

Sustainable Restoration of Degraded Landscapes Improves Soil Glomalin Content

Bakhytzhan Yelikbayev

Satbayev University

Eduardo Correa

Empresa de Pesquisa Agropecuária de Minas Gerais, Unidade EPAMIG ITAC

Neimar Duarte

Instituto Federal de Minas Gerais

Marcela Pagano (✉ marpagano@gmail.com)

Instituto Federal de Minas Gerais

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Abstract

The rising use of plantations for wood and cropping systems for food, the agronomic pressures, and resource overexploitation, in addition to climate change constraints, need urgent efforts for restoration of the different vegetal covers. Thus, research in ecosystem restoration is rising worldwide as the perception that global change negatively affects ecosystem services as well as the above and belowground biodiversity. Among microorganisms, mycorrhizae and *Rhizobia* benefit and contribute to the natural regeneration of plant communities. Consequently, the study of mycorrhizas in natural ecosystems supplement with relevant information the restoration plans. The understanding that most plants are mycotrophic influences the rehabilitation efforts of varied vegetation types. We show here the ecosystem restoration with respect to the benefits of plant symbiosis. As an index of soil stabilization in the different sites glomalin content (Undisturbed = Restored > Disturbed), was the best predictor of restoration success.

1. Introduction

Due to the rising use of plantations for wood, the increase of the agronomic pressures, and resource overexploitation, in addition to climate change constraints, urgent efforts are needed for restoration of the different vegetal covers. Previous reports (Aronson et al., 2011) highlighted the need for more scientific information on restoration practices of strategic priority areas in tropical biomes. Thus, research on restoration of degraded ecosystems is rising worldwide as the perception that global change continues to negatively affect the above and belowground biodiversity and ecosystem services, which has driven to deeply research more indices of restoration success. Plantations affect the soil physical and chemical properties, modifying the number, diversity, and activity of the associated microbiota, including symbiotic fungal populations that rely in the host plants rhizospheres, favoring restoration.

Together with the understanding of biodiversity values (Brockerhoff et al. 2008, Scherr and McNeely 2008) different compartments such as soil seed banks (Bakker et al. 1996, Thompson et al. 1997, Reis et al., 2010) and soil microbes (Matias et al. 2009, Ohsowski et al. 2012) were focused in restoration ecology practices. Therefore, pronounced research on the interactions of plant associated microorganisms in undisturbed ecosystems (Heneghan et al. 2008, Harris 2009, Pagano, 2012) was developed. Moreover, non-nutritional effects of this symbiosis, such as reducing plant diseases, modifying water relationships (Mathur and Vyas, 2000) or stabilizing soil structure (Rillig and Mummey, 2006) are the focus of recent research as both soil properties as well as the plant species cover need to be studied to obtain gains from AM fungi in restoration projects. Additionally, Burri et al. (2011) have showed that AM fungi can increase soil resistance to wind erosion.

The AMF effectively intermediate soil aggregation (Rillig et al. 2000), as the stability of soil macroaggregates depends on the growth and decomposition of roots and mycorrhizal hyphae, which realize glomalin (Singh et al., 2020).

Evidence that restoration can be enhanced by reintroducing mycorrhizal fungi from the native plant microbiome, was explained (Koziol et al. 2018, Buisson et al., 2020).

Mycorrhizae were an instrument in restoration due to their benefit in increasing plant growth (Chaudhary and Griswold, 2001). However, attention on AM fungi was then motivated on their contribution to soil structure and erosion control due to their involvement in soil aggregate stabilization (Rillig and Mummey, 2006). AM reinforce soil structure in both a physical and chemical way. The extraradical hyphae of AM fungi involve soil and root particles and produce proteins (such as glomalin) which stabilize soil aggregates (Rillig et al., 2007). Biotic (disease, herbivory and/or the presence of competitors) as much as abiotic stresses (nutrient deficiency and drought) can affect both plant and microbial fitness. As soil microorganisms are also influenced by abiotic conditions (soil type and soil water content).

The study of arbuscular mycorrhizas (AMF) as an index of restoration of degraded ecosystems, supplies relevant information for restoration programs; However, there are few reports on this topic despite the potential uses of this symbiosis. Moreover, research necessary for understanding the benefits of plant microbiomes in restoration is necessary. In the present study, the occurrence of AMF in different preserved and restored ecosystems has been investigated. We show that glomalin content and soil aggregation were indicative indices of soil stabilization in restored ecosystems.

2. Material and methods

2.1. Study sites. The study area, located in Minas Gerais state, Brazil, presents mean annual precipitation and temperature of 142.5 mm and 19.8°C, respectively. The climate of the region is Cwa type according to Köppen (humid subtropical with dry winter and hot summer, with well-defined dry seasons from May to September and rainy seasons from October to April. Representative field sites): (1) undisturbed protected areas of Atlantic forest, selected for this study, located in the plain of the São Francisco River basin, in Brazil, were reported previously (Pagano et al. 2012, 2022). Soil properties were as follow: neutral pH 6.8 and OM: 0.77%, high Base saturation and moderated P content, average temperature of about 23°C, predominant precipitation in summer and dry winter. Soil samples were collected in the different vegetation types.

2.2. Data

Morphological and chemical methods were applied in this study to distinguish sites. Data on undisturbed, restored, and degraded areas, AMF propagules, soil aggregation, and glomalin content were analyzed for the period of 2007–2009 to 2019–2020 for each vegetation type.

2.3. AMF occurrence

AMF spores occurring in the soil samples (100g soil) were extracted by wet sieving (Gerdemann and Nicolson, 1963) and identified, their diversity were estimated.

2.4. Aggregate-size distribution

Dry aggregate size distribution was determined by the standard dry-sieving method (Amaro Filho et al., 2008). Briefly, 3g of air-dried, undisturbed sample was sieved through sieves having 2 and 0.25mm square openings so two aggregate size classes are obtained (>2 and >0.2mm).

2.5. Soil glomalin content

Glomalin was extracted from 1-g soil samples with citrate buffers using the method of Wright and Uphadyaya (1996, 1998). Extracts were autoclaved at 121 °C for 30 and 60 min, respectively; then centrifuged to remove soil particles. Protein in the supernatant was determined by the Bradford dye binding assay using bovine serum albumin as the standard.

3. Results

3.1 AMF occurrence

In the soil samples, 14 AMF species could be distinguished, totalizing six families and ten genera (Table 2). Four species belonged to the genera *Acaulospora* and two to *Racocetra*, while the genera *Claroideoglomus*, *Gigaspora*, *Scutellospora*, *Dentiscutata* and *Ambispora* were represented by a single species each. The species *Acaulospora capsicula*, *Sclerocystis rubiforme*, *Sclerocystis taiwanensis* and *Racocetra verrucosa* were exclusive to the undisturbed sites, whereas *G. margarita* was observed only in adjacent cultivated areas e.g. maize field. Some (Four) spore types could not be readily distinguished at the species level.

3.2. Aggregate-size distribution

Macro and microaggregates were affected by land use (Fig. 4 and 5, Table S1) macroaggregates were associated to the restored site, and microaggregates were associated to the undisturbed sites.

3.3. Statistical Analysis

Principal component analysis (PCA) was evaluated on the three sampling sites using data from soil aggregation. The significance of differences between field sites in glomalin content was tested by using analysis of variance (ANOVA) one-way. Tukey post hoc test was used to conduct pairwise tests for significant effects. Principal component analysis (PCA) was performed with PAST 4.08 (2021).

4. Discussion

Among soil microorganisms, the multiple symbiosis of AM fungus and *Rhizobium* is crucial for restoration as mycorrhizae provide P to the plant, potentializing N fixation), the resulting tripartite symbioses create an efficient strategy to accelerate soil restoration (Chaer et al. 2011). Nevertheless, compatibility between native rhizobial strains and AM species or isolates help the specific fertilization requirements of native plantations. Additionally, restoration demand a complete understanding of plant life histories. Native AMF can colonize plants in natural conditions but the loss of these fungi with

disturbance require appropriate management. Thus, highly dependent plant hosts are selected over mycorrhizal- independent plants. AMF are an important biotic component (Pagano 2012, Stürmer et al. 2012, Pagano et al., 2022) that should be included in restoration programs. Inoculating AM to plants in degraded soils with the objective of promoting plant growth, accelerate ecological succession, attaining desired plantations (Renker et al. 2004) require a better understanding of successional processes (Quesada et al. 2009), the soil microbiota succession must also be studied.

To perform soil analysis is crucial to predict the microbial establishment and design possible long-term interactions. A diagnostic procedure and diagnostic protocols help to recognize and remove anthropogenic disturbances in degraded sites (Aronson et al., 2011). Then, to characterize, ameliorate soils and control invasive species, weeds, leaf cutter ants and problematic grazer fauna is crucial for the success of restoration. Diagnostic protocols identify possible barriers to ecological succession and the necessities for different approaches for restoration., the presence of different types of plant functional groups as well as different plant symbioses (such as mycorrhizal types, *Rhizobium*, etc.) and their combination is crucial for the development of restoration practices. The use of selected strains of rhizobia as well as isolates of AMF can influence plant growth, seedling survival, rhizospheric effect, etc. Of them, plant survival is an important parameter for species selection with restoration purposes. Propagules of native AMF (extraradical hyphae, spores), other soil organisms, organic matter, fine roots, and native seeds are present in the topsoil. However, if topsoil is not accessible, thus, inoculation of AMF may be added to disturbed soils using other procedures. Commercial inoculum, which usually contains only one species of AMF(*Glomus intraradices*), or a few species, being of low quality (Vahter et al., 2023), is generally applied; however, the establishment of local collections to develop site specific(e.g. On-farm) native AM inocula is more commonly used (Chaudhary and Griswold, 2001) and more appropriated. In this regard, it has become the custom to use spores or rhizospheric soil from cultivated host plants as inocula (Read 2002). For monitoring restored sites the determination of indices such as abundance of AM spores and percent root colonization can be evaluated to compare the restored with reference sites and assess restoration with time. Suitable ecological attributes for restoration (plant cover, abundance, mycorrhization) can support rehabilitation efforts; however, tolerance to projected future climate change (Gelviz-Gelvez et al. (2015) has been pointed in potential plant species for restoration projects included in semiarid regions, based on species that would not tolerate environmental modifications caused by climate change, which are inappropriate for use in long-term ecological restoration.

In the restoration of Brazilian forest, efficient strains were selected and used as rhizobial inocula for native legumes, and selected AMF species (*Acaulospora scrobiculata*, *Gigaspora margarita*, *Glomus brohultii*, and *Scutellospora cerradensis*) were added for double inoculation, which was benefic for survival of the plant species along time (Pagano et al., 2022). A diverse community (Three AMF families) (Table 1) was found in the rhizospheric soils from those plant species used in restoration (Table 3) No Gigasporaceae species were found in the degraded areas, contrasting with the preserved site. In the restored area Acaulosporaceae species (Fig. 2) predominated, while Gigasporaceae and Glomeraceae

species were common. Gigasporaceae family was not present in the degraded areas but was characteristic of the preserved site. In contrast, Glomeraceae species were found in the restored site.

At least, seven species of AM fungi were detected in the restored fields (Table 1). The lower AMF diversity was found in the degraded area and the highest richness was found in the preserved undisturbed sites (Fig. 2). Only a species of *Scutellospora*, but not *Gigaspora* was found in the degraded areas, thus contrasting with the preserved sites.

Principal component analysis (PCA), evaluated on the three sampling sites (Fig. 1) showed how the restored site samples, associated with macroaggregates, diverged from the undisturbed (associated with microaggregates) and from disturbed sites (Fig.1).

5. Conclusions

Environmental changes, agronomic pressures, and resource overexploitation affect plant-soil associated microorganisms either in number, diversity, or activity; however, restoration practices minimize soil disturbance, increase soil organic matter or improve soil structure for sustaining biological communities associated to selected plant species, which permit plants to perform better under stressful and unfavorable conditions. Efforts to increase benefits from mycorrhizae for restoration were reported to achieve maximum understanding of soil microorganisms and their associations as well as the selection of specific microbiota during plant succession, which is imitated in ecological restoration. During this period, glomalin content in the different sites (Undisturbed = Restored > Disturbed) and aggregate stability increased, promoting sustainable land restoration, which allows to be used as an index of soil stabilization and restoration success, under tropical conditions.

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Tables

Table 1. Arbuscular mycorrhizal families found at the different ecosystems

AMF family	Undisturbed site	Restored site	Disturbed site
<i>Glomeraceae</i>	7 [#]	6	7
<i>Acaulosporaceae</i>	3	6	5
<i>Gigasporaceae</i>	-	2	8
Total spore number	10	14	20
Total genus richness	4	3	3

[#]spores/100g soil. Based on Pagano et al. (2019, 2022) . Values are means of three samples.

AMF produce glomalin, protein that improves the soil physical properties, carbon sequestration, nutrient content, and microbial activity, through its effects on soil establishing a substrate for microbes, being a gluing agent for soil aggregate formation (Singh et al. 2020)., besides stabilization of pollutants. Thus, higher glomalin content improves ecological restoration as significant differences were detected between restored and degraded sites (Fig. 3).

Tables 2 and 3 are not available with this version.

Figures

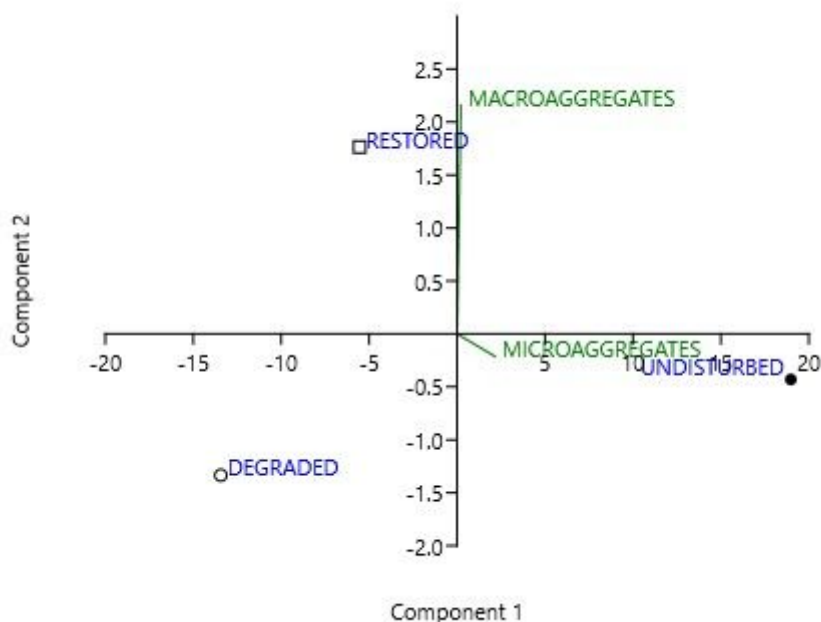


Figure 1

PCA ordination diagram of the soil samples. The three sampling sites are: Undisturbed forest (Black dot), restored(square), and disturbed (dot). Macroaggregates (ø 0.2–2mm) and microaggregates (ø 0.05–

0.2mm). Size and orientation of vectors represent correlation among them and with the axes.



Figure 2

Undisturbed site associated with mycorrhizas and AMF spores recovered from rhizospheric soils). Clockwise, from upper left: undisturbed vegetation, AMF spores (*Scutellospora* and *Acaulospora* spp.) recovered from rhizospheric soils (photos by M. Pagano).

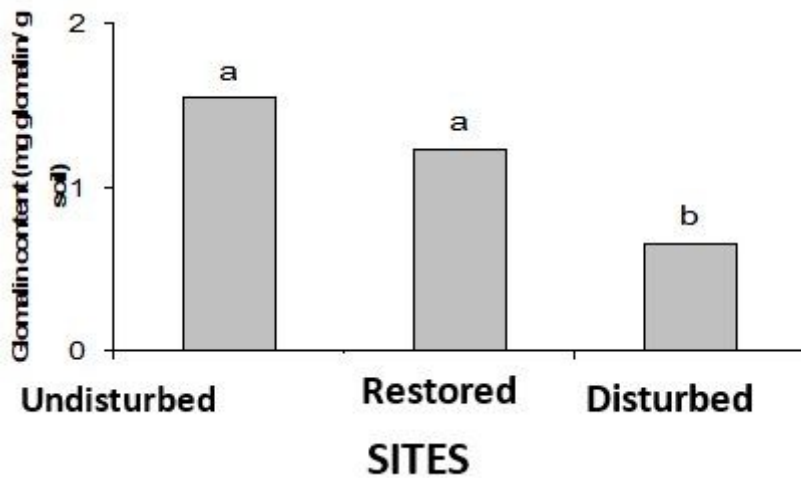


Figure 3

Bradford-reactive soil protein (Glomalin) content in relation to soil disturbance. For each histogram bar, $n = 5$. Histogram bars labeled with the same lower-case letter are not significantly different at $P < 0.05$. Undisturbed Forest – restored and disturbed sites. Based on Pagano et al. (Unpublished) and Pagano et al. (2022). Values are mean of five samples.

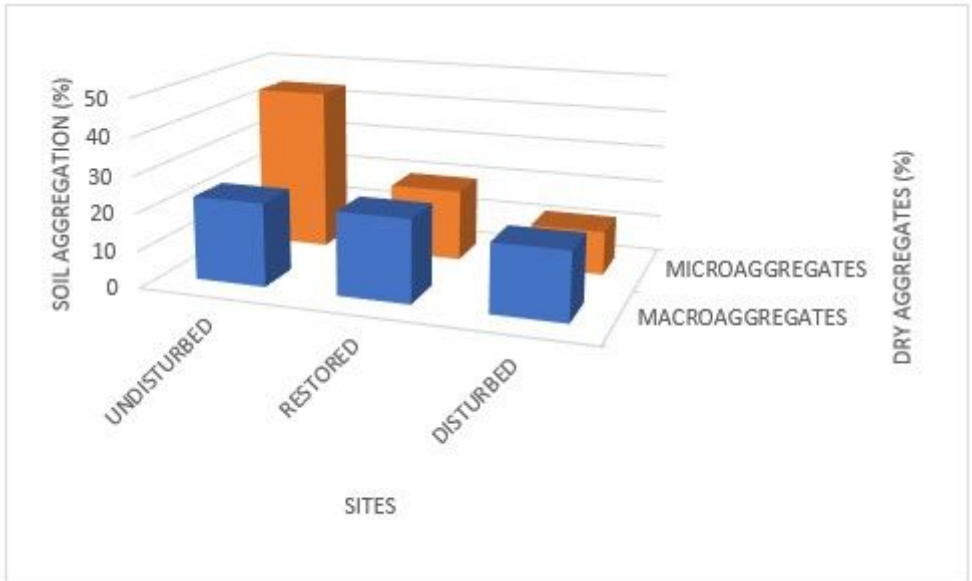


Figure 4

Aggregate size distribution of the soil from the sampling sites.

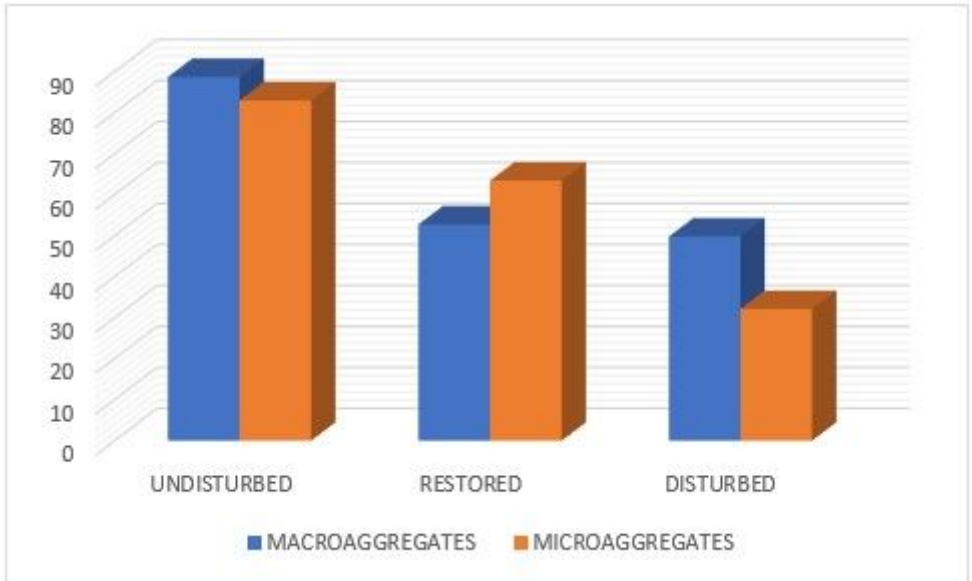


Figure 5

Aggregate stability of the different soils.

Supplementary Files

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- [TableS1.docx](#)