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Q2

Morphological and molecular diversity of arbuscular mycorrhizal fungi in revegetated iron-mining site has the same magnitude of adjacent pristine ecosystems

Q4 Q3 *Caroline Krug Vieira*[1], *Matheus Nicoletti Marascalchi*[2], *Arthur Vinicius Rodrigues*[3], *Rafael Dutra de Armas*[4], *Sidney Luiz Stürmer*[2,*]

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A B S T R A C T

Arbuscular mycorrhizal fungi (AMF) are important during revegetation of mining sites, but few studies compared AMF community in revegetated sites with pristine adjacent ecosystems. The aim of this study was to assess AMF species richness in a revegetated iron-mining site and adjacent ecosystems and to relate AMF occurrence to soil chemical parameters. Soil samples were collected in dry and rainy seasons in a revegetated iron-mining site (RA) and compared with pristine ecosystems of forest (FL), canga (NG), and Cerrado (CE). AMF species were identified by spore morphology from field and trap cultures and by LSU rDNA sequencing using Illumina. A total of 62 AMF species were recovered, pertaining to 18 genera and nine families of Glomeromycota. The largest number of species and families were detected in RA, and *Acaulospora mellea* and *Glomus* sp1 were the most frequent species. Species belonging to Glomeraceae and Acaulosporaceae accounted for 42%–48% of total species richness. Total number of spores and mycorrhizal inoculum potential tended to be higher in the dry than in the rainy season, except in RA. Sequences of uncultured Glomerales were dominant in all sites and seasons and five species were detected exclusively by DNA-based identification. Redundancy analysis evidenced soil pH, organic matter, aluminum, and iron as main factors influencing AMF presence. In conclusion, revegetation of the iron-mining site seems to be effective in maintaining a diverse AMF community and different approaches are complementary to reveal AMF species, despite the larger number of species being identified by traditional identification of field spores. Q5

Introduction

Iron (Fe) is one of the most common metal used in industry and found in granite and basalt rocks. Open air iron mining is an extremely impacting activity and drastically transforms the landscape, as iron ore deposits are accessed after stripping ironstone outcrops and biota followed by excavation that can reach up to 300 m in depth (Skirycz et al., 2014). This process

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results in changes on landscape relief, altering soil physical structure, decreasing soil organic matter stocks and nutrient availability to plants, and impacting soil biota community (Klauberg-Filho et al., 2002). The iron quadrangle region represents one of the most important geological areas in Brazil due to its rich mineral deposits of iron, manganese, and gold (Costa et al., 2014) and accounted for 68.4% of the total iron brazilian production in 2014 (Brasil, 2016). The region also harbors an endangered ecosystem associated with superficial iron crust known as canga or banded iron formation, whose soils are shallow, acid, poor in nutrients and with toxic levels of aluminum and heavy metals (Skirycz et al., 2014).

Restoration of mining areas is a legal requirement in Brazil to obtain mining permits and it includes several steps to be implemented, including topsoil removal and storage, landscape architecture to minimize landslides, revegetation with native plant species, and monitoring to assess restoration progress (Skirycz et al., 2014). During revegetation, growth and nutrition of appropriate plant species can be achieved by a combination of fertilizer management and microbial inoculation, including arbuscular mycorrhizal fungi (AMF) and nitrogen fixing bacteria (Mendes Filho et al., 2010). AMF are soil fungi belonging to phylum Glomeromycota that form a monophyletic group of *ca.* 288 described species (Öpik and Davison, 2016). These fungi establish the arbuscular mycorrhizal (AM) association with roots of 80%–90% of vascular plants and provide an array of benefits to ecosystems (Smith and Read, 2008). The ecological significance of these fungi and the AM association can be fully appreciated when analyzed under the provision of soil ecosystem services: promotion of plant growth and nutrition, increased plant resistant to biotic and abiotic stresses, improvement of water retention and soil aggregation, and increased plant quality for human health (Gianinazzi et al., 2010). AMF diversity and spore density can decrease in areas contaminated by heavy metals or polluted by mining activities (Aggangan et al., 2015; Klauberg Filho et al., 2002; Mergulhão et al., 2010; Yang et al., 2015; Zarei et al., 2010). However, revegetation processes to recover mining-impacted areas can recover AMF communities as verified by increases in species richness and sporulation (Caproni et al., 2003, 2005; Teixeira, 2015).

Most studies assessing AMF diversity and community structure in Brazilian mining areas have relied solely on morphological identification of species, which can underestimate AMF diversity as environment can affect sporulation, a reliable identification depends on expertise of an AMF taxonomist, and AMF species colonizing roots might not be found as spores in the soil (Sanders, 2004). Conversely, molecular methods to assess AMF diversity can overcome some of these problems as they do not rely on spore-based identification (Sun et al., 2016). Molecular markers commonly used in AMF ecological studies include SSU and LSU rRNA genes, the former being widely used despite its poor resolution for some lineages in Glomeromycota and the latter being more phylogenetically informative with its use increasing in recent years (Hart et al., 2015). Despite the fact that ribosomal operon (the Krüger fragment) allows alignment over all lineages in Glomeromycota (Krüger et al., 2011), a marker for barcoding or species recognition is still missing for the phylum (Öpik et al., 2014). A combination of morphological and molecular techniques has been used more often in ecological studies of AMF to measure species diversity and richness.

Several studies in mining sites and natural ecosystems have explored the influence of soil chemical parameters upon AMF communities. In a global scale, soil pH and organic soil carbon were important drivers of AMF community composition (Davison et al., 2015). Yang et al. (2015) observed that in heavy metal contaminated soils, some phylotypes were associated with total Cu and Cd concentrations in soils. Concentration of arsenic (As) (Sun et al., 2016) and antimony (Sb) (Wei et al., 2015) in soil was negatively correlated with AMF species richness. Total As concentration was positively correlated with the presence of some genera like *Ambispora* and *Septoglomus* (Sun et al., 2016) and species like *Acaulospora morrowiae* (Schneider et al., 2013). In mining soils contaminated with zinc (Zn) and lead (Pb), AMF diversity was negatively correlated with concentration of these two metals and the phylotype *Glomus 3* was detected exclusively in sites with high levels of soil heavy metals (Zarei et al., 2010). These studies indicate that AMF spore abundance and species richness are decreased by soil contamination with metals but some few AMF species or phylotypes are able to adapt and tolerate different levels of metals in the soil. Results of these studies have practical implications as metal-tolerant AMF should be isolated and inoculated in plants used for revegetation processes of areas affected by mining.

In this study, AMF communities in an iron mining site undergoing revegetation with grasses and adjacent pristine sites occupied by different types of vegetation were surveyed using different approaches as morphological identification of field and trap culture collected spores and sequencing of bulk soil (Öpik et al., 2014). The aim of this study was to determine AMF species richness occuring in an iron mining site and adjacent ecosystems and to relate the occurrence of AMF species to soil chemical parameters. We tested the following hypotheses: 1) AMF species richness is lower in revegetated area after iron mining compared to adjacent pristine ecosystems, and 2) the number of AMF species identified increases using the LSU rDNA as a molecular marker relative to the use of morphological techniques.

1. Material and methods

1.1. Study area

The benchmark area was located in the Centro de Tecnologia de Ferrosos (Vale S.A. company) located in the municipality of Nova Lima and Brumadinho, Minas Gerais state. Mean annual precipitation and temperature are 142.5 mm and 19.8°C, respectively (Table 1). Climate is Cwa type according to Köppen (humid sub-tropical with dry winter and hot summer) (Alvares et al., 2013) with well-defined dry seasons from May to September and rainy seasons from October to April.

Soil samples were collected in the following sites: (a) semidecidual seasonal forest (FL) in secondary stage of succession, (b) canga (NG) — an ecosystem associated with superficial iron crust, (c) cerrado (CE) — a savanna type of vegetation, and (d) revegetated iron-mining area (RA) — an area undergoing environmental restoration after extraction of iron ore. The restoration process included artificial revegetation through the transplanting of woody species and sowing with a mix of grasses species, including *Melinis minutiflora* P. Beauv., *Urochloa brizantha*

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