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Active Restoration of Degraded Contaminated Sites Using Glomeraceae

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Abstract

Degraded and contaminated ecosystems require specific vegetal covering after restoration or revegetation and for this purpose, selected plants and soil microbes need to be studied by their role in this process, besides environmental constraints, interactions plant- soil and its associated microorganisms are critical for plant establishment. Thus, microbial management (inoculation of bacteria and mycorrhiza) were developed in modern restoration programs. New strengths have increased worldwide since most plant species, including grasses, associated with diverse microorganisms mainly mycorrhizae, Thus, a high percentage of restored areas, which were degraded due to the increasing conventional agriculture, need to be repaired. This study shows how to manage the restoration of degraded contaminated landscapes for a successful repair of key characteristics using arbuscular mycorrhizae from Glomeraceae.

Keywords: Degraded Lands; Mycorrhizas; Contaminated Soils; Ecological Restoration

Abbreviations: AMF: Arbuscular Mycorrhizal Fungi; SOM: Soil Organic Matter; SOC: Soil Organic Carbon

Introduction

Increasing deforestation, grazing and land contamination have as consequence the restoration of those ecosystems not sufficiently managed. Restoration aimed to establish improved ecosystems, integrating native species, creating some ecosystem functions to restore biodiversity or some functions and services. To restore contaminated ecosystems [1] for returning structure and function of the ecosystem to predisturbance situations, baseline conditions are determined before restoration activity or identification reference sites and conditions. This is required to mitigate soil erosion, flooding, and to increase pollination, among other ecosystem services. Restoring landscapes with ecologically sustainable plant covers is crucial. Plants affect the soil physical -chemical characteristics, modifying the soil microbiota (free, symbiotic fungal and bacterial microorganisms). With increased mining and ecosystem degradation, mechanisms for restoration of degraded ecosystems have been renewed [2,3]. With increasing estimation of biodiversity [4,5]. microorganisms associated with native plants in both undisturbed (reference) and degraded ecosystems (to be restored) were investigated [6]. The AMF symbiosis can be manipulated for restoration of degraded ecosystems, collaborating in restoration programs. Ecological restoration has developed from phytocentric, disregarding belowground microbiota [7] to the intricate current ecology [8]. The short-term restoration projects are generally not enough to monitor the vegetal growth, and changes in soil microorganisms as well as soil characteristic throughout years [5]. Difficulties in restoration practices such as saving wild species, needing more scientific data from them, for ecological and economic purposes, being relevant for conservation. [9]. Additionally, native plant species can promote native fauna, which disperse propagules, maintaining wildlife. The conservation of trees and related organisms increases forest values [2] is also used in agroforestry. The AMF symbiosis manipulated in the management of restored sites supplying scientific information [10].

Thus, restoration needs effective strategies for conservation of biodiversity, including endangered endemic species, single landscapes, hotspots, and biota with relevant ecosystem services [11]. Primacy areas for conservation and for habitat restoration were established and habitat indicators for forest and grasslands have been more examined. We surveyed here the current information on contaminated ecosystem´s restoration, with respect to research focusing on plant symbiosis. More scientific information on restoration practices of specific sites and strategic priority areas is needed for forest repairing. [12] have showed the situation of ecological restoration in Brazil, and, previously, [13] integrated two reference sites for comparing restored ecosystems. As reference sites are needed to evaluate the restoration success. In this sense, researchers continue to debate on the effective techniques for ecological restoration. Regarding natural regeneration in degraded tropical forests, it was stressed the proliferation of pioneer plants and fall of the shade-tolerant woody plants in several fragmented forest landscapes.

In this sense, the increase of disturbance-adapted native organisms, such as pioneer tree species, has a key role in the biodiversity of fragmented landscapes, where plant native species growth can be increased using wider spacing. However, little is known about the growth of native species besides the selection of fast-growing legume trees and their mycorrhizal and rhizobial symbioses [14] (Pagano et al., 2022) which has resulted successful [15], mined sites need urgent restoration as its vegetation, degraded rupestrian fields, need restored plantations. These low resilience fragmented sites with restricted potential for autogenic restoration need biologically viable restored sites providing persistent biodiversity. Additionally, the use of AMF in restored sites in Brazil, benefited from this association. Soil aggregation is also an important factor to be evaluated in restoration approaches as the stability of soil macroaggregates is influenced by the growth and decomposition of roots and mycorrhizal hyphae, which influences soil aggregation. Moreover, Indicators of soil health in restored soils include soil organic matter (SOM), soil organic carbon (SOC), soil nutrients, and soil microbial communities, especially AMF.

Restoration of contaminated sites

After the contaminant source ceased, restoration relays on natural physical, chemical, and biological processes to isolate, destroy, or reduce the bioavailability or toxicity of contaminants [16] accelerating the recovery. Early reports on the AMF present in two hydrocarbon polluted soils from Argentina and Germany, analyzed by [17] showed the root colonization and AMF (structures in stained roots), besides the AMF infective propagules, which were also analyzed, and trap cultures established with soils. Root colonization by AMF was higher in plants grown in non-polluted soils than in both polluted sites. In addition, the proportion of arbuscles to vesicles was higher in plants grown in non-polluted soil than in those grown in polluted soils. This was consistent with larger populations of AMF propagules with potential to colonize roots in non-polluted soils. Nevertheless, AMF propagules from polluted soils also had a high colonization capacity. *Glomus aggregatum and Glomus mosseae* were isolated and identified from both polluted and non-polluted soils, the occurrence of *Glomus* predominated.

Plants for restoration

Increasing native species' richness is a viable strategy for restoration, which may benefit from herbaceous species. Moreover, the need to better understand the interactions between vegetation, fauna, soil microbiota, and soil quality are crucial for plant species establishment and stability; however, key ecological engineer organisms remain unknown. Restoration can be improved by reintroducing mycorrhizal fungi from the native plant microbiomes present in natural ecosystems (Koziol et al., 2018) Despite the great demand for restoration of different ecosystems in Brazil [12], few analyses were carried out in their different and megadiverse biomes [18]. Plantations provide goods and services, improving financial benefits, from wood. and other products. Regarding the benefits of soil microbiota and amendments, it has been shown that AMF are supported by most plants in natural ecosystems being of high interest for restoration and conservation of natural ecosystems.

AMF can increase the uptake of phosphorus (P), nitrogen and other nutrients by plants. [5] have collected the potential of soil amendments (compost, biochar, and Arbuscular mycorrhizal fungi (AMF) for restoration of disturbed sites, signifying the rhizosphere "hotspot" of microbial activity, Thus, linking plant and soil microbiota is fundamental for efforts in most biomes including semiarid forests where most trees have mycorrhizal symbionts. Moreover, non-nutritional properties of this symbiosis, such as reducing plant diseases, stabilizing soil structure are the focus of recent research. As AMF are specialized in both their biotic and abiotic environments, both soil properties and plant species need to be studied to obtain advantages from AMF in restoration projects. However, improving the management of AMF in field situations is needed. Originally, mycorrhizae were commonly used in restoration benefiting plant growth; however, the contribution to soil structure, improving soil aggregation [19], thus, reinforcing soil structure, as the extraradical hyphae involve soil particles. A diagnostic procedure aids identifying anthropogenic disturbances in degraded sites [12].

Then, to enhance soils and control invasive species, weeds, leaf cutter ants and grazer fauna increase the success of restoration. Using diagnostic protocols barriers to ecological succession and the necessities for restoration of degraded soils in Brazil. [20]. proposed to check the suitability of the local micro-sites for native plant establishment and growth; Presence of barriers, such as soil or substrate degradation, exotic aggressive grasses, and/or intense herbivory and seed predation; besides presence of satisfactory soil seed bank, sprouts, seedlings and saplings of native tree and shrub species; Also, relative abundance levels of life forms (e.g., herbs, shrubs, trees), successional groups (pioneer or non-pioneer) and presence of adjacent forest fragments and effective seed rain can be evaluated. It was also pointed the

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necessity to include different life forms, restoration techniques and studies using reference areas to measure restoration success [18].

In this sense, the presence of different types of plant functional groups as well as different plant symbioses (such as mycorrhiza, *Rhizobium*, etc.) and their combination is crucial for the development of restoration practices. The use of rhizobia as well as AMF can influence plant growth, seedling survival and rhizospheric effect. However, Plant survival is of most importance to select species for restoration objectives. However, there are other fungal species living in soil presenting good capacity for soil restoration such as *Trichoderma* sp., Aspergillus spp., *Mucor* sp., and some mushrooms. *Trichoderma* being potentially more effective in degrading organic contaminants. Regarding ecological restoration in Brazil, the treated biome of Atlantic Forest is the most investigated (with the highest number of papers reported). This is due to its high biodiversity and priority for restoration. The reintroduction of native grasses and forbs via seeding or transplants is crucial for the restoration of grassy biomes [21], however, confused understanding about the ecology, conservation values of the grassy vegetation exist. (2) as the economic output of agricultural lands makes them expensive to reforest, which will degrade biodiversity and compromise ecosystem services [22].

The steps followed to add AM fungi to disturbed soils where previously tested. Ihe best approach to inoculate a restoration site is with topsoil (stored in adjacent sites). As has been shown, propagules of native AMF (extraradical hyphae, spores, auxiliary cells), other soil organisms, organic matter, fine roots, and native seeds are present in topsoil. On the other hand, if topsoil is not accessible e.g. due to storage space restrictions, AMF may be added to disturbed soils using other procedures. Commercial inoculum, which usually contains only one species of AMF, *Glomus intraradices*, is generally applied; however, to develop site specific native AMF inoculum is more commonly considered and appropriate. In this regard, using spores or soil as inoculum is common. For monitoring restored sites, the determination of infective propagules including spores recovered from rhizospheric soils and roots (stained for AM colonization) of plants growing in the degraded and reference soils, are required.

Among other indices, abundance of AMF spores and percent root colonization can be evaluated to compare the restored site with reference sites and, after statistical analyses, would assist restoration success with time. Usually, cluster analysis, PCA) based on the presence of AMF species in the studied sites, can show changes in AM composition with site Interestingly, some ecological attributes (plant cover, abundance, sociability, mycorrhization and tolerance to future climate changes), for use in long-term ecological restoration. Moreover, AMF and specific rhizobia plant inoculation can increase plant growth and survival, when inoculated. In Brazil, efficient strains were selected and used as rhizobial inoculum for several native species. This double inoculation improved the survival of those plant species. In general, three or more AMF families can be found in the soils of the rhizosphere. Some tree species such as in the legume tree *Anadenanthera peregrina* [23] (figure 1).

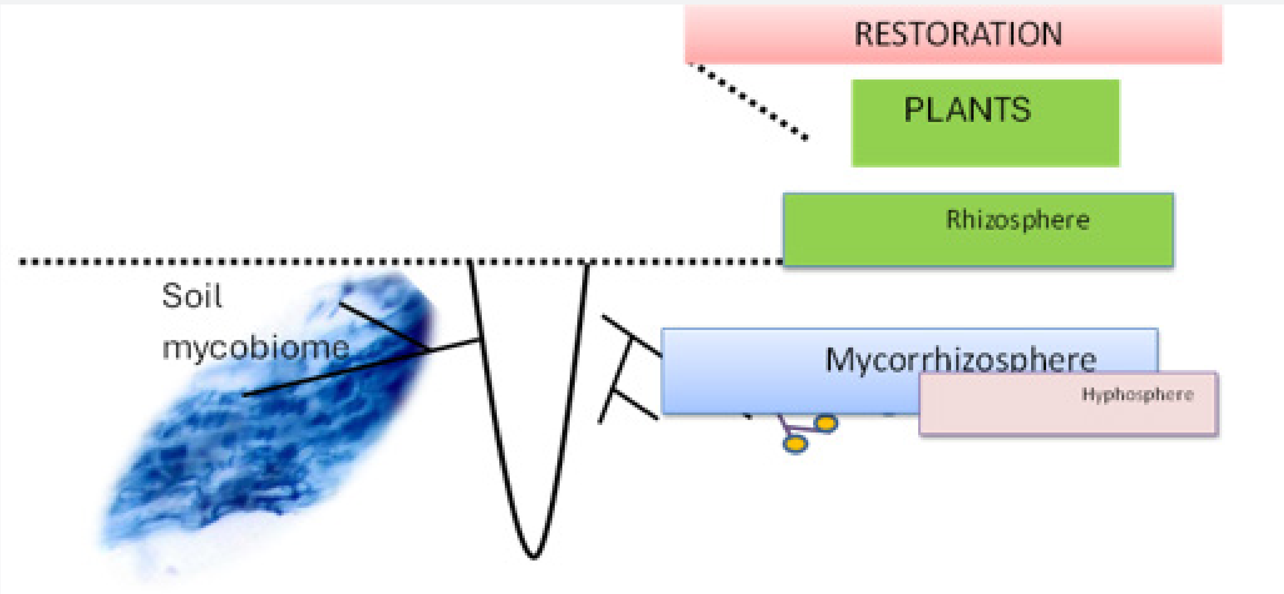

Figure 1: Conceptual diagram of restoration of degraded contaminated ecosystems using AMF for restoring contaminated ecosystems. After evaluation of diagnostic of a contaminated site and establishment of 2 undisturbed sites, restoration can be achieved by the introduction of topsoil or plantations. AMF inoculation can be performed on host plants or by inoculation (c). Monitoring the restored sites: determination of infective propagules including spores recovered from rhizospheric soils (d) and roots of plants growing in the degraded and reference soils stained for AM colonization (e) (Photo credit M. Pagano).

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However, the reported studies used only a reference area for comparing restoration success, and the most examined organisms were plants (81%), (trees > fungi > birds, invertebrates, mammals, and reptiles [18]. Interestingly, in dry deciduous woodlands most trees associate with mycorrhizas, pointing out their value for restoration efforts [24].

The multiple symbiosis of AMF - rhizobium in restoration

AMF can improve the N_2-fixing symbioses providing P to the plant, potentializing N_2 fixation), the resulting tripartite symbioses create an efficient strategy to accelerate soil reclamation and ecological restoration [25]. Nevertheless, compatibility between native rhizobial strains and AM species /isolates can be tested in greenhouse conditions Nowadays, requirements of native plantations need to be more studied to formulate specific soil fertilization. Additionally, reduced fertilization in the presence of symbionts must be tested. Both restoration and revegetation demand understanding of plant life histories including traits related to AM formation

Therefore, the choice of plant species would have great implication in the manipulation and conservation of mycorrhizal species. Native AM can colonize plants in natural conditions but the loss of these fungi with disturbance will require specific management. Thus, highly dependent plant hosts should be selected over mycorrhizal- independent hosts. The need for more information on how restoration practices influence mycorrhizal fungi development and function was highlighted. Some researchers [15]. stressed that management of AMF in soil can take in count a higher fungal sporulation to increase C fixation in soil as spores present a higher C contain.

AMF as an important component that should be included in restoration programs. Detailed works are invaluable and will provide important evidence of the fungal and plant diversity association. Shows tree plantations associated with mycorrhizas in riparian forests of Brazil and AMF spores recovered from their rhizospheric soils. Thus, only recently restoration specialists have started inoculating AM to degraded soils with the objective of promoting plant growth, accelerating ecological succession, or attain desired plantations [26]. As restoration efforts require a better understanding of successional procedures, soil microbiota succession must also be studied. Regarding AMF and ecological succession, there are controversial reports [12]. have discussed the state of art of ecological restoration in Brazil (Table 1). Regarding AMF, aseptate intra and intercellular hyphae, vesicles, were observed in most of plant samples. Arbuscules or hyphal coils were less frequent. Root colonization varied from about 77% in the revegetated area, 58 to 60 % in the degraded area and 66 % in the preserved area. AM colonization was higher in the preserved and revegetated areas.

Table 1: Recent papers dealing with ecological restoration in degraded contaminated sites.

Reports on AMF and plant restoration		References
Restored environments	Brazil	Pagano (2012)
Soil samples	Argentina, Germany	[17]
Mine spoil restoration	Brazil	Souza et al. (2011)
AMF in degraded land restoration	Brazil	Soares and Carneiro (2010)
Restoration grasslands		Lehmman and Parr (1991)
History restoration restoration		[27]

Microorganisms for restoration

Ecological restoration has been underpinned on the cultivation of native plant species, which performed better when inoculated with appropriate bacteria and fungi. Usually, uninoculated or inoculated transplants are used but soil microbes are increasingly focused for benefit restoration ecology to test the interactions between plants and microorganisms. Among soil microorganisms, mycorrhizas constitute essential symbiosis together with *Rhizobium in legumes,* facilitating the plant establishment under stressful and unfavorable circumstances.

Restoration procedure

Ecological restoration is a multi-phase procedure, with different stages: where to assess the site and conditions, actions to identify and analyze the causes of disturbance and methods for stopping them, are evaluated. It is also required to prepare objectives for the biota, and microbiota and continuously comparation to reference sites (similar pristine adjacent ecosystems). It is also required to check for historical pre-disturbance communities, and species best adapted to changing climate conditions; besides eliminate sources of disturbance. It is also needed to control livestock and invasive plant species in riparian areas, despite checking soil properties (pH, Organic Matter, Base saturation, P content, which are crucial to facilitate nutrient cycles [27-45] (Figure 2,3).

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Figure 2: Vegetation of restored iron mined sites in Brazil, inoculated seedlings AMF spores recovered from rhizospheric soils of native vegetation (*Scutellospora* and *Acaulospora*) recovered from rhizospheric soils (photo credit M. Pagano).

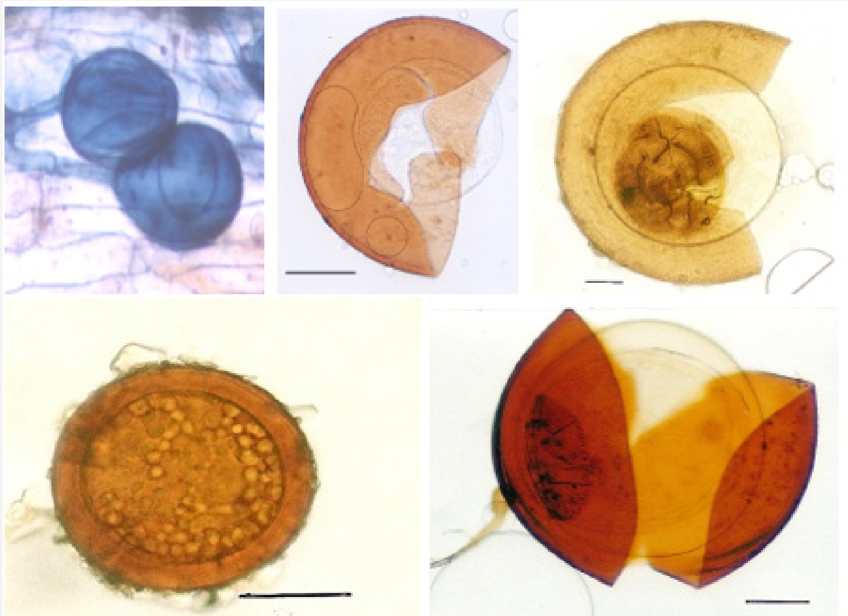

Figure 3: Mycorrhizas in restored lands. native plantation, colonized root by AM, and spores recovered from *campo rupestre rhizospheric* soils*:* *Scutellospora* and *Acaulospora* (Scale bar 10 μm photo-credit M. Pagano).

Conclusions

Contaminants affect plant-soil associated microorganisms either in number, diversity, and activity, however, restoration efforts mitigate such disturbances, increase soil organic matter improving soil structure maintaining soil communities associated to plant species. Soil microbes, and especially the interactions between plants and microorganisms such as mycorrhizas symbiosis together with *Rhizobium* in legumes, protect the plant under stressful hostile conditions. Several efforts to enhance advantages from mycorrhizae in restoration were reported. to achieve maximum understanding of soil microorganisms and their associations as well as the selection of benefic microbiota during plant succession.

AM occurrence in different preserved and restored ecosystems, and questions related to ecological succession Consequently, further research is necessary on plantations for restoration of degraded sites, especially in riparian, dry forest and many threatened ecosystems.

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