

# Chapter 21

## Mycorrhizas and Ecological Restoration in South America



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### Contents

21.1	Introduction.....	432
21.2	Ecological Restoration and Mycorrhiza: A General View.....	433
21.3	Ecological Restoration and Mycorrhizas: State of the Art in South America.....	434
21.4	Study Cases for South America.....	435
21.4.1	Role of Arbuscular Mycorrhiza in the Ecological Restoration of Venezuelan Degraded Ecosystems.....	435
21.4.2	Effects of Individual and Consortia of Arbuscular Mycorrhizal Fungal Species in the Quality Index of the Endangered Conifer from Chile ( <i>Araucaria araucana</i> ).....	437
21.5	Ectomycorrhizas and Ecological Restoration with a Focus in the Neotropical Region.....	439
21.6	Conclusion.....	440
	References.....	441

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## 21.1 Introduction

Restoration ecology is a discipline that aims to return a degraded ecosystem to an historical trajectory, in order to sustainably persist for a long term, by using the natural history knowledge of a particular ecosystem as well as the ecological theory (Huxel and Hastings 1999; Hobbs and Harris 2001; Van Andel et al. 2012). Currently, internationally exists a growing development of this area of knowledge, with the existence of journals such as *Restoration Ecology*, which publish several investigations about ecological restoration, from different regions of the world with the support of the Society for Ecological Restoration (SER; [www.ser.org](http://www.ser.org)).

The concern of restoration ecology is highly ambitious, since it requires previous ecological knowledge and the participation of different disciplines such as ecology, botany, zoology, mycology, and edaphology, among many others, and to have a reference ecosystem, which is the one with the characteristics that are expected to reach after the restoration actions. However, the ecosystem dynamic is probabilistic; hence, it can develop many different trajectories. Many of them can occur at the same time in different parts of the spatial dimension since the landscape can have environmental heterogeneity. Based on this, it can be acknowledged that usually it is not appropriate to perform only one restoration plan. Therefore, a restoration plan should have several sub-plans that consider the heterogeneity of the reference ecosystem.

There are two types of ecological restoration strategies: (a) passive restoration, which is the action that implies preventing, controlling, or modifying the degradation factors of a certain ecosystem, such as tree felling, hunting, wildfires, and livestock, among others, in order to allow the recovery of biodiversity and ecological functioning through natural succession (Becerra et al. 2018); and (b) active restoration, which are the actions performed when a component of the ecosystem or an ecological process cannot be recovered, those actions might be, for example, habitat management, sowing of propagules, trees, shrubs or herbs, planting, watering, fertilization, using artificial shadow, among others (Becerra et al. 2018). Which type of restoration is used depends on the site conditions, and any restoration plan should

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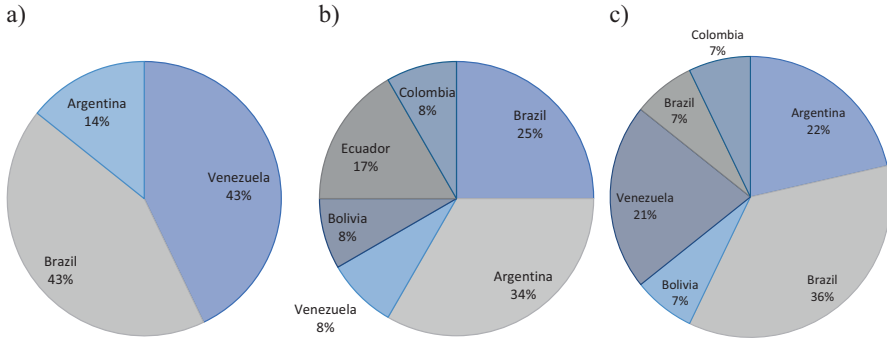
evaluate, a priori, the type of restoration needed, as well as where and when the restoration should be performed, following what is known about the focal ecosystem.

In the recent years, new conceptual elements and theoretical frameworks have led to generating restoration proposals by using natural resources from the same site that will be restored. In this context, nature-based solutions (NbS) arise, improving restoration effectiveness and generating several contributions of nature to people, through the recovery and maintenance of ecosystems. Those benefits can reduce the negative socioeconomic and environmental impacts of the different change precursors of ecosystems. Given that any strategy or sustainable action that aims to be effective and scaled to a social level should incorporate the sociocultural context of the communities (Bartels et al. 2013), the effectiveness of the NbS significantly increases when a co-production of a NbS approach is followed, in where the communities knowledge and experiences are enriched with the science-technical knowledge (Lavorel et al. 2020).

## 21.2 Ecological Restoration and Mycorrhiza: A General View

Mycorrhizal associations have benefits for both partners that establish the symbiosis, since the mycobiont helps the plant with the uptake of nutrients and water from the soil and receives carbohydrates in return (Smith and Read 2008). Also, the mycorrhizal symbiosis allows the plant to tolerate biotic and abiotic stresses, such as drought and pathogens (Delavaux et al. 2017). These functions escalate to population, community, and ecosystem levels, since these enhance plant recruitment, increase plant diversity and ecosystem productivity, and drive carbon and nutrient cycling (van der Heijden et al. 2015). It is also a matter of high importance to consider mycorrhizal symbioses in the context of climate change and its mitigation. Plants with mycorrhizal associations are responsible for significantly higher carbon sequestration compared to non-mycorrhizal plants (Soudzilovskaia et al. 2019). Therefore, since ecological restoration is one of the main mitigation solutions for climate change, through the logic of increasing carbon sequestration (Bastin et al. 2019), mycorrhizal symbiosis consideration will support and upgrade the above-mentioned solution (Soudzilovskaia et al. 2019). In consequence, several studies around the world have considered the use of mycorrhizal fungi to improve the outcomes of ecological restoration.

A relatively recent global meta-analysis found that when mycorrhizal symbiosis is considered and applied in restoration contexts, plant biomass increases, with the greatest effects observed in N-fixing woody plants, C<sub>4</sub> grasses, and plants growing at low soil availability of P, and that the increment of biomass also increases in the first 3 years after inoculation (Neuenkamp et al. 2019). Species richness of the restored communities is also higher when mycorrhizas are used and the restored communities being much more similar to the reference ecosystem in the same contexts (Neuenkamp et al. 2019).



**Fig. 21.1** (a) Proportion of field experiments by country in contexts of ecological restoration. (b) Proportion of nursery experiments by country with an interest in ecological restoration. (c) Proportion of descriptive studies by country searching for mycorrhizal behavior in contexts of ecosystem recovery

### 21.3 Ecological Restoration and Mycorrhizas: State of the Art in South America

In the valuable work of Neuenkamp et al. (2019), as usually occurs with meta-analyses, they do not consider all the available information, mainly due to fit selection criteria for the particular questions of the study. In fact, in the general patterns found by Neuenkamp et al. (2019), only two studies from South America were considered. It is acknowledged that South America can show different ecological patterns compared to those found in the northern hemisphere, especially in terms of mycorrhizal symbioses (Bueno et al. 2017). Thus, searching deeper for other studies conducted in South America might show some deviation of this region from the general patterns already found. This can have important consequences when an ecological restoration experiment within the South American region might be developed. Consequently, for the present chapter, data search was performed in the Web of Science (WoS), using the keyword string *mycorrhiza\** AND (restoration or reclamation or rehabilitation) and between the years 1980 and 2022. This search identified 1509 studies. Then, within the results of the first search, a filter by South American countries was applied, one by one: Chile, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, Suriname, French Guiana, Brazil, Paraguay, Uruguay, and Argentina. Among them, only seven published studies involved the use of arbuscular mycorrhizal (AM) symbiosis in field experiments in contexts of ecological restoration were found from Venezuela, Brazil, and Argentina, (Fig. 21.1a). In the same search, other studies indirectly related to ecological restoration were found, such as 11 studies in nursery conditions focused to apply the obtained results in field ecological restoration contexts that were also found for South American countries (Fig. 21.1b). Finally, 14 studies explored what occurred with mycorrhizal symbiosis either in space, time, or both after some years of restoration in perturbed sites (Fig. 21.1c).

It is worth highlighting the pioneer studies in the topic of ecological restoration in South America. Those come from Venezuela and as such, they will be further mentioned in the next section. Then a new study from Chile will be presented, and even though is a nursery experiment, the same experiment was replicated in the field, and data are currently under analysis to be further published as the first case of a field experiment of ecological restoration by using arbuscular mycorrhizal fungi (AMF) in Chile.

## 21.4 Study Cases for South America

### 21.4.1 *Role of Arbuscular Mycorrhiza in the Ecological Restoration of Venezuelan Degraded Ecosystems*

Five years after the restoration of a small area (0.16 ha) of a tropical dry forest at Península de Macanao (Venezuela) (Fajardo et al. 2013), Fajardo et al. (2015) compared the AMF communities present in restored plots with those found in plots with 5 years under natural regeneration. The results showed that restoration may have promoted a greater richness and diversity of AMF, in particular, in those plots where a hydrogel was applied (the more effective treatment), although the differences between restored and no restored plots were not significant. It is possible that 5 years after restoration were not enough to define these trends. However, the species composition of AMF between restored and no restored plots was different with the presence of species belonging to the Gigasporaceae in the restored plots, which is a family usually found in undisturbed habitats.

It has been widely reported that the successional rate of degraded ecosystems could be faster through inoculation of plants with AMF of interest or management of their populations (Janos 1980; Allen 1991). In this regard, the use of AMF as a bioinput to propagate tree species to recover disturbed areas is a practice that, in Venezuela, has been increasingly implemented in recent years.

Early works using this important biological interaction for the restoration and rehabilitation were carried out in the oligotrophic savannas of southern Venezuela (Cuenca et al. 2002, 2003). More recently, Cáceres et al. (2014) evaluated the effect of inoculation with AMF from different successional localities and a xeric scrub (reference ecosystem) on the growth and survival of two legumes species with potential to be used in the restoration. For *Piscidia carthagenensis*, the experiment consisted of the application of four treatments that combined three soil types and four inoculation conditions. At the greenhouse, inoculum from two successional sites (2 and 20 years old of abandonment) produced the greatest effects on height, total biomass, and leaf area when plants were grown in the successional soil, but not when grown in the scrub soil (Kalinhoff 2012). In the field conditions and after a drought season, it was found that *P. carthagenensis* plants grown in successional soil inoculated with native AMF showed a survival rate higher than 80% compared

to non-inoculated plants (Cáceres et al. 2014). For *Coulteria mollis*, two soil types and three inoculation conditions were combined. The growth response (height and total biomass) of inoculated plants that were grown in the scrub soil was significantly higher than those grown in the successional soil regardless of the inoculation received (Cáceres et al. 2014). It is possible that functional compatibility between different arrays of fungi and plants associated, in turn, with the AMF diversity of each inoculum used, explains the differential responses observed between both tree species (Cáceres et al. 2014). These authors concluded that the production and use of native inoculum of AMF is a favorable strategy for assisted restoration.

Continuing with the activities of recovery of dry forest areas destroyed by sand mining, in 2014, it was decided to incorporate AMF as an additional treatment to improve the propagation of several tree species and leave, as a fixed treatment, the application of hydrogel for its proven effectiveness (Fajardo et al. 2013). To do this, it was selected two distinct areas for planting. One of them consisted of an area under natural regeneration (SA) that began once sand extraction ceased, and the other one was an area where sand mining had finished recently, and hence it was an area with practically bare soil (DA). Regarding plant species, it was selected four native tree species: *Prosopis flexuosa*, *Parkinsonia praecox*, *Coulteria mollis*, and *Bulnesia arborea*. In the nursery, 75 pre-germinated seeds, of each species, were sown in two soil types— one of them collected from the degraded area (DA) and the other from the successional one (SA)—and subjected to 3 inoculation treatments, non-inoculated control (C), inoculation with a mix of AMF from the degraded area (DI), and inoculation with a mix of AMF from a no disturbed area (NI), 25 seeds for each treatment. The mycorrhizal inoculum had been produced 4 months earlier. After 6 months of growth in the nursery, a total of 432 individuals were planted in 9 plots established both in DA and SA. The height and survival of all individuals planted were recorded three times a year.

In general, in the DA, 50% of all plants that were inoculated with DI had survived, while 45% of all no inoculated plants remained alive. Only 39.7% of all plants inoculated with NI survived. Regarding plant species, *P. praecox* had the higher number of live individuals in all treatments, especially when it was inoculated with the NI. Meanwhile, *B. arborea* had the highest mortality. *P. flexuosa* and *C. mollis* had an intermediate survival percentage, highlighting the fact that *P. flexuosa* might not need to be inoculated judging by the results. In the SA, total survival was below 10% at the end of the evaluated period, and the majority of live individuals were inoculated with NI. Again, *P. praecox* was the plant species with the high percentage of survival, especially when it was inoculated with NI.

With respect to growth, which could only be determined in the DA, both *P. flexuosa* and *C. mollis* showed the highest height values in the control treatment, while *P. praecox* grew slightly more when it was inoculated with NI, though the differences with the other treatments were not significant. In summary, neither *P. flexuosa* nor *C. mollis* would not be necessary to inoculate them, while it would be recommended to do so for *P. praecox*.

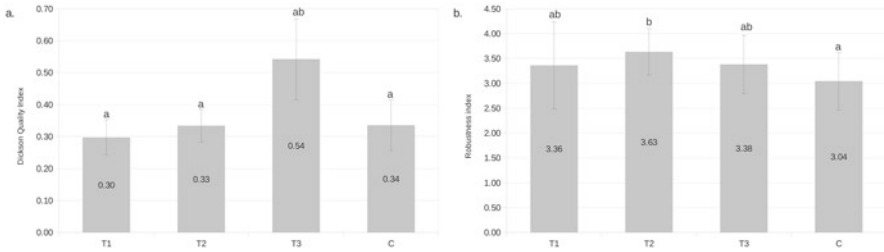
Planting mycorrhizal trees or shrubs in degraded areas is a strategy that favors the creation of the so-called fertility island. These plants (also considered as nurse

plants), besides contributing to recovering the natural inoculum of soil lost during degradation processes, which will favor the incorporation of other mycorrhiza-dependent plant species, would also act as elements generating wind turbulence around the individual that would produce an increase of the propagule recruitment both from other plant species and AMF (Cuenca 2015). This would contribute to reactivate and accelerate the natural regeneration (succession) that could otherwise be extremely slow, which could lead to further degradation of the site.

#### **21.4.2 Effects of Individual and Consortia of Arbuscular Mycorrhizal Fungal Species in the Quality Index of the Endangered Conifer from Chile (*Araucaria araucana*)**

In the coastal Nahuelbuta mountain range (37° 40'S to 37° 50'S) in south-central Chile, the temperate forests of the conifer *Araucaria araucana* are classified as endangered (MMA 2020) as a result of the expansion of the agricultural frontier and an increase in the frequency and intensity of forest fires, product of climate change (Garraud et al. 2020). This makes it necessary to generate nursery seedling production tools, to strengthen future ecological restoration plans. In this context, it has been suggested that the underground microbiological and plants' metabolic processes (and their effects in the overall ecosystem functioning) would be more adapted to in-site and local conditions in a scenario of ecological restoration using microbial inoculum (Godoy and Marín 2019). Plants and their rhizosphere, including their AM fungal symbionts, constitute a close spatial-temporal connection with ecosystem age, reflecting the state of ecological succession on a large scale. Previous research has also shown that in such restoration programs, applying consortia of AMF works better regarding plant growth and survival than applying single species inoculum (Aguilera et al. 2017; Godoy and Marín 2019).

In order to test such predictions, an experiment to assess the effects of single and mixed-species commercial AM fungal consortia, as well as native AM fungal consortia in the quality index of *A. araucana* seedlings, was performed in Nahuelbuta National Park, south-central Chile. For this, *A. araucana* seeds were collected from the same park (and stored at 4 °C) to maintain the genetic variability of the species and not alter the ecosystem processes of the rhizosphere in the experiment (Fernández et al. 2020). Prior to germination, seeds were disinfected with 1% NaOCl for 1 min and rinsed twice with distilled water before sowing. Germinated seeds were arranged in 2 L plastic pots with composted bark substrate. In addition of a control (C) treatment without inoculum, three treatments with 1 g of inoculum material per seedling (which contained approximately 900 AMF spores) were established: T1, single AMF species (*Claroideoglossum claroideum*) from a commercial inoculum; T2, four AMF species (*Acaulospora laevis*, *Scutellospora calospora*, *Clariodeoglossum etunicatum*, and *Rhizoglossum intraradices*) from a commercial



**Fig. 21.2** Average values and standard deviations for Dickson's plant quality index (a), and robustness index (b) for *Araucaria araucana* seedlings ( $n = 25$ ) in treatments with different mycorrhizal inoculations (T1, singular commercial AMF species; T2, four commercial AMF species; T3, native soil containing 17 AMF species) and control (C). Different letters above the mean value indicate statistically significant differences ( $p < 0.05$ ), according to the Games-Howell test

inoculum; and T3, corresponded to native soil containing 17 AMF species (Becerra 2019). A total of 100 seedlings were evaluated (25 per treatment). After 11 months of the bioassay under controlled conditions in the nursery, the evaluation was carried out by harvesting the seedlings for each treatment and the control, to calculate the Dickson quality index and the robustness index (Rueda Sánchez et al. 2018).

The Dickson quality index was significantly higher in T3 (0.54) than in the other treatments (0.30–0.34), which did not differ among them (Fig. 21.2a). In contrast, the robustness index was significantly higher in T2 (3.63), followed by T3 (3.38) and T1 (3.31), and the control (3.04) was significantly lower than the rest (Fig. 21.2b). There is scarce research in the use of the rhizosphere and native symbionts (and their interactions with plants), as restoration tools for native Chilean flora (Godoy et al. 1995; Godoy and Marín 2019; van Galen et al. 2021). A study by Godoy et al. (1993) using the AMF species *Rhizoglyphus intraradices* as inoculum for four native conifer species under greenhouse conditions (20 weeks) shows that all morphometric variables analyzed had higher values than the non-inoculated control, indicating compatibility and efficiency of the mycorrhizal inoculation. In this experiment, it is shown that consortia of AM fungal species lead to better results as compared to single-species inoculum and the control. But the evaluation of the inoculation effects depends on the metric used; as here, different indexes were higher under different treatments. Native AMF species inoculum increased the overall plant quality, while AMF commercial consortia increased their robustness, probably indicating differential roles and/or functional redundancy of the particular AMF species involved. Future forest ecological restoration plans in altered ecosystems should include the use of native mycorrhizal inoculates, to increase plant adaptation and survival, ultimately and hopefully recovering tree populations. In this sense, currently, a field experiment with the same setting is being analyzed.



## 21.5 Ectomycorrhizas and Ecological Restoration with a Focus in the Neotropical Region

Currently, at a regional scale, it was possible to find one short-term study, and outside the WoS search previously mentioned, on the use of ectomycorrhizas (ECMs) in a field experiment in contexts of ecological restoration, with positive effects on plant quality index when ectomycorrhizas are used to inoculate plants (Godoy and Marín 2019). At a global scale, few restoration studies include soil microbiota, and research that explores the landscape does not look so much different. Effects of ECMs in plant success are rare, especially in the neotropical region. Research about restoration has grown in the last decades, but studies usually explore ecological theories that consider only plants. Considering other organisms will allow a better understanding of the plant communities and avoid unsuccessful restoration. How communities assemble, both naturally and artificially (via restoration), has received worthy attention (Belyea and Lancaster 2012; Temperton and Hobbes 2004), but only a few studies have evaluated belowground patterns and the role of soil microbiota (e.g., mycorrhizal fungi) in the restored communities (Sun et al. 2017). Belowground interactions may play an important role in restoration projects, especially in ecosystems that deal with environmental challenges such as high irradiance, salinity, nutrient-poor soils, or contaminated soils (Barcelos et al. 2012).

Inoculation of arbuscular mycorrhiza (AM) has been used as a biological tool for plant establishment and development in agriculture (Solaiman and Mickan 2014) and in restoration (de Moura et al. 2022) practices. However, little is known about the role of ECMs and whether they can facilitate restoration in the same way AMF does. The studies on tropical ECMs are fairly recent (Corrales et al. 2018; Vanegas-León et al. 2019), and the role of ECMs in the neotropical region is not very well known. The use of ECMs in the restoration of native forests in the neotropical region deserves more attention.

Ectomycorrhizas are considered pioneer associations during the colonization of plant communities (Lindig-Cisneros et al. 2019). Introduced native seedlings (via restoration) may not succeed if the native microbial communities are not introduced as well, especially those associated with initial colonization. Restoration studies often involve controlling invasive species (Weidlich et al. 2020), which may depend on more (Moyano et al. 2020) or less (Vogelsang and Bever 2009) on mutualistic species. Very little is known about the consequences of having exotic soil microbiota in areas where exotic plants have been removed. Since plants actively alter the soil microbiome to their benefit in a species-specific way (Huang et al. 2019), aboveground biological invasions also impact the underground. Specifically, for ECMs, interactions between native plants and exotic fungi do not necessarily cease after the removal of exotic plant species (Lofgren et al. 2018), and this fact adds an unknown competition problem between native and introduced fungi.

In the neotropical region, there are records of ectomycorrhizal fungi (Singer et al. 1983; Sulzbacher 2013; Roy et al. 2016) even though not much is known about their function in forests. It is safe to say that ECMs provide ecosystem services, act

in processes that help to recover native environments, and, therefore, promote restoration (Ortega et al. 2004). It is assumed this must be the case for forests in the neotropical region as well. Specific ectomycorrhizal fungi enhance the survival rates and early growth performance of several plant species (Carey 2016). Inoculation of nurse seedling species with appropriate fungal partners is one of the most efficient environmental approaches, especially in disturbed ecosystems. The presence of endemic symbionts in an ecosystem is an indicator of a well-diversified community (Mueller and Halling 1995) either above- or belowground. Ectomycorrhiza formation could be one of the reasons species become established and survive in harsh environments. The benefits of these interactions may go beyond single individuals. It is believed that nurse plants facilitate other plant species to become established by (1) improving microclimatic conditions on-site, (2) changing the soil microbiota to favor other ECM-forming plants, or (3) acting as a hub tree, directly transferring resources through ectomycorrhizal connections. Ongoing research in southern Brazil is studying if part of these complex interactions can be attributed to ECMs (Weidlich et al. 2020).

It is, therefore, essential to know more about the diversity and ecophysiology of ECMs in tropical regions and find out which are the ECM hosts and how frequently these associations occur. Also, it is necessary to investigate how the neotropical ECM function and what are the benefits to the partners. This is challenging because it forces us to look outside the box, searching for undescribed patterns. A better understanding of ECMs in the neotropical and tropical regions will allow us to infer how it can be included in restoration projects in the same way arbuscular mycorrhizae have been used.

## 21.6 Conclusion

The experiments from South America are scarce and come from Venezuela, Brazil, Argentina, and Chile most of them showing positive effects in plants as well. The scarce evidence from South America comes mainly from arbuscular mycorrhizal symbiosis, with one exception for ectomycorrhizal symbiosis. Also, it was not possible to find evidence of the role of orchid and ericoid symbioses in experiments from South America. More field experiments are needed in countries such as Perú, Ecuador, Colombia, Guyana, Suriname, French Guiana, Uruguay, and Paraguay, and it is also urgent to explore the effects of orchid and ericoid mycorrhizas in plants in the context of ecological restoration.

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