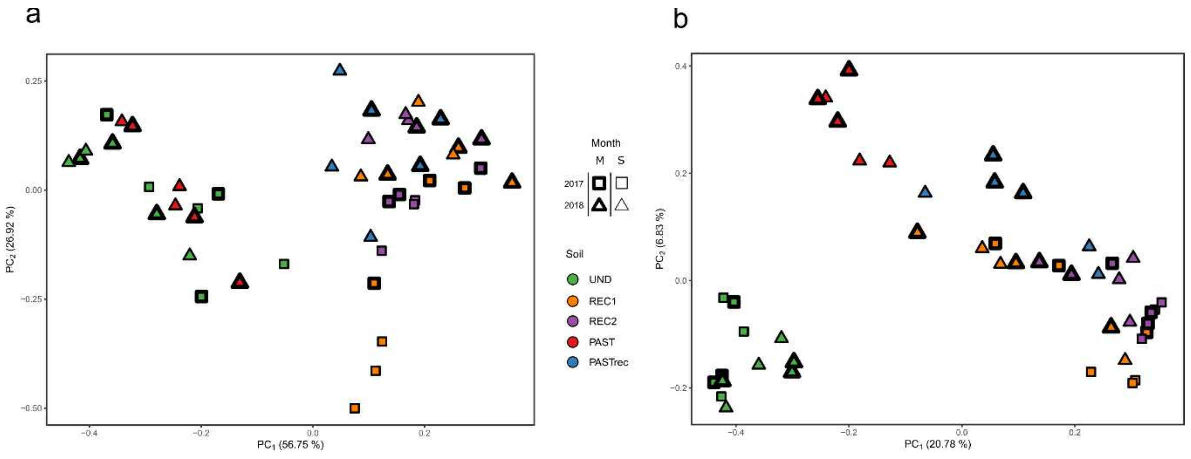

Fig. 3. PCoA plot based on Bray-Curtis distance from (a) Chemical and (b) fungal sequencing data. Different colors and shapes correspond, respectively, to areas and sampling periods. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018. Percentage values in parenthesis refer to the explanation of each axis.

3.3. AMF composition

Mycorrhizal colonization was observed in all areas. No relation was found between spore and colonization analysis. UND presented higher spore density in all samplings, followed by PAST. REC areas presented lower SD (Table 4). Colonization rates were higher in March (rainy season) than in September (dry season), in both years, with exception of REC1, where sugar cane was implemented in Sep/2017 (Table 1 and 4).

Table 4

Percentage of root colonization (RC), spore density (SD) and Chao1 index of DGGE analyses of arbuscular mycorrhizal fungi. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018.

Year	Period	Area	RC	SD	Chao1
2017	Mar	UND	67.7 a	206 b	22 a
		REC1	37.3 b	39 e	9 d
		REC2	76.7 a	45 e	20 b
	Sep	UND	24.3 c	300 a	15 c
		REC1	49.0 b	104 d	11 c
		REC2	24.7 c	56 e	14 c
2018	Mar	UND	70.7 a	189 b	25 a
		REC1	57.3 a	62 e	25 a
		REC2	71.3 a	32 e	5 d
		PAST	64.3 a	128 d	27 a
		PASTrec	66.3 a	27 e	17 b
	Sep	UND	52.3 b	158 c	23 a
		REC1	69.3 a	89 d	23 a
		REC2	47.0 b	60 e	24 a
		PAST	47.3 b	108 d	18 b
		PASTrec	59.0 a	26 e	19 b

The data followed by the same lowercase letters in the same columns do not differ by Scott-Knott test ($p < 0.05$).

Sporulation was affected by the chemical soil conditions imposed by IOMW. High sporulation was correlated with high OM (2.8 – 4.6), lower pH (4.5 – 6.0) and lower P (1.1 – 4.0) (Supplementary Fig. 2; Table 2).

AMF are dispersed in all areas and samplings (Fig. 4; Table 4). Greater richness was observed in REC areas in Sep/2018. Similarity among the areas in 2017 was approximately 40 %, with exception of REC2, in September (Fig. 4). In 2018, UND and PAST were closely grouped in

March (60 %); and 40 % of similarity was observed among UND, PAST and REC1 in both samplings. REC2 and PASTrec were grouped separately in March, but higher similarity was observed between them in September (>40 %) (Fig. 4).

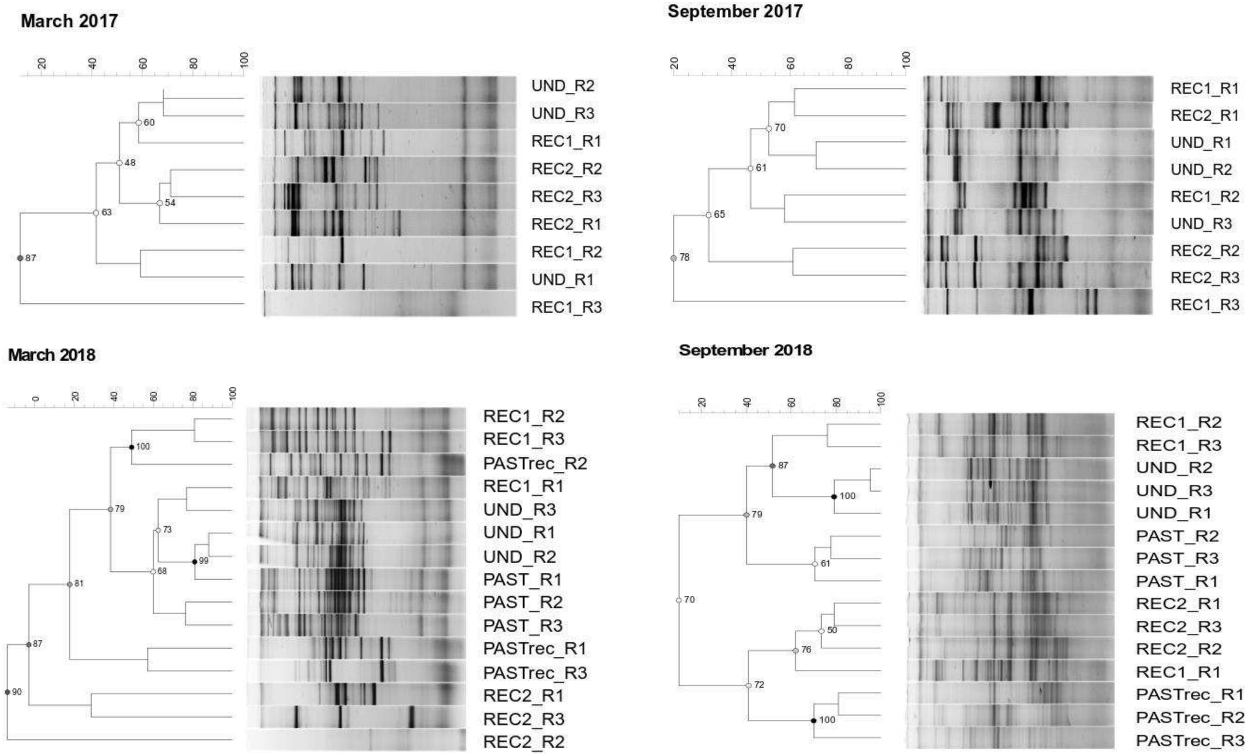

Fig. 4. Dendrogram following the DICE WARD analysis obtained from the DGGE band patterns of the AMF population, which shows the groupings of the areas during the samplings. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), in March and September 2017 and 2018; R1, R2, R3: sampling replicates.

3.4. Monitoring over 3 years

All chemical soil data and biological features were evaluated from 2016 to 2018 and 31.93 % of variations was captured (Fig. 5). UND and PAST formed distinct groups and each area presented similar distribution over time, for both rainy and dry seasons. All areas under IOMW showed similar distribution. A migration of REC microbiota has been observed over the years (Fig. 5) but it is not possible to accurately indicate the direction.

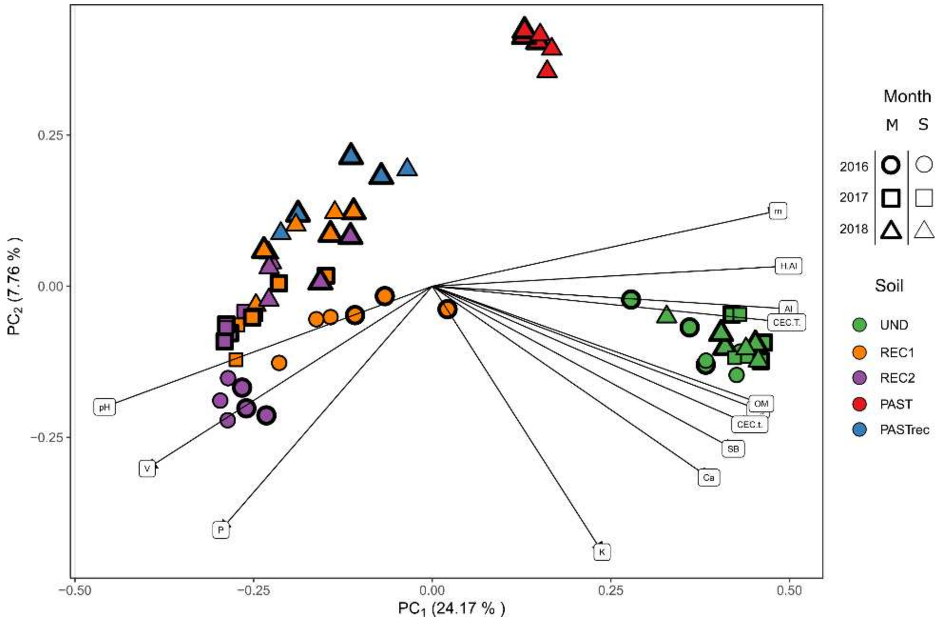

Fig. 5. PCoA plot for all sampling performed since the collapse of the dam, based on Bray-Curtis distance from sequencing data. The chemical data vectors were added in the analysis by the *envfit* function available on Vegan (2.5.6 version). Different colors and shapes correspond, respectively, to areas and sampling periods. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec) in March and September 2016, 2017 and 2018. Percentage values in parenthesis refer to the explanation of each axis.

The separate analysis of the PCoA from chemical and biological features revealed the different effect of each datum on the distribution, over the years. The PCoA from chemical data capture most of the variance of soil data (84.79 %) but only separated IOMW and non-IOMW affected sites. UND and PAST presented similar distribution (Supplementary Fig. 3), as already demonstrated (Fig. 3a). REC1 presented different distribution in Sep/2017 but remained similar to the REC areas.

The PCoA from biological data presented all the OTU information, responded to 24.87 % of the variance and showed higher ability to separate the areas than the chemical data. UND and PAST presented distinct community. In 2016, REC2 presented distinct fungal community distribution; in 2017 and 2018, this area was similar to other REC areas. Over the samplings, REC areas approximated to PAST (Supplementary Fig. 3b).

Chemical and AMF features from REC and UND were evaluated and compared over the three years (Fig. 6). Chemical data analysis showed decreased similarity in the last year of sampling. The analysis of AMF features showed increased similarity over the years (Fig. 6) between REC areas and UND (Fig. 6).

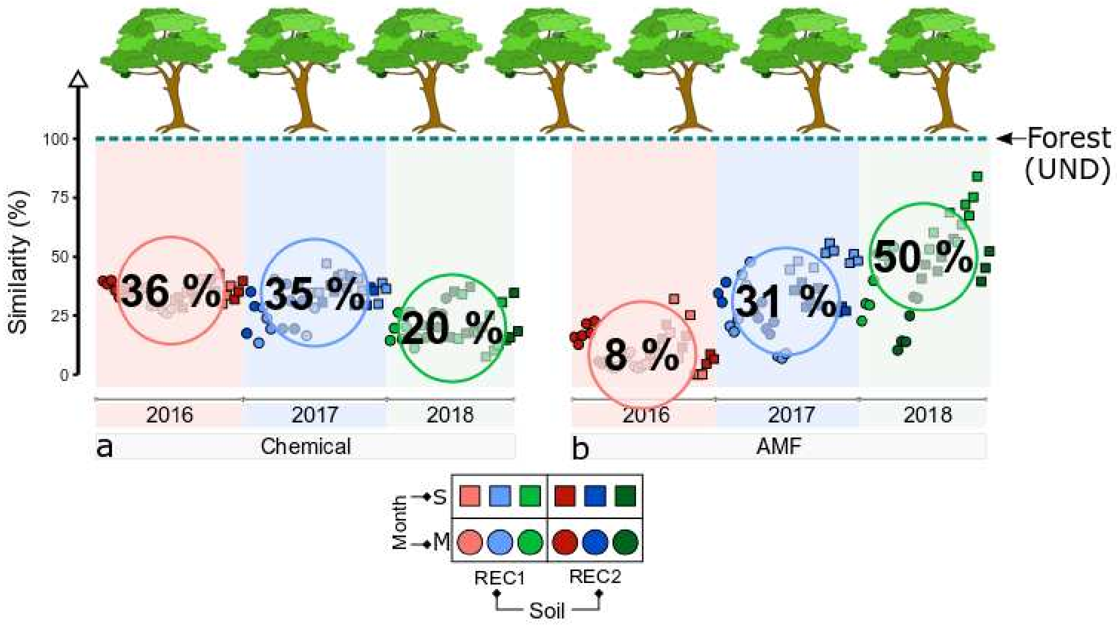

Fig. 6. Progress of (a) chemical factors and (b) AMF features over 3 years (2016, 2017, 2018), in March and September. Each point refers to the similarity between the recovery areas (REC1 REC2) and undisturbed Forest. The similarity was calculated by the formula S= (1 -D_{bray}), where: S = similarity; D_{bray} = Bray-Curtis distance.

4. Discussion

From the comparisons among areas in the process of revegetation (REC areas) and non-IOMW affected areas (UND and PAST) the question remains: does the implemented revegetation process in REC areas aim only to revegetate the area or does it seek environmental recovery?

IOMW deposition can drastically damage soil and biota composition (Table 1, 2). The soil became more compacted (Jordão, 2018), which hinders plant development (Correa et al., 2019). Significant loss of microbiota abundance and structure has been observed in areas with high soil compaction (Hartmann et al., 2014). These physical disturbances of the soil induce deep and lasting changes in the soil microbiome and associated soil functions (Hartmann et al., 2014). Not only the soil structure, but also chemical factors can affect plant development and the availability of other elements in the soil (Margenot et al., 2018). The difference in soil pH between IOMW and non-IOMW affected sites was significant (Table 2) and can be explained by iron ore processing, which maintains the flotation pH range between neutral and slightly alkaline (Fouchee et al., 2016). The higher pH observed in soil under IOMW can hinder plant development and favor specific groups of microorganisms that tolerate this condition. Despite the progress in the vegetation of the affected sites (Prado et al., 2019), pH is a limitation for plant establishment (Mensah, 2016), and affects nutrient availability (Rashid et al., 2016). Regarding the symbiosis, low P and N fertilization systemically induces a physiological state of plants favorable for AMF association (Bonneau et al., 2013). In IOMW sites, high concentrations of phosphorus (5.4 – 11.9) were found (Table 2), which may have reduced the number of AMF spores (Table 4). Nutrient availability was also reported to affect fungal community and root colonization (Bonneau et al., 2013; Liu et al., 2016).

A significant loss of OM in IOMW-affected sites (Table 2) indicates that the disturbance was highly detrimental to the OM pools and resulted in the depletion of Mg and K (Weil and Brady, 2016) (Table 2). Biological functionality and nutrient cycle became disturbed, with low organic matter contents and unfavorable physical-chemical and microbiological characteristics, as observed in REC areas (Table 2). The presence of plants in a soil with low nutrient concentration may increase microbial diversity due to the input of diversified sources of carbon and nutrients, such as carbohydrates, exudates and plant debris that may be degraded by soil microbiota, recycling mineral nutrients. Besides, plant and root development create new niches, favoring the establishment of more diverse microbial communities (Jacoby et al., 2017).

The fungal community differs between IOMW and non-IOMW affected sites (Fig. 3b) and increased richness was not necessarily associated with diversity. Fungi can help aggregate stabilization, maintaining suitable structural conditions for cultivation and porosity for crop growth (Jordão, 2018), help in the decomposition of plant material, increase the bioavailability of nutrients through nitrogen fixation, phosphorus in exchange of carbon and the mobilization of essential nutrients in a symbiotic relationship with many plants (Rashid et al., 2016). Considering the role of fungi and the associations established in the environment, the high-throughput sequencing technologies offer immense possibilities (Nilsson et al., 2019 b). Microorganisms play an essential role in the biogeochemical cycling in the soil and clarify the relation between the taxonomic and functional diversity of the microbial communities in IOMW-affected sites (Crognale et al., 2017).

According to reports, some species are more represented in certain sites, due to vegetation composition (Bourceret et al., 2016) or for suppression and domination by soil microbiota (Svenningsen et al., 2018). Microbial diversity, abundance and distribution are depressed in affected soils compared to undisturbed soils (Macdonald et al., 2011). These results are associated with location, type of contaminant, recovery strategies employed, dominant vegetation and time after recovery (Mummey et al., 2002). Studies carried out in the same area showed that some plants affected directly by the sediments did not resist the impact and gradually disappeared after the disaster (Santos et al., 2019). Plant mortality may be a result of nutrient deficiency in the soil and the effect of salt and alkali stress on plant physiology (Sun et al., 2019). They also pointed out that most native species do not regenerate, and some leguminous species, as well as the pioneer species *Ricinus communis* L., were dominant and this species presents high demand for N-NH_4^+ (Santos et al., 2019).

Ascomycota, the prevalent fungal phylum, is associated to compacted soils, which suggests its positive effects on saprobic fungi (Hartmann et al., 2014). Basidiomycota was the second most representative phylum in disturbed fragments (Rosales-Castillo et al., 2018). The prevalence of some genera or species can be determined by different factors and it was observed that vegetation cover is an important regulator (Schimann et al., 2017). High rates of *Fusarium oxysporum* were observed in all areas, except in UND, where its density was low. *Fusarium* includes most plant pathogens of crops and *F. oxysporum* is related with fusarium wilt. Studies on the suppression of *Fusarium* wilt revealed that oscillations are smaller in organic soil (Bruggen et al., 2015), which indicates that the resistance and resilience of the microbial community can be higher in UND. Among the species found in non-IOMW affected sites, *Mortierella horticola* and the genus *Trechispora* represent groups of saprotrophs in soil, found on decaying leaves and other organic materials (Wagner et al., 2013; Vanegas-León, 2019). A soil microbiota plays indispensable roles in an ecosystem and can be used as a soil health indicator (Soils, 2018). This balance among microbiota species is important to maintain ecosystem services. Fungi influence and shape ecosystems by cycling organic matter and are characterized as major ecological players in both terrestrial and aquatic environments (Nilsson et al., 2019 b).

Soil chemical data can clearly distinguish IOMW from non-IOMW. Considering only the chemical soil factor, small changes were observed in IOMW-affected sites over the samplings (Fig. 3a). The difference presented in REC1 in September/2017 through the analysis of the chemical data could be affected by the introduction of sugar cane. The analysis of fungal OTU and chemical data presented changes in the distribution pattern of the areas, besides a different dynamic population among areas and greater sensitivity of biological composition (Fig. 3b). Except for the implementation of sugar cane in REC1, no changes were observed in the revegetation process, without the introduction of other plant species, such as native trees. Therefore, the similarity between OTU composition over time does not suffer major changes. Initially, the establishment of vegetation improves the aesthetics of the area. However, over the years, it is necessary to diversify, ensure restoration, increase soil fertility and accelerate ecological succession (Sheoran, 2010).

Despite the low occurrence of Glomeromycota in sequencing data, other analyses were performed to characterize this group and it is possible to observe changes throughout the samplings (Fig. 6). The fewer changes of soil chemical-physical characteristics documented in this area (Jordão, 2018), suggest the importance of mycorrhizal association for the ecosystem recovery process and that increased AMF community is affected by the revegetation process (Prado et al., 2019). The diversity in AMF and glomalin contents was correlated to improved soil conditions and can be considered a good indicator of the rehabilitation of affected soils (Jordão, 2018; Leal et al., 2016). Therefore, the IOMW-affected soil remains under imbalanced nutritional condition when compared to UND (Table 2).

In some environments characterized by mineral nutrient deficiency and various abiotic stress conditions, such as REC areas, mycorrhizal plants present selective advantage over non-mycorrhizal (Chen et al., 2018), which explains the occurrence of mycorrhizal symbiosis in all REC areas (Table 4). Decreased mycorrhizal colonization between rainy and dry season, except for REC1, can be associated with water availability in the soil. This higher root colonization observed during the rainy season corroborates the findings of Oliveira and Oliveira (2005), who showed a relation between AMF colonization and sporulation with seasonal, host plant species, pluvial precipitation, soil moisture content and soil chemical composition. Many factors can drive the AMF distribution (Xu et al., 2017). The DGGE analysis showed greater similarity between the non-IOMW sites in both samplings, in 2018 (Fig. 4), as observed in PCoA chemical analyses (Fig. 3a). In REC1, the introduction of sugar cane in Sep/2017 resulted increased AMF colonization. The higher similarity between the proximity areas, UND and REC1, in 2017 and 2018 (Fig. 4) and in the firsts samplings (Prado et al., 2019) may have resulted from the dispersion process. The increased AMF similarity among areas, as observed in Sep/2018 (Fig. 4), was related to the revegetation process and led to the establishment of mycorrhizal association. This increase in the similarity of the AMF community is also seen in comparison to the forest (Fig. 6). The indicative of 50 % may be due to the local vegetation, but the increase in this similarity may be restricted to the vegetal species present in REC that are less diversified. The information related to this microbiota can be used for further comparisons and the development of strategies to apply it as inoculum. The use of AMF inoculum promotes the growth and health of young plants, their fitness and survival after planting, besides improving plant growth in contaminated soils (Kimura and Scotti, 2016: Chen et al., 2018). Inoculation allows decreasing the amount of fertilization without reducing plant production (Chen, 2018). It also has the potential to restore the fertility of degraded soils (Rashid et al., 2016). However, the introduction of sugar cane in REC1 limited the process of recovering floristic diversity due to the competition of propagules and seeds from the forest with the sugar cane, making the regeneration process delaying. This shows that this activity will only contribute with a vegetation cover and not to the recovery of the area and the ecosystem functions.

The revegetation process is a good strategy to improve the conditions of impacted areas, which, even before the dam collapse, had already suffered with erosion, due to grazing and lack of vegetation cover, as PAST area (Fig. 1). Researchers and experiments conducted in this affected site stands out the introduction of native tree species. Forests with greater diversity present greater capacity to recover from disturbances, better nutrient cycling, greater attractiveness to the fauna, greater soil protection from erosive processes and greater resistance to pests and diseases (Martins, 2018). Over the time, the biological data have been changing since revegetation was implemented (Fig. 5; Supplementary Fig. 3). To date, REC areas have not presented chemical and biological values close to those of the references areas, but our results suggest that soil exploitation by microorganisms in reclaimed ecosystem will promote significant changes and the recovery of soil functionality. The change in the microbiota distribution in REC can indicate that these areas are increasing the similarity with non-IOMW affected, mainly with PAST (Fig. 2 and 5).

Recovering areas are unlikely to exhibit soil characteristics like those of undisturbed or conserved areas in record time, but microbiota activity on this soil may improve soil condition. Time plays a key role in soil changes during restoration (Fig. 5), as observed in a post-reclamation age effects study (Adeli et al., 2019; Gastauer et al., 2019). Our data corroborate the idea that the chronosequence role is important, since deeper understanding about soil microbial community and functional relationship regulation requires spatial and temporal scale analysis (Mummey et al., 2002). From this perspective, it is important to observe the physical, chemical and biological characteristics of the soil, and focus on the microorganism potential as allied to the recovery process. Therefore, the changes in soil biological properties of these areas must be monitored (Guerra et al., 2017; Segura et al., 2016).

This analysis portrays with greater accuracy how the restoration process works. Besides, a long-term monitoring is required for further knowledge. Our data indicates that revegetation is the initial path of restoration and helps in the restructuring of microbiota and soil properties, but a follow up is required. According to what we have observed so far, the way the process has been conducted will not be possible to restore it, or at least to reduce the pressure of the Atlantic Forest ecosystem so threatened. The data indicate that the revegetation process goes towards a condition, structure and landscape closer to the pasture than to the reference ecosystem UND. Without the control, systematic interventions to prevent degradation by negative human influence or grazing, restoration progress will never be sustainable and will stagnate. In addition, it is mandatory the introduction of tree species for more robust and complex functions in the soil. Otherwise, the process will not progress. Many issues remain to be clarified and the research must continue to seek urgent responses to mitigate environmental damage and restore the functioning of ecosystems.

5. Conclusions

This study reveals that besides soil chemic-physical factors, the microbiota should be considered in soil recovery strategy.

Soil revegetation process produced a positive effect on soil fungal diversity and AMF community structure and these REC areas are becoming more similar to PAST area.

Among the fungal phyla found in the sequencing analysis, Glomeromycota showed increased REC, since the collapse of the dam.

The microbiota was very sensitive, and, in the chronosequence of three years, it was possible to observe changes in its composition in the sites directly affected by IOMW.

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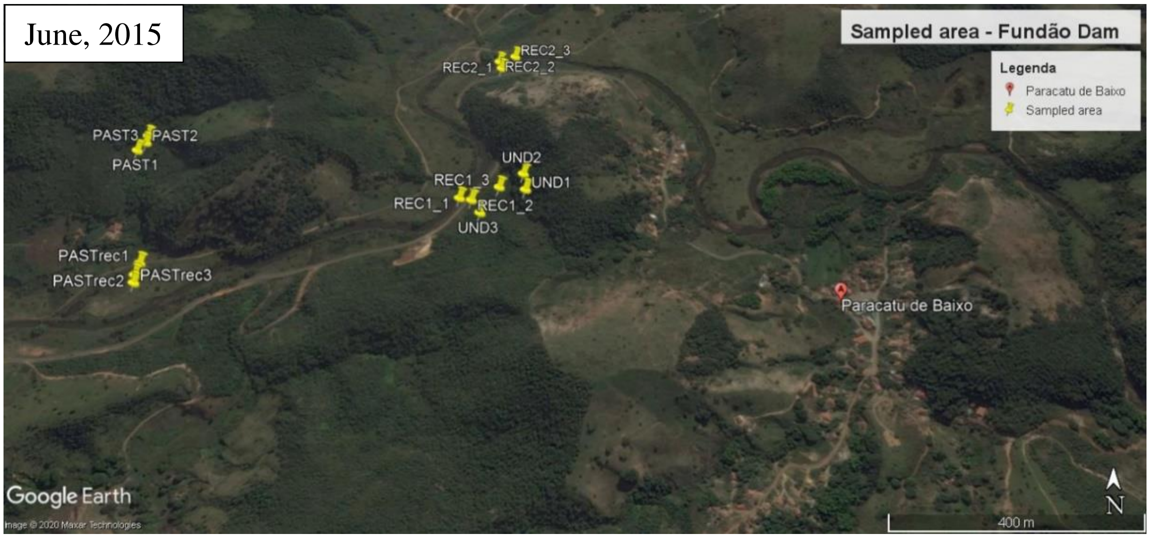
SUPPLEMENTARY MATERIAL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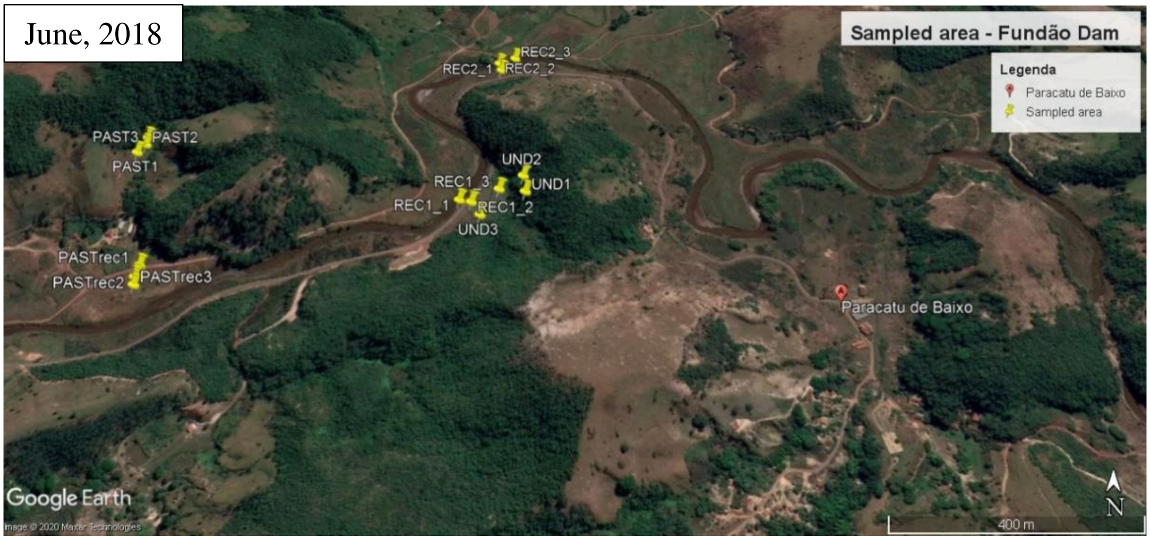
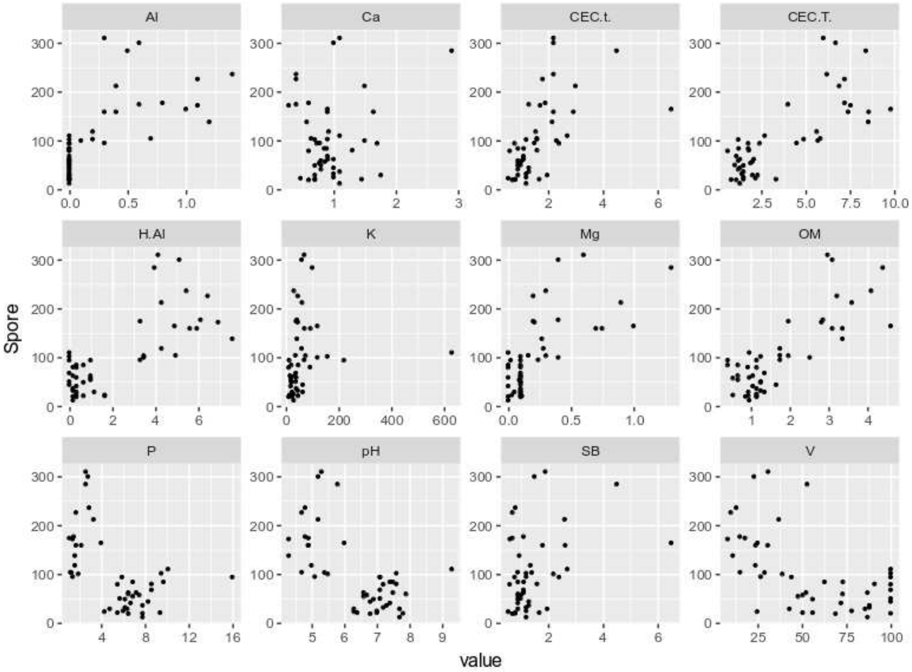
April, 2016

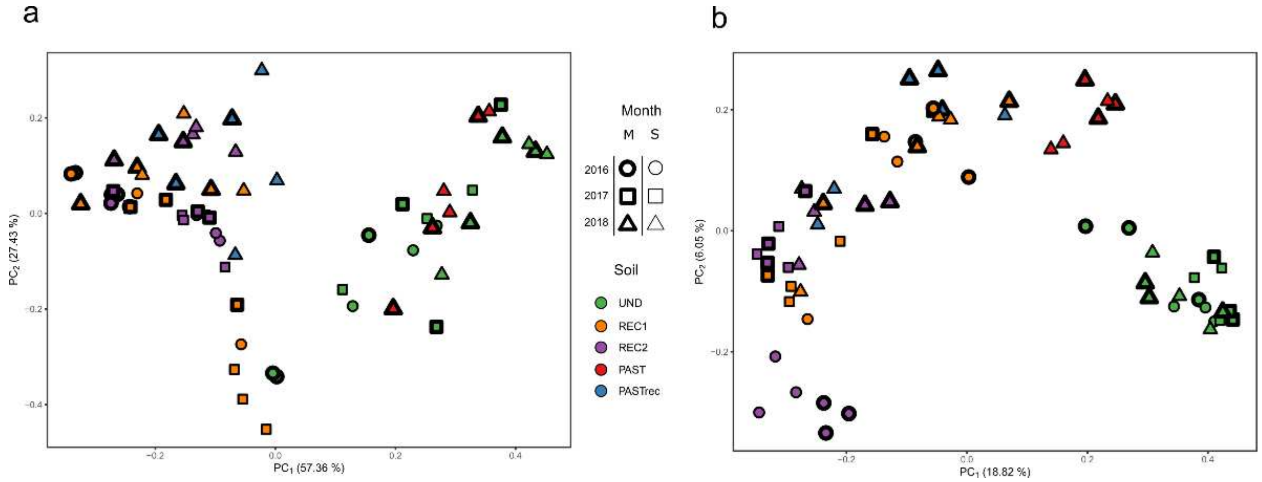

June, 2017



Supplementary Fig. 1. Map of the study area showing the study sites. Google earth background in 2015 (before the collapsed of Fundao Dam), 2016, 2017 and 2018. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec), and the respective sampling replicates (1, 2 and 3) in March and September 2016, 2017 and 2018. (Google earth Pro).



Supplementary Fig. 2. Correlations between spore density of AMF and soil chemical features. Each plot corresponds to one chemical information. Al, aluminium; Ca, calcium; CEC(t) effective cation exchange capacity; CEC(T) total cation exchange capacity; H+Al, Potential acidity; K, potassium; Mg, magnesium; OM, soil organic matter; P, phosphorous content; pH; SB, Sum of exchangeable bases; V, base saturation index.



Supplementary Fig. 3. PCoA plot for all the samplings dates (2016, 2017 and 2018) based on Bray-Curtis distance from (a) Chemical and (b) fungal sequencing data. Different colors and shapes correspond respectively, to areas and sampling dates. Sampled areas: undisturbed forest (UND), pasture (PAST) and areas affected by iron ore mining waste under recovery process (REC1, REC2, PASTrec) in March and September 2016, 2017 and 2018. Percentage values in parenthesis refers to the explanation of each axis.

4. CONCLUSÕES GERAIS E PERSPECTIVAS

A diversidade da comunidade fúngica nas áreas afetadas pelo rejeito de mineração de ferro foi alterada pelo processo de revegetação e foi observado um aumento na diversidade total de fungos, bem como de FMA ao longo das amostragens.

O monitoramento da comunidade fúngica e dos FMA na área em recuperação mostrou-se mais sensível que os fatores químicos, evidenciando diferenças ao longo do período analisado. Em função da sensibilidade, esses microrganismos podem ser usados como bioindicadores auxiliando em programas de reabilitação de áreas afetadas pelo rejeito de mineração.

O acompanhamento das alterações nas áreas impactadas pelo rejeito de mineração, principalmente dos dados biológicos, tem se mostrado imprescindível no delineamento e condução de experimentos visando a recuperação dessas áreas, sendo necessária a continuidade das amostragens.

Este projeto deixa uma reflexão sobre as políticas de recuperação ambiental ao relacionar a diversidade microbiana e saúde ambiental. Pontua a necessidade de uma progressão no processo de revegetação e reflorestamento como a introdução de espécies vegetais, a exemplo de arbóreas. Além dessa preocupação em avançar com o processo de revegetação é necessário que a comunidade seja integrada nesse processo como protagonistas e assumam a responsabilidade de colaborar com o processo evitando assim um retrocesso na recuperação ambiental.

A partir deste estudo será possível selecionar microrganismos que auxiliarão no manejo e no restabelecimento da vegetação. Esses microrganismos poderão ser utilizados como inóculo na produção de mudas a serem introduzidas na área ou no preparo de um mix com o consórcio microbiano, podendo este ser aplicado durante a semeadura direta no campo como uma medida de restruturação do meio ambiente. Coloca também em evidência a necessidade de introdução de espécies nativas nas áreas em recuperação para aumentar as relações ecossistêmicas estabelecidas no solo.

As medidas de recuperação levam em consideração a caracterização da área, da biodiversidade desse ecossistema e uma avaliação da qualidade do solo e de seus possíveis usos, como também das águas que circundam as regiões que passaram por esses programas de revegetação. Desta forma, o processo de revegetação será mais eficiente e ambientalmente adequado, uma vez que serão utilizados microrganismos já adaptados ao ambiente em estudo.