ER 412
Galiano Field Course
Olga Tkachenko
V00051631

# Mycorrhizal Connections at the Galiano Conservancy Association:
## Working with existing fungal diversity to improve restoration success

ER 412
Galiano Field Course
Olga Tkachenko
V00051631

**Summary**

The application of lessons learned from studies of mycorrhizal fungi and plant symbioses as they pertain to restoration has been slow and inconsistent, despite evidence that considering mycorrhizae is vital to ecosystem success, as well as to the conservation of fungal diversity (Markovitch et al., 2023). To assist in incorporating some of these lessons, I briefly describe the current state of knowledge on mycorrhizal fungi application in restoration. I also describe a simple randomization protocol for introducing mycorrhizal fungi to restoration projects at the Galiano Conservancy Association.

**Introduction**

One difficulty of restoring degraded ecosystems rests in the recovery of soil. Soil communities and health of soil ecosystems can largely determine restoration success, especially where historical soil communities have been degraded or changed beyond recognition (Wubs et al., 2016). A measure that has been shown to increase the success of restoration of degraded sites is inoculation with mycorrhizal fungi, important components of the soil microbiome that form symbiotic associations with the roots of vascular plants (Maltz & Treseder, 2015; Wubs et al., 2016; Brundrell & Tedersoo, 2018).

As many as 90% of vascular plant have an association with mycorrhizae (Brundrell & Tedersoo, 2018; Brundrell & Tedersoo, 2020). The benefits to both fungal and plant partners are numerous, including carbohydrates, habitat, and deep-water access for the mycorrhizal partner, and increased water and nutrient uptake for plants (Brundrell & Tedersoo, 2018). The latter results in increased plant success, especially in later successional stages, when plants more likely to be obligate symbionts with mycorrhizae (Greipsson, 2010, p. 195; Brundrell & Tedersoo, 2018). Mycorrhizal inoculation has been shown to increase plant biomass, especially in nitrogen-fixing plants, as well as in ecosystems with low bioavailability of phosphorus (Neuenkamp et al., 2019). Additionally, mycorrhizal fungi can break down complex molecules in the soil, making them bioavailable for plant uptake (Read et al., 2004). Association with mycorrhizae has been shown to be protective to plants in times of stress, such as in degraded environments and those with high salinity (Yu et al, 2022). Mycorrhizal fungi can improve plant defences against pests and pathogens and help prevent invasions by introduced plants (Greipsson, 2010, p. 194; Tao et al., 2017; Yu et al., 2022).

## Goals and Objectives

*Goal*

The goal of this paper was to create a protocol for the application of mycorrhizal fungi in ecosystem restoration for use by the Galiano Conservancy Association staff, without the need for expert mycologist knowledge, while providing important background information on the current state of knowledge in the field of mycorrhizal fungi and restoration.

*Objectives*

1) Summarize the current gaps between knowledge of the importance and ubiquity of mycorrhizal fungi to ecosystems and application in restoration projects.

2) Identify possible difficulties, knowledge gaps, and future directions in application of mycorrhizal fungi to restoration and how that pertains to the GCA.

ER 412
Galiano Field Course
Olga Tkachenko
V00051631

3) Create a simple protocol for the application of mycorrhizal inoculant to restoration sites at the GCA that does not require expert mycologist knowledge or extensive experimental design experience to implement.

**Background**

Mycorrhizal fungi represent several different fungal lineages that co-evolved with vascular plants, likely emerging 450 million years ago (Brundrell & Tedersoo, 2018). There are four main types of mycorrhizae: what was formerly called endomycorrhizae, now referred to as arbuscular mycorrhizae (AM); ectomyccorhizae (EcM), comprising species of Basidio- and Ascomycetes; ericoid mycorrhizae (ErM), which associate with members of the Ericaceae plant family, and orchid mycorrhizae, associated with the Orchidaceae family (Brundrett & Tedersoo, 2018).

AM are known for forming structures called "arbuscules" within the plant cell and are associated with 72% of vascular plants (Brundrett & Tederso, 2018). EcM form structures called mantles and Hartig nets and are found in 2% of vascular plants (Brundrett & Tedersoo, 2018; Brundrett & Tedersoo, 2020). Ericoid and orchid mycorrhizae are associated with 1.4% and 10% of all vascular plants, respectively (Brundrell & Tedersoo, 2018). Notably, many tree species in temperate and boreal forests form symbioses with EcM, including those in the family Pinaceae (*Tsuga*, *Abies*, *Pseudotsuga*, and *Pinus*), as well as *Arbutus*, *Alnus*, *Quercus* and *Acer* species (Policelli et al, 2020; Brundrett & Tedersoo, 2020). Ericoid mycorrhizal fungi (ErM) form associations with plants in the family Ericaceae, of which Salal (*Gaultheria shallon*), is a member (Brundrett & Tedersoo, 2020).

ER 412
Galiano Field Course
Olga Tkachenko
V00051631

**Inoculation via commercial vs. whole-soil inoculant**

Studies in lab settings and in the field have demonstrated that mycorrhizal inoculation can increase plant biomass and survival, but that application of commercial mycorrhizal inoculants yields little to no improvement in plant growth results compared to whole-soil inoculation – the addition of a sample of soil from an intact soil community – which results in much greater improvements in plant growth (Paluch et al., 2012; Lance et al., 2019; Markovchick et al., 2023).

In a 2015 meta-analysis, Maltz & Treseder found that inoculation with soils from reference sites with intact mycorrhizal communities was more successful in introducing mycorrhizae to restoration sites than using single mycorrhizal species isolate as inoculant or applying commercial inoculants. In particular, inoculation efforts in restoration sites yielded the most success where the original mycorrhizal community was degraded and levels of mycorrhizal diversity low, and where diverse plant communities were introduced that can support mycorrhizal diversity similar to that of the reference sites (Maltz & Treseder, 2015). Lance et al. (2019) found that application of whole soil inoculant yielded superior results even to applying a ten times greater volume of commercial inoculant than recommended by the manufacturer. The additional benefit of inoculation with whole soil samples is that intact beneficial microbe communities may be introduced to the degraded site, whereas inoculating with a commercial mycorrhizal inoculant introduces only a limited number of mycorrhizal species, some of which may not be specific to the restoration site (Maltz & Treseder, 2015).

Although mycorrhizae have been shown to migrate from sites with intact mycorrhizal communities to nearby disturbed sites over time, this migration is slow and not simultaneous with plant reintroduction, resulting in less diversity of mycorrhizal species at degraded sites than that found at nearby reference sites (Markovchick et al., 2023a). This finding suggests that there is a place for mycorrhizal re-introduction from intact soil areas, especially in restoration of sites severely degraded by heavy metal contamination, clearcut logging, mining, and fire (Markovchick et al., 2023a).

**Why consider mycorrhizae?**

One issue faced by restoration practitioners attempting to apply the lessons learned from the last two decades of research is the gap between current knowledge and research findings in a controlled setting and large-scale application by land managers and restorationists (Markovchick et al, 2023). Markovchick et al. (2023) found that few land management plans in the United States reference fungi and mycorrhizae, with only one such plan out of the 130 assessed referencing mycorrhizae in forest management.  Similarly, a review by Policelli et al. (2020) found that of 140 papers that mentioned ectomycorrhizae in restoration, only 13 studies manipulated ectomycorrhizal communities to meet restoration goals. It appears studies that discuss the effects of plant invasions on ectomycorrhizal communities are more common than studies that address recovery of ectomycorrhizae following invasions (Policelli et al., 2020). Clearly, there is a gap between the current state of knowledge of the benefits of mycorrhizal conservation and reintroduction to restoration efforts and actual application.

Markovchick et al. (2023) made several recommendations for incorporating mycorrhizal fungi management in forest restoration. Some of these suggestions include actively managing mycorrhizal communities and focusing on restoring and conserving these communities, specifically in sites that may act as refugia for mycorrhizal species; committing to managing and restoring mycorrhizal diversity in extremely degraded sites, with particular attention to restoring diverse mycorrhizal communities consistent with local plant communities; and including mycorrhizal restoration as part of any future ecosystem restoration plan, while addressing concerns such as introduction of pathogens, degradation of intact reference sites from which soil inoculum is sourced, and providing staff with training for appropriate handling of reference and restoration sites (Markovchick et al., 2023). The latter suggestion requires the expertise of a professional mycologist, which may be difficult to source and fund for a small non-profit organization such as the Galiano Conservancy Association. Nevertheless, there are some ways that I suggest the GCA may implement mycorrhizal restoration and considerations of mycorrhizal diversity into restoration planning and implementation.

To help bridge this gap between studies and practice, I propose that the Galiano Conservancy Association trial a pilot project for incorporating mycorrhizal restoration in most terrestrial restoration projects. By introducing this project and carefully documenting the actions taken, I propose that the GCA can both improve the success of both plant and fungal restoration efforts and create an accessible and replicable protocol that may be employed by other restoration efforts to improve the success of their restoration planting.

**NGOs applying mycorrhizal restoration lessons**

A brief literature search turned up some evidence of small-scale efforts to apply mycorrhizal inoculation in restoration efforts. One such initiative is the Whitebark Pine Restoration project, which has used applications of a spore slurry blended from *Suillus sp.* and other endomycorrhizal species' spores to inoculate whitebark pine seedlings, finding that this increases seedling success by 11% after four years of growth when compared to uninoculated controls (Cripps, 2018; Goldfarb, 2015). Some of these inoculated seedlings were planted in Waterton Lakes National Park in Alberta in 2010 (Goldfarb, 2015). Another finding was a blog by a professional ecologist in Portland, Oregon describes not only using mycorrhizal inoculation in restoration projects, but also advocates for mycorrhizal community conservation and restoration (Query, 2023).

**Proposed protocol for mycorrhizal restoration at the Galiano Conservancy Association**

The Galiano Conservancy Association could initiate a pilot project the introducing mycorrhizal inoculant via introduction of soil from reference sites suspected to contain rich diversity of mycorrhizal species. There are several concerns and questions around this practice that I will attempt to address below: how is one to determine what makes an appropriate reference site from which to source inoculant?; how can one avoid introducing pathogens or degrading the reference sites in the process of collecting inoculant?; what is the process of inoculating soil in the field or in the nursery setting?; and, how does one measure the response and success of the pilot project?

One way to avert the issue of finding the appropriate inoculant for the restoration site is to source whole soil inoculant from reference sites known to have well-established forest ecosystems similar to those proposed for the restoration site (Maltz & Treseder, 2015). The soil can be collected to a depth of up to 10 cm to ensure that a variety of both EcM and AM species are collected, with approximately 50 grams of soil applied in prior similar experiments (Lance et al., 2019). When conducting whole soil collection from a reference site, one must use caution to avoid creating significant disturbance (Maltz & Treseder, 2015). Some studies have addressed this by selecting a site that is within 10 meters of the target plant species, but not in an area that is vegetated (Lance et al., 2019). Soil can be taken from several small areas in a reference site, dried, and then later homogenized with a sieve to avoid taking too much soil from one area, as well as to hopefully increase the diversity of mycorrhizal species in the inoculant (Lance et al, 2019).

Another consideration is the possibility of whole soil inoculation introducing plant pathogens from reference sites to restoration sites. One possible solution is to source soil inoculant from areas with no know plant pathogen concerns. It should be noted that mycorrhizal fungi species compete with some pathogenic fungi, so it is possible that introducing mycorrhizal species that are appropriate to the ecosystem at the site, with corresponding plant partners, will reduce the risk of pathogenic fungal invasion (Alves Cardoso Filho, 2023). A final significant concern is that there is no way to confirm that the target mycorrhizal species are present in the inoculant or in the reference site without conducting microscopic studies or laboratory testing that is expensive and requires specialist mycology expertise to conduct. However, it is possible to measure the success of soil inoculation by creating a simple randomization protocol and measuring the differences in response of plants inoculated with whole-soil samples expected to contain mycorrhizae and controls.

Taking these considerations into account, I have proposed a simple sample protocol (see Appendix A on page 15) implementing a simple randomization design that can be used at the GCA's restoration sites.

*Knowledge Gaps and Future Directions*

It is important to consider the possible impact of moving soils between sites, both on the reference site from which soil is removed and the restoration site or nursery plant to which it is applied. Concerns about transmission of plant pathogens between sites via the transfer of soil are also valid. It is also unclear how applying lessons learned from the literature will transfer to real-world restoration practices on Galiano Island, where clearcut logging impacts are felt to this day.

As has been noted by Markovchick et al. (2023), professional mycologist expertise is required to definitively assess and quantify mycorrhizal diversity in soils using microscopy or laboratory methods. As a result, it is impossible to confirm that any findings are directly a result of impacts of mycorrhizal inoculation rather than other factors. Nevertheless, reducing other confounding factors via the proposed simple randomization method may allow to at least narrow down any improvements to introduction of whole soil inoculant, if not to specific mycorrhizal community improvements.

ER 412
Galiano Field Course
Olga Tkachenko
V00051631

**References**

Alves Cardoso Filho, J. (2023). Mycorrhizal Association and Plant Disease Protection: New perspectives. *Arbuscular Mycorrhizal Fungi in Agriculture - New Insights*.

Brundrett, M. C., & Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, *220*(4), 1108–1115.

Brundrett, M. C., & Tedersoo, L. (2020). Resolving the mycorrhizal status of important northern hemisphere trees. *Plant and Soil*, *454*(1–2), 3–34.

Cripps, C. (2018, October 4). *Mycorrhizae*. Whitebark Pine Ecosystem Foundation of Canada. Retrieved on 14 July 2024 from https://whitebarkpine.ca/mycorrhizae/

Goldfarb, B. (2015, December 21). *To save a pine tree, researchers fight fungus with fungus*. High Country News. Retrieved on 14 July 2024 from https://www.hcn.org/issues/47-22/to-save-a-pine-tree-researchers-fight-fungus-with-fungus/

Greipsson, S. (2011). *Restoration ecology*. Jones and Bartlett Publishers.

Lance, A. C., Burke, D. J., Hausman, C. E., & Burns, J. H. (2019). Microbial inoculation influences arbuscular mycorrhizal fungi community structure and nutrient dynamics in temperate tree restoration. *Restoration Ecology, 27(5)*, 1084–1093.

Maltz, M. R., & Treseder, K. K. (2015). Sources of inocula influence mycorrhizal colonization of plants in restoration projects: A meta-analysis. *Restoration Ecology*, *23*(5), 625–634.

Markovchick, L. M., Carrasco-Denney, V., Sharma, J., Querejeta, J. I., Gibson, K. S., Swaty, R., Uhey, D. A., Belgara-Andrew, A., Kovacs, Z. I., Johnson, N. C., Whitham, T. G., & Gehring, C. A. (2023). The gap between mycorrhizal science and application: Existence, origins, and relevance during the United Nation's decade on Ecosystem Restoration. *Restoration Ecology*, *31*(4).

Markovchick, L. M., Schaefer, E. A., Deringer, T., Kovacs, Z. I., Deckert, R. J., Yazzie, J., Dixit, A., Propster, J. R., Patterson, A., Hultine, K. R., Grady, K., Allan, G. J., Whitham, T. G., & Gehring, C. A. (2023a). Postrestoration colonization suggests slow regeneration, plant translocation barriers, and other host/symbiont lessons during the United Nations' decade on ecosystem restoration. *Restoration Ecology*, *31*(5).

Neuenkamp, L., Prober, S. M., Price, J. N., Zobel, M., & Standish, R. J. (2019). Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and Time. *Fungal Ecology*, *40*, 140–149.

Paluch, E. C., Thomsen, M. A., & Volk, T. J. (2012). Effects of resident soil fungi and land use history outweigh those of commercial mycorrhizal inocula: Testing a restoration strategy in unsterilized soil. *Restoration Ecology*, *21*(3), 380–389.

Policelli, N., Horton, T. R., Hudon, A. T., Patterson, T. R., & Bhatnagar, J. M. (2020). Back to roots: The role of ectomycorrhizal fungi in boreal and temperate forest restoration. *Frontiers in Forests and Global Change*, *3*.

Read, D. J., Leake, J. R., & Perez-Moreno, J. (2004). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, *82*(8), 1243–1263.

Query, T. (2023, March 31). Partnering with fungi and soil for better futures. The Nature of Cities. https://www.thenatureofcities.com/2023/01/16/partnering-with-fungi-and-soil-for-better-futures/

Tao, L., Hunter, M. D., & de Roode, J. C. (2017). Microbial root mutualists affect the predators and pathogens of herbivores above ground: Mechanisms, magnitudes, and missing links. *Frontiers in Ecology and Evolution*, *5*.

Wubs, E. R., van der Putten, W. H., Bosch, M., & Bezemer, T. M. (2016). Soil inoculation steers restoration of terrestrial ecosystems. *Nature Plants*, *2*(8).

Yu, L., Zhang, W., Geng, Y., Liu, K., & Shao, X. (2022). Cooperation with arbuscular mycorrhizal fungi increases plant nutrient uptake and improves defenses against insects. *Frontiers in Ecology and Evolution*, *10*.

ER 412
Galiano Field Course
Olga Tkachenko
V00051631

**Appendix A: Suggested protocol for inoculation with mycorrhizae in a simple randomization design**

1) Select a reference site with known intact forest ecosystems composed of species similar to that planned for the restoration site. Selecting a site with similar plant species that are thriving implies a healthy soil community, including mycorrhizae (Lance et al., 2019).

2) Follow a simple protocol for the collection of soil inoculant from reference sites. I have adapted the protocol described by Lance et al. (2019) below.

3) Collect soil up to a depth of 10 cm from the reference site, ideally a short distance from the same species of trees or shrubs that are to be inoculated with the whole soil sample, as this ensures appropriate mycorrhizal species transfer (Lance et al., 2019).

4) Soil can be collected from multiple distinct areas and homogenized for later application to avoid collecting too large of a sample from one area and creating disturbance at the reference site (Lance et al., 2019).

5) Soil can be dried for a few days to one week in a cool dark area, then sieved together into a more fine homogenous mass (Lance et al., 2019).

6) Lance et al. (2019) applied 50 grams of soil to the surface of soil around the plant. The soil surface can be gently abraded by hand or tool. The surface of the soil should be immediately watered (Lance et al, 2019).

7) This protocol can be applied directly in the field during planting, or in the nursery when applied directly to plant pots or seeds.

8) Plants should be randomly selected to either receive the treatment or not, with 50 % of plant receiving no treatment (control) and 50 % receiving soil inoculation. To avoid confounding factors, it should be noted that assigning plants to treatment should be randomized via a coin flip or another simple randomization measure, and the treated and control plants labelled. In a nursery setting, this can be easily done by writing on the plant pot, while in the field the use of flagging or tags can be implemented to label treatments.

9) Measurements of plant height and stem width, for example, can be collected at planting (baseline), as well as regularly for a pre-determined period of time (i.e. 6 months post-planting, 1 year post-planting, 2 years post-planting). The survival of each plant should also be noted, as increasing survival rates may be one of the goals of introducing mycorrhizal inoculation to the nursery program or restoration planting.

ER 412
Galiano Field Course
Olga Tkachenko
V00051631