Untangling the role of soil fungi in eucalypt dieback to enhance revegetation outcomes

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In October 2022, the CSIRO, in partnership with Greening Australia and volunteers from Upper Snowy Landcare Network (USLN), launched a large-scale seedling microbial inoculation experiment at Gegedzerick Travelling Stock Reserve, near Berridale. The aim of the experiment is to test whether inoculating ribbon gum (*Eucalyptus viminalis*) seedlings with beneficial soil fungi, known as mycorrhizae, improves the performance of seedlings when planted into woodland areas experiencing severe dieback.

This experiment is part of a broader collaboration between CSIRO, USLN, Greening Australia and the Australian National University around understanding the possible links between ribbon gum dieback and changes to the diversity and composition of beneficial soil microbes that many plants, including eucalypts, depend upon for healthy population growth and reproduction.

The project is generously supported through a grant from the NSW Environmental Trust: https://www.environment.nsw.gov.au/news/eucalypt-dieback-gets-research-funding

Soil microbes provide valuable ecosystem services

It is well known that many plant species form mutualistic associations with soil microbes that improve plant health and ecosystem function. Mycorrhizae represent one such association – these are networks of fungi that form intimate connections with a host plant's root system, and provide those plants with protection from pathogens, improved resistance and resilience to drought and access to micronutrients and water. These services are provided to the plant in exchange for sugars that the plant produces by photosynthesis. Mycorrhizal networks have also been shown to allow plants to communicate with one another in the so-called "wood-wide web", as discussed beautifully in this article: https://www.nytimes.com/interactive/2020/12/02/magazine/tree-communication-mycorrhiza.html

There is growing evidence that landscape disturbances from agricultural activity, such as overgrazing, land clearing, nutrient enrichment, and soil erosion can seriously disrupt the connections that plants make with these mutualistic soil fungi and other microbes. Depletion of beneficial microbes can, in turn, limit how well native vegetation can recover from those disturbances. A recent article by Dr Adam Frew and colleagues from the University of Southern Queensland provides an excellent overview of the importance of mycorrhizae for the recovery of vegetation communities following disturbance: https://theconversation.com/how-fungis-knack-for-networking-boosts-ecological-recovery-after-bushfires-132587

Testing the benefits of soil microbial inoculation for eucalypt revegetation

The rollout of our large and complex inoculation experiment at Gegedzerick has been a great success. It all began in March 2022 at the Greening Australia depot in Canberra. CSIRO researchers first collected soil from various healthy patches of ribbon gum woodland across the Snowy Monaro with no (or only very little) signs of canopy dieback. Those sites also had a very rich understorey of native grasses, forbs, shrubs and thriving eucalypt seedlings. This field soil was then integrated with potting mix and placed in black forestry grow tubes over

which were sprinkled ribbon gum seeds (see image 1a below). All seeds germinated well, and the emergent seedlings have since thrived (image 1b). After six months, many of the tubes containing the healthy live soil developed fruiting bodies (that look like mushroom caps) of known mycorrhizal fungi – a sure sign that the eucalypt seedlings had begun forming the connections with desirable fungi (see image 2 below of orange mycorrhizal fungi within the genus *Laccaria*).

Now that the seedlings have been planted at Gegedzerick (see image 3), we will evaluate the long-term benefits of microbial inoculation for revegetation through careful monitoring of seedling survival and growth over the next two years. We will also periodically harvest some seedlings and undertake detailed microscopic analysis to ascertain the presence of mycorrhizae connecting with the plant root systems.

Is there a link between ribbon gum dieback and soil microbes?

The CSIRO is using next generation DNA analysis to evaluate if the widespread dieback of ribbon gum woodlands across the Snowy Monaro region is associated with a depletion of beneficial soil microbes from the soil. Indeed, if we do find that areas suffering severe ribbon gum dieback have reduced diversity of beneficial soil microbes such as mycorrhizae, that may go some way to explain why those dieback-affected areas are slow to recover (or cannot recover at all), even under ideal climate conditions for eucalypt growth such as now.

Landcare, Greening Australia, and landholders invest considerable effort in rehabilitating dieback landscapes, through a combination of natural regeneration of good quality woodlands and planting nursery-grown seedlings. Planting seedlings is time and labour intensive and requires long-term investment in seed collecting, propagation in greenhouse facilities, preparing a site for planting, fencing and ongoing plant maintenance, such as watering seedlings during periods of drought. Despite our best efforts, not all planted seedlings will survive.

We predict that the establishment and growth of mycorrhizal plant species will be hampered if planted into degraded habitats that are depleted in those beneficial soil fungi. This is the rationale for our large inoculation experiment. If we can reconnect the eucalypt seedlings with beneficial mycorrhizae prior to planting at a dieback affected area using various inoculation treatments, those plants may have higher rates of survivorship and long-term growth.

There are efforts underway across Australia to harness the natural ecological benefits of mycorrhizae. Development of effective ways of reconnecting seedlings with beneficial microbes prior to planting in the field will be a game changer for revegetation of degraded landscapes. A recent article about similar research undertaken by fungal ecologists at Western Sydney University highlights that "*cracking the riddle of how trees rely on fungi to thrive and communicate*" will be a "*a boon for the nursery industry and bush regeneration efforts*": https://www.abc.net.au/news/science/2022-08-03/trees-and-fungi-how-they-communicate/101276836

Value of volunteer participation in microbial research

The experiment was enabled through generous time contributions from Greening Australia and Landcare volunteers who assisted with preparing the inoculation treatments in the glasshouse in March 2022 and planting the seedlings at Gegedzerick in the following October. There will be ongoing opportunities for volunteers to assist with monitoring and evaluating the outcomes of the experiment for seedling survival and growth. CSIRO is excited to be undertaking this cutting-edge research with community partners. By working together, we can achieve enhanced restoration outcomes in the beautiful Snowy Monaro region.

For further information about the project and to discuss opportunities for engagement, please contact Ben Gooden at the following email address: Ben.Gooden@csiro.au

What are mycorrhizae?

Mutualistic soil fungi that form symbiotic associations with the roots of host plants, including eucalypts. Mycorrhizae facilitate enhanced uptake of water, nutrients and defend against enemy attack. In return, the host plant provides the fungus with sugars produced through photosynthesis.

Eucalypts can form two main types of mycorrhizal associations – arbuscular mycorrhizae and ectomycorrhizae (see **image 4**). Ectomycorrhizae also produce fruiting bodies, many of which look like the classical toadstool mushroom.

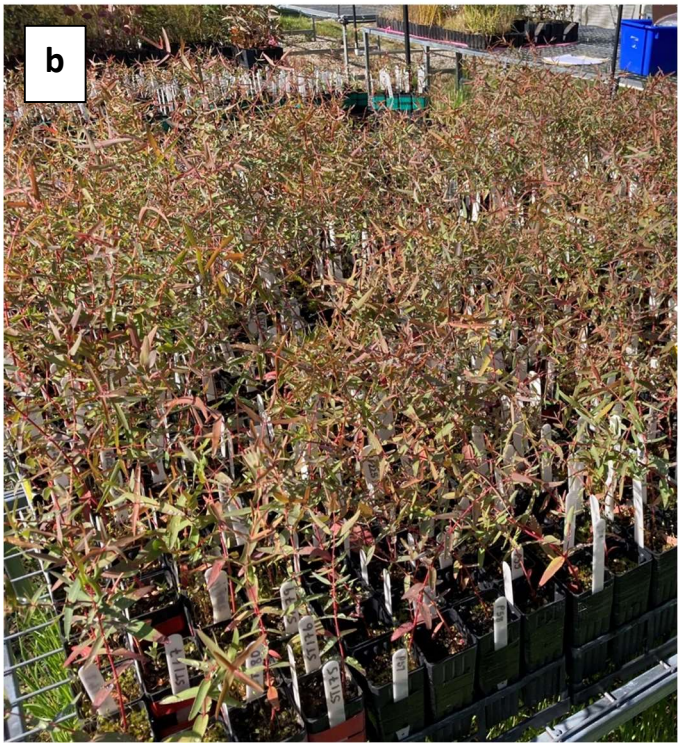

Image 1 – (a) Greening Australia volunteers sowing ribbon gum (*Eucalyptus viminalis*) seeds in grow tubes filled with field soil collected from healthy eucalypt woodlands; (b) ribbon gum seedlings thriving after 6 months, prior to planting at the Gegedzerick revegetation site.

Image 2 – Fruiting body of a fungal species within the genus *Laccaria*, a gorgeous ectomycorrhizal fungus that can form intimate mutualistic associations with eucalypt roots. Several fruiting bodies were detected in pots containing field soil.

Image 3 – Researchers planting the ribbon seedlings inoculated with the healthy soil containing diverse microbes, including beneficial fungi, at the Gegedzerick TSR.

For further information

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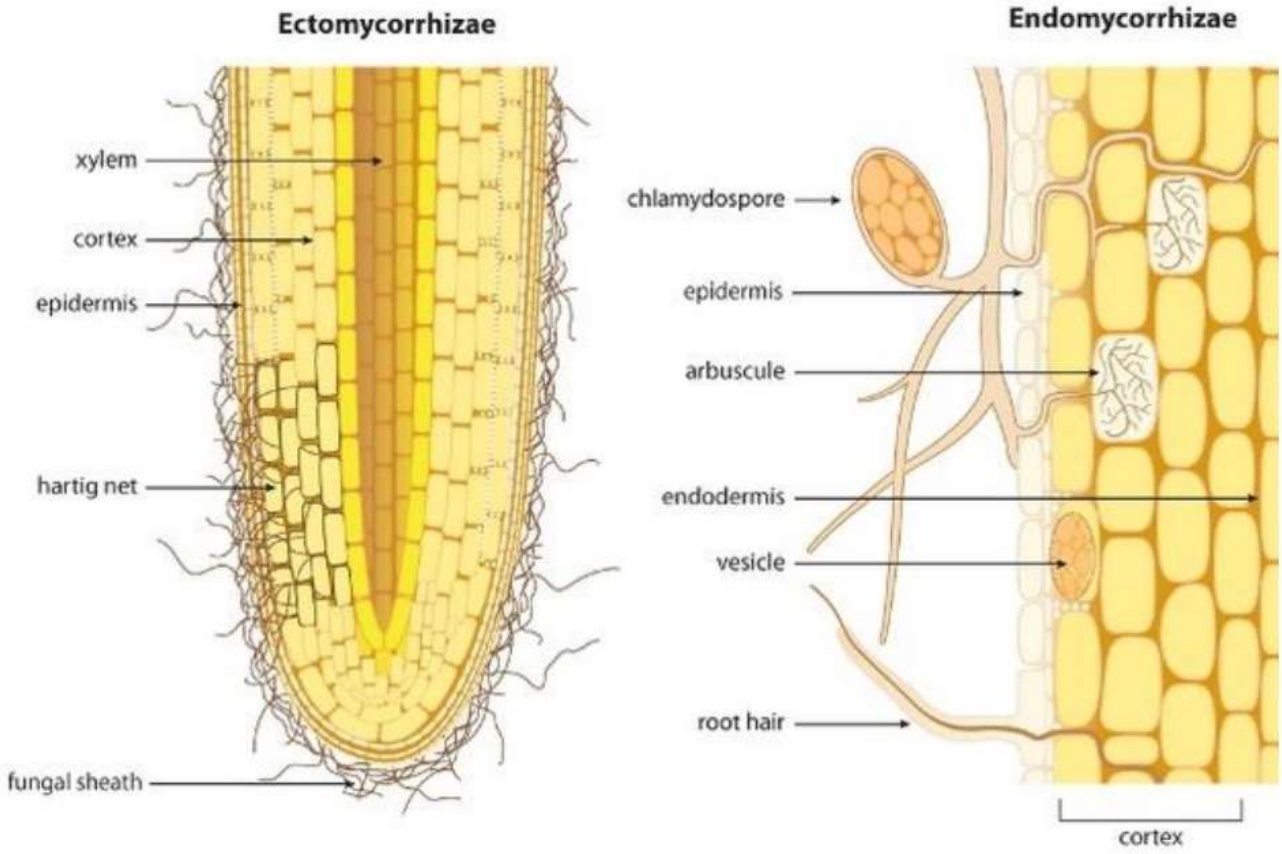

Image 4 – Schematic showing the difference between ectomycorrhizae and endomycorrhizae colonization of plant roots. © 2013 Nature Education. Citation: McNear Jr., D. H. (2013) The Rhizosphere - Roots, Soil and Everything In Between. Nature Education Knowledge 4(3):1. www.nature.com/scitable