



INTEGRATING FUNGI

TO IMPROVE CONSERVATION, RESTORATION
& RESILIENCE AT
MARY CAIRNCROSS SCENIC RESERVE

SEPTEMBER 2024



Report for the Sunshine Coast Council

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We acknowledge the Jinibara Traditional Owners and recognise their continuing connection to land, waters and community. We pay our respects to the Jinibara people, their cultures and the Elders past and emerging.

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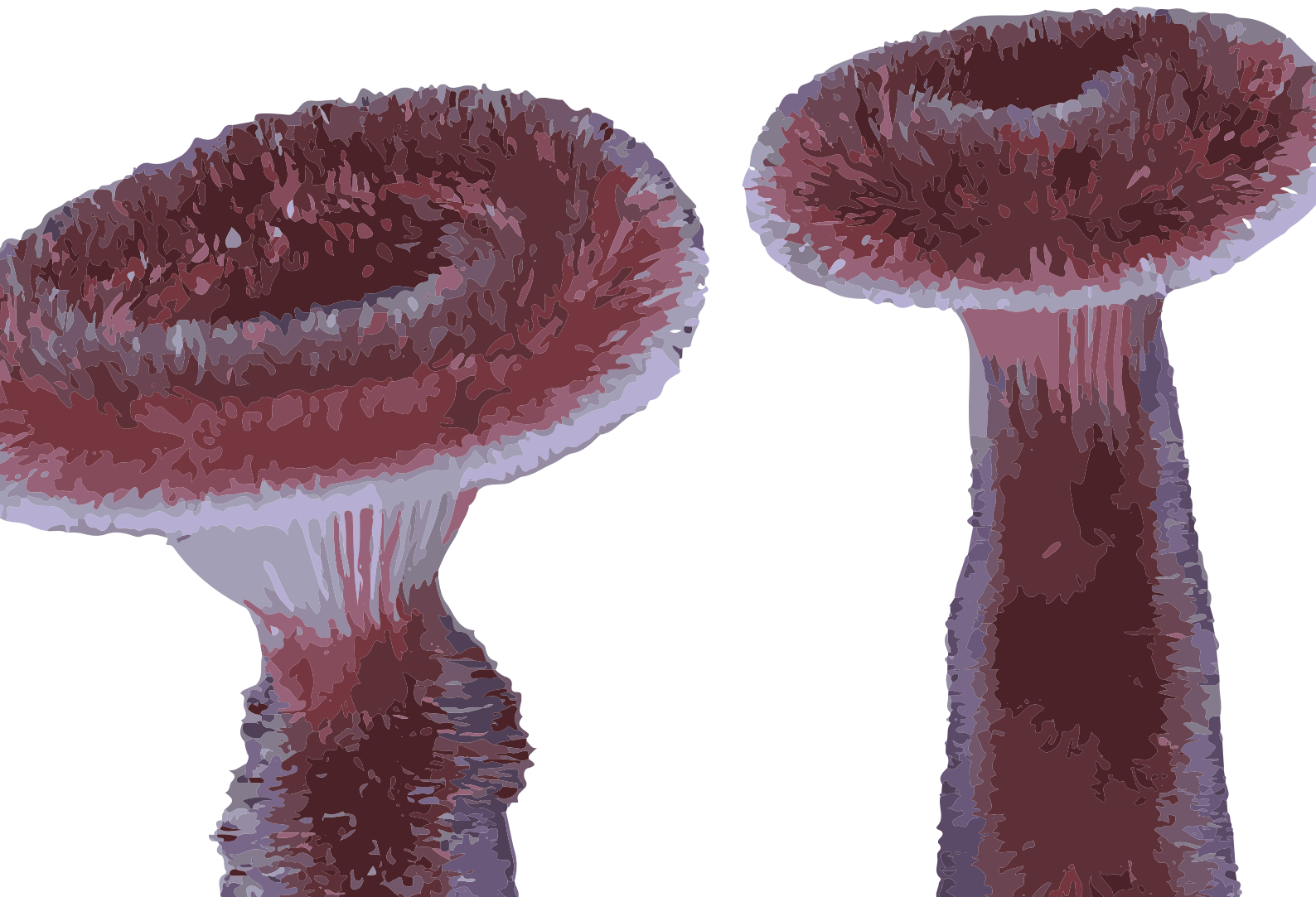
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1. EXECUTIVE SUMMARY

Hairy Oyster.

Panus lecomtei.





HEALTHY

ecosystems are supported not just by the species within them, but by the numerous, often unstudied interactions between species that allow them to survive and reproduce in balance. The most successful environmental management plans consider the full spectrum of species, including the three F's: Flora (plants), Fauna (animals) and Fungi.

Fungi fill a broad array of roles in any ecosystem; from gut fungi that assist animals' digestion to mycorrhizal fungi that help plant roots access water and nutrients.

Mary Cairncross Scenic Reserve (MCSR) is in a good position to better manage native biodiversity as it has some historical data on the macrofungi of the site (McMullan-Fisher 2010a) and additional fungi have been identified in the past decade. This includes eye-catching, photogenic fungi that draw in tourists and volunteers, but also some problematic fungi that threaten other species.

Systematic modification across landscapes for agriculture, forestry and urbanisation has caused the highest extinction rates ever recorded. These impacts have decreased ecosystem function across the globe and contributed to catastrophic global warming. Fungi are also threatened by these processes, although their extinction rates and importance to conservation efforts are less well recorded.

Without fungi, native species like the Australian Bush Rat may also be at risk of extinction from the site.

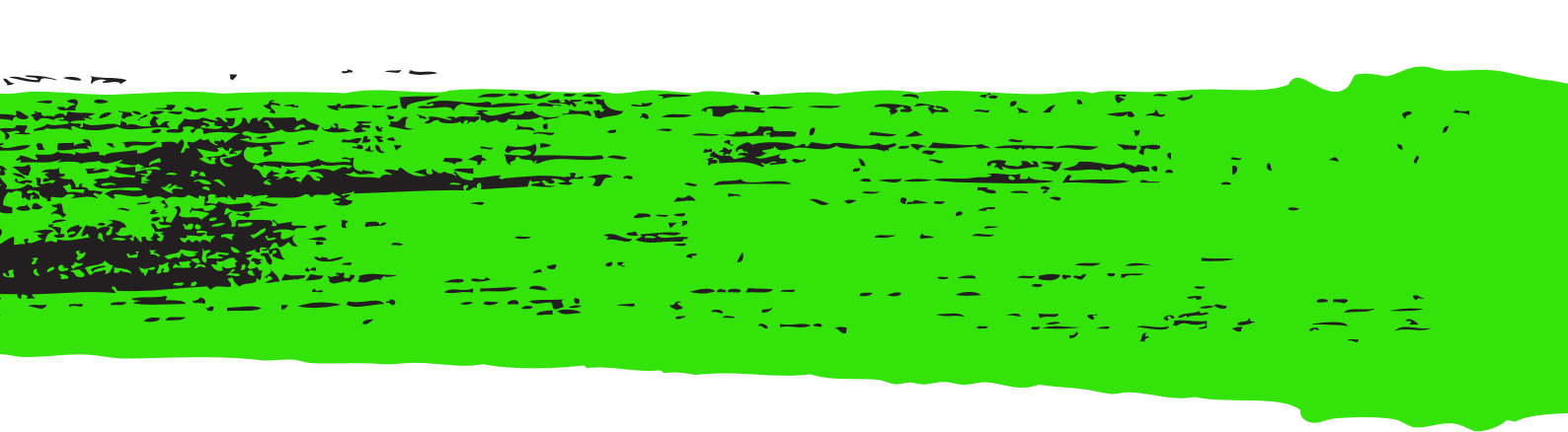
Integrating fungi into environmental management plans, community engagement and biodiversity research at MCSR will help us restore, maintain and conserve an important natural resource and habitat. We will also provide a more fulfilling experience for community members and tourists.

2. WHY FOCUS ON FUNGI?

Green Pepe.

Mycena chlorophos.





AS knowledge of fungi expands, their role in ecosystems becomes clearer. From decomposition to healthy soils to food sources for threatened species, fungi are a crucial part of a healthy environment.

And yet, Queensland state conservation lists do not include fungi.

By focussing on fungi now, MCSR will help drive more understanding of the delicate webs that keep our natural spaces thriving.

2.1 SITE BACKGROUND

MCSR is a site that has been protected from the large-scale clearing and associated damage from most human disturbances. This has been actively managed with conservation and survival of indigenous species at the site. The site is dominated by Complex Notophyll Vine Forest (CNVF, Regional Ecosystem 12.8.3) with corridors of gallery rainforest (Notophyll Vine Forest, RE 12.3.1) along the existing creek drainage lines. A patch of swamp forest with dominants of *Eucalyptus robusta*, *E. grandis* and *Melaleuca salicina* (open forest to woodland, RE 12.3.4) also occurs in the Reserve. There are ecotonal areas between these ecosystems, particularly for ectomycorrhizal fungi around host Eucalypt and other ectomycorrhizal dominant host trees.

MCSR is an important remnant of local habitats that has become fragmented and isolated since settlement. The area will be expanded to include some of the adjoining Ecological Park to the north of MCSR. Some of the adjoining areas will be restored to the appropriate local ecosystems.

Council, staff, volunteers and Friends of MCSR work together to manage the site, protect the species that live there and provide a refuge and engaging experience for local people and tourists alike. The existing knowledge of current stakeholders provides an important base to learn more about the local fungi and their interactions.

To date, different biodiversity groups within MCSR appear to be considered separately. A full biodiversity review would help highlight trends, connections, and synergies of how species are interacting and surviving across the site.

2.2 FUNGI & THEIR ROLES

Fungi (including lichens) are a whole kingdom of our biodiversity. Different groups of fungi have different ecological roles and together with other organisms help sustain healthy and functional ecosystems. This report also includes some fungus-like organisms, previously grouped with fungi but now recognised as phylogenetically different (e.g. slime moulds and Oomycetes).

Fungi have critical roles in maintaining ecosystem functions:

-  Fungal reproductive structures, hyphae and yeasts are important food sources for arthropods, molluscs, small terrestrial mammals, some birds and reptiles.
-  Decomposer fungi assist in many processes, such as nutrient recycling, forming tree hollows and even cleaning water sources.
-  Parasitic fungi can support natural selection, trap nematodes or parasitise arthropods to act as population controls.
-  Fungi-like slime moulds are also important in decomposition, food webs and nutrient cycles.
-  Mycorrhizal fungi (symbiotic partners of most terrestrial plants) support the health of plants through access to water and nutrients.
-  Other fungal plant symbionts like endophytes, common in the tissues of most plants, can improve drought tolerance.
-  Gut symbionts assist digestion in macropods and many arthropods, particularly beetles.
-  Lichens capture carbon, provide habitat for micro-fauna and are indicators of air quality.
-  Cyanolichens contribute important biologically available nitrogen to nutrient cycles.
-  Nematophagus fungi prey on nematodes common in soils and wood.
-  Entomopathogenic fungi, sometimes called zombie fungi, prey on arthropods.

Fungi at different stages of their life cycle may utilise different nutritional strategies. Some fungi may spend their life cycling between being considered saprotroph, beneficial symbiont, neutral biotroph and parasites. Thus, to fully understand what fungi belong to

each of these groups can be ambiguous in some circumstances (Kabbage et al. 2015; Zeilinger et al. 2016).

For example, many endophytes spend most of their life unseen working inside their plant hosts (Mapperson 2014). However, at the end of the decay of their host plant, the endophytes become visible as reproductive structures like the bright cups of Burgundy Cups (*Phillipsia subpurpurea*). These are commonly seen on wood on the ground in MCSR, their spores released at the floor of the forest where they need to find seedlings to partner with.

This report tries to include as many fungal groups as possible and highlight the interconnected nature of many fungi.

Fig. 1: Bright magenta-centred Burgundy Cups (Phillipsia subpurpurea) are often seen on decomposing wood but are also a known endophytic partner of plants.



2.3 PROJECT PRIORITIES

These four priorities for the report were developed in consultation with MCSR staff:

-  **Understanding existing fungal knowledge.** Review and collate existing knowledge of fungal species within the MCSR, define species that are useful in citizen science projects and define ecological function of species.
-  **Building a fungal molecular library.** Suggest procedures and priorities for development of a fungal molecular library with a focus on threatening fungi, functional fungi, uncommon fungi and exploring the use of specific primers developed for Loop-mediated isothermal amplification (LAMP) to facilitate locating priority species.
-  **Outline threats to fungi.** Review known and likely impacts of climate change, habitat and species loss and known problematic fungi and fungal weeds.
-  **Exploring potential fungal ecological projects.** Compare soil fungal communities, review roles of fungivorous animals in fungal dispersal, suggest hygiene and monitoring procedures.

2.4 SUNSHINE COAST COUNCIL ALIGNMENT

This report's priorities align with Sunshine Coast Council strategies and plans in a number of ways. The following were considered:

-  Environment and Liveability Strategy 2017
-  Environmental Reserves Network Management Plan 2021-2027 (Volumes I-III)
-  Back to Nature - the Sunshine Coast Ecological Park Master Plan – 2023

The Environment and Liveability Strategy 2017 has a strong focus on integration with natural and built environments. Healthy natural areas support healthy communities. Integrating fungi into the research, education, conservation activities and management context will help preserve and enhance our natural environment, while supporting opportunities for the community to benefit sustainably from the associated products and ecosystem services.

Inclusion of fungi in biodiversity management will strengthen and expand the conservation management of these areas. Improving information on fungi will increase managers' understanding of how to better support important conservation and recreation areas.

MCSR's subtropical rainforest is an amazing ecological asset. Improving biological connectivity by increasing the area to include some of the adjoining Ecological Park will help ameliorate some of the negative consequences of the area being surrounded by urban and agricultural dominated areas. It will help provide sustainable public access while still supporting ecosystem health and resilience by protecting ecosystem processes and biodiversity and implementing restoration actions where needed.

The *Environmental Reserves Network Management Plan 2021-2027* recognises that data should inform management and actions should be guided by a consistent set of principles. Improving knowledge and data about the fungi at the site will allow managers to better understand what fungal assets are surviving in the old growth areas and track how these return into restored areas. Similarly, it will inform managers of any problematic fungi that may threaten the ecosystem health or health of specific species.

It will be possible to know if fungi need conservation or are important supporting partners for other species of conservation importance. This will allow managers to consider more active restoration work that would help heal the site.

For example, Land Mullet (*Bellatorias major*) periodically feeds on fungi and the population has become extinct at MCSR, so is a priority species for rewilding into the site. Understanding the diet and food availability for these animals will improve their successful reintroduction and long-term survival.

Many local people already visit MCSR for the amazing fungi that appear unexpectedly, delighting nature lovers and nature photographers. The photogenic nature of some fungi are an opportunity to engage visitors in citizen science research at the site.

There are a number of mycologists in the region and across the country who could be engaged in various projects to better understand the local fungi and how they interact with different species. With support these natural history stories can be shared to the local community, visitors, and the scientific community. This report contains many potential projects that would increase the mycological literacy of locals, volunteers and staff to support conservation and ecological restoration.

Most of the values of the Sunshine Coast Council's 2023 Back to Nature plan would be enhanced if fungi were actively included in engagement, education, research and management of the site but it closely aligns with these values:

 **Value 1:** An ecologically valuable, resilient and connected landscape – fungi are

important for ecological health and resilience.

- 🍄 **Value 2:** A place that welcomes people to learn, play and contribute to the future – can be achieved by having educational materials about fungi and projects that let people engage and contribute to fungal research.
- 🍄 **Value 3:** A place to be inspired by nature – many people are already inspired by the fungi at the site.
- 🍄 **Value 5:** A place of unexpected and spontaneous discovery – the unpredictable nature of fungal reproduction contributes to the experience of people engaging with fungi. There is the real possibility that new fungi species will be discovered.
- 🍄 **Value 6:** A place that shares the stories of local people and the natural history of the region – there are great opportunities to learn more about the natural histories of fungi and their discovery.

2.5 DATA ANALYSIS & MONITORING FOR ADAPTIVE MANAGEMENT

Active adaptive conservation management (Wilhere 2002; Westgate *et al.* 2013) endeavours to make the best conservation management decisions based on available data at any given time while dealing with uncertainty and working within limited budgets. There are statistical tools and frameworks that try to help guide decision makers (McCarthy and Possingham 2007; Keith *et al.* 2011). Many managers are trying to integrate adaptive management and evidence based frameworks (Gillson *et al.* 2019).

We want to stress the importance of regular monitoring of at least some of the biodiversity, ideally across all major functional biological groups (Stephenson *et al.* 2022). Fungi have historically been left out of management and the limited amount of reliable historical data means we need to build baseline data on which to help make decisions.

Much restorative conservation work uses benchmarks to measure and guide management decisions about the relative health and diversity of areas being managed for conservation (Sutherland and Peel 2011; McNellie *et al.* 2020). For example, the regional ecosystem framework (Queensland Government 2014) used to define vegetation was based on data that included reference sites that define geology and vegetation for communities. This data is often used when considering restoration sites as a basis to help decide what species to try to restore.

Although there has been discussion of the need for baseline data in Australia to facilitate conservation management of fungi (Pouliot and May 2010) there has been little inclusion of fungal data in state benchmark data, so local areas need to build up their own data.

Surveys for biodiversity are shifting to integrate data collection of how many of the species that are interacting. For example, data collection can now focus on three-way connections between healthy plants that are supported by a diverse community of mycorrhizas, which depends on healthy populations of fungivorous animals.

Macrofungi are not the only fungi that are important for ecosystem function, but are a starting point to build fungal biodiversity knowledge.

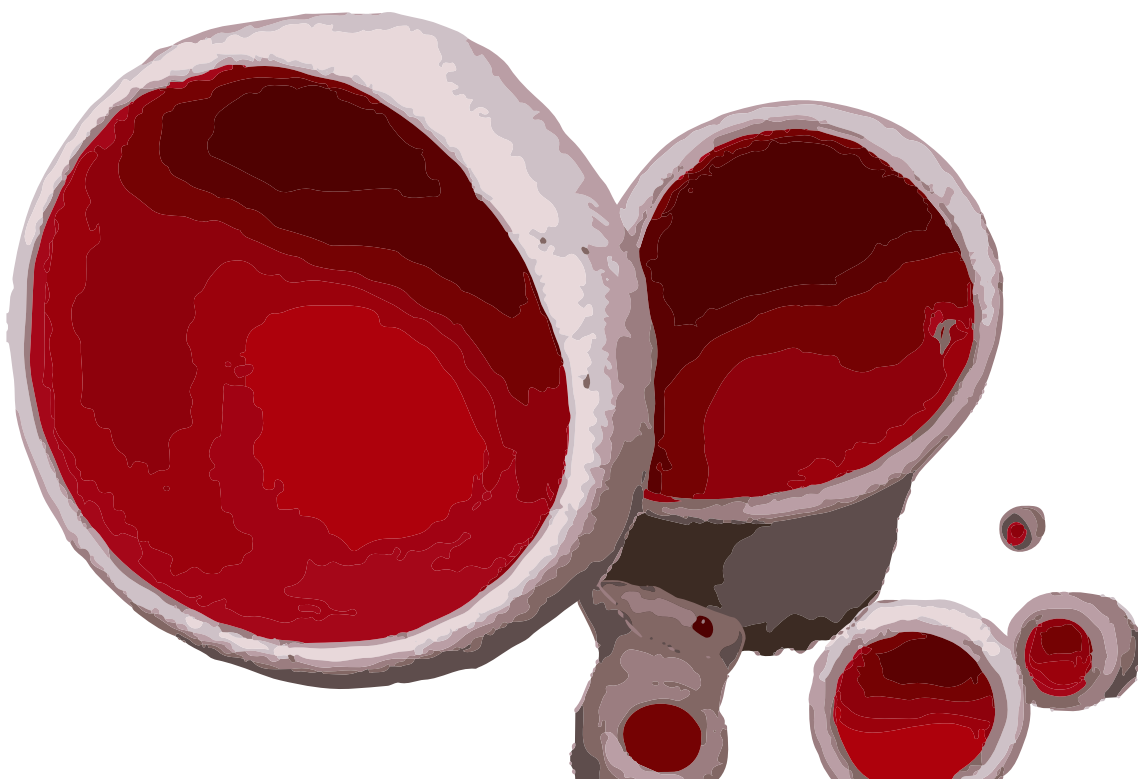
We advocate for ongoing monitoring to collect data, but also regular data analysis so that population trends are understood. This report suggests ways to integrate some of the fungal data collection with the data collection of the other organisms, particularly animals and plants. Building a fungal knowledge base and integrating fungi into ongoing data collection will facilitate better understanding of the site's ecology.

The Health and resilience of ecosystems depends on healthy populations of organisms living together so that they all live and reproduce. Understanding the interconnections and components like food webs will improve the ability to make management decisions that reduce harm to the site.

3. PRIORITY A: EXISTING KNOWLEDGE

Burgundy Cups.

Phillipsia subpurpurea.





WHAT

we currently know about fungi in MCSR are summarised in this section. This includes a fungal species list, known biological attributes, knowledge gaps and suggestions to improve species knowledge via stakeholder engagement.

MCSR is in a good position to better manage fungal components of native biodiversity as it has some historical data on the macrofungi of the site (McMullan-Fisher 2010a) and collections to begin a molecular reference library. This survey and report were undertaken during a previous La Niña event when there were more macrofungi reproducing than would be expected to be found in an average rainfall year. This report grouped fungi into broad morphological and phylogenetic groups with field surveys recording 130 macrofungi, including two slime moulds. Of these, 46 macrofungi were identified to species level with a further 29 taxa likely to be species equivalent but did not yet have scientific names. One of the limitations with macrofungal identification is that in Australia about half of the macrofungi are yet to be formally named. Many of these macrofungi have not been recorded since the 2010 survey period.

Most of these macrofungi were decomposers and some were parasitic. Only a single ectomycorrhizal species, *Laccaria lateritia*, was recorded. Since this report we now know that some of the ascomycetes recorded, Burgundy Cups (*Phillipsia subpurpurea*) and *Hymenoscyphus* sp., may be decomposers when reproducing on wood and also endophytic inside plants.

This report supplied lists for three of the regional ecosystems separately. The relative abundance of different taxa was considered, producing a list of the ten most recognisable macrofungi that are suitable for easy monitoring. These collections have already contributed to the improved taxonomy of local fungi. The specimen *H. coralloides* SMF2536 was sampled for molecular barcode by Jerry Cooper and the resulting phylogeny confirm this is the new species Cascading Icicles (*Hericium novae-zealandiae*).

A number of weedy and problematic fungi were considered as well (McMullan-Fisher 2010a), including additional parasitic mushroom *Cyclocybe parasitica*.

The largest functional group at the site were recycler fungi including 56 mushrooms, 42 polypores, eight leathers, six patches and paints (*Corticod* spp.), six fans (*Campanella* spp. *Chaetocalathus* sp., *Marasmius equicrinis* and *Panellus luxfilamentus*), four jellies (*Auricularia* aff. *thailandica*, Ear fungus (*Auricularia cornea*), *Auricularia delicata* complex and Snow fungus (*Tremella fuciformis*)), two puffballs (*Lycoperdon* sp. 'stem, brown with warts' and *Lycoperdon purpurascens*) and one icicle (*Pterula fascicularis*).

A number of recycler fungi may also be parasitic including three mushrooms (*Armillaria fumosa*, *Cyclocybe parasitica* and *Xerula* sp.), five polypores (*Fomitopsis lilacinogilva*, *Ganoderma australe*, *Ganoderma steyaertanum* and two *Phellinus* spp.) and Cascading Icicles (*Hericium novae-zealandiae*). Problem fungi include *Pyrrhoderma noxium* and Weedy *Favolaschia* (*Favolaschia claudopus*).

There are a further 203 macrofungi that have been recorded from the Blackall range, many of which are ectomycorrhizal. This spreadsheet has not been updated for name currency nor records since 2010 for sites other than MCSR.

Some of these macrofungi are not likely to occur at MCSR as they are associated with habitats and host organisms that are not found at the site.

As yet, Queensland state conservation lists do not include fungi. The following likely threatened fungi should therefore be a priority for finding and listing:

- Waxcaps (family Hygrophoraceae): Particularly *Hygrocybe bolensis* and *Humidicutis arcohastata* (Near Threatened IUCN Global Red List) known from the Blackall Range
- Mushroom: Australian Shiitake (*Lentinula lateritia*)
- Fan: *Crepidotus innuopurpureus*
- Toothed fungi: *Phellodon* known from the Blackall Range
- Icicles: Cascading Icicles (*Hericium novae-zealandiae*), and *Pterula fascicularis*
- Club fungi and other ascomycetes: *Xylaria grammica* and *Entonaema* sp.

There are several ways to increase the understanding of which fungi at the site need specific

Many Australian soils are low in natural fertility, particularly in rainforests. Most nutrients are limited and decomposition rates are rapid in wet periods. There is some preliminary soil data from an honours thesis (Curran 2022) that shows average soil fungi diversity of 301 species per sample. There is good fungal diversity in the soils in MCSR and Ecological Park (EP) However, this study had some limitations that are discussed further later in this document.

Nonetheless this research confirmed some of our assumptions about restoration trajectories from managed pastures to rainforests. Local expertise confirms that if weeds and grasses can be suppressed while local rainforest plants return, canopy tree and shrub restoration improves. This was seen in the principal component analyses (Curran 2022) for aboveground vegetation that showed there was low grass cover where there was high plant diversity in the forested areas and similarly there was much greater soil compaction in the pasture areas.

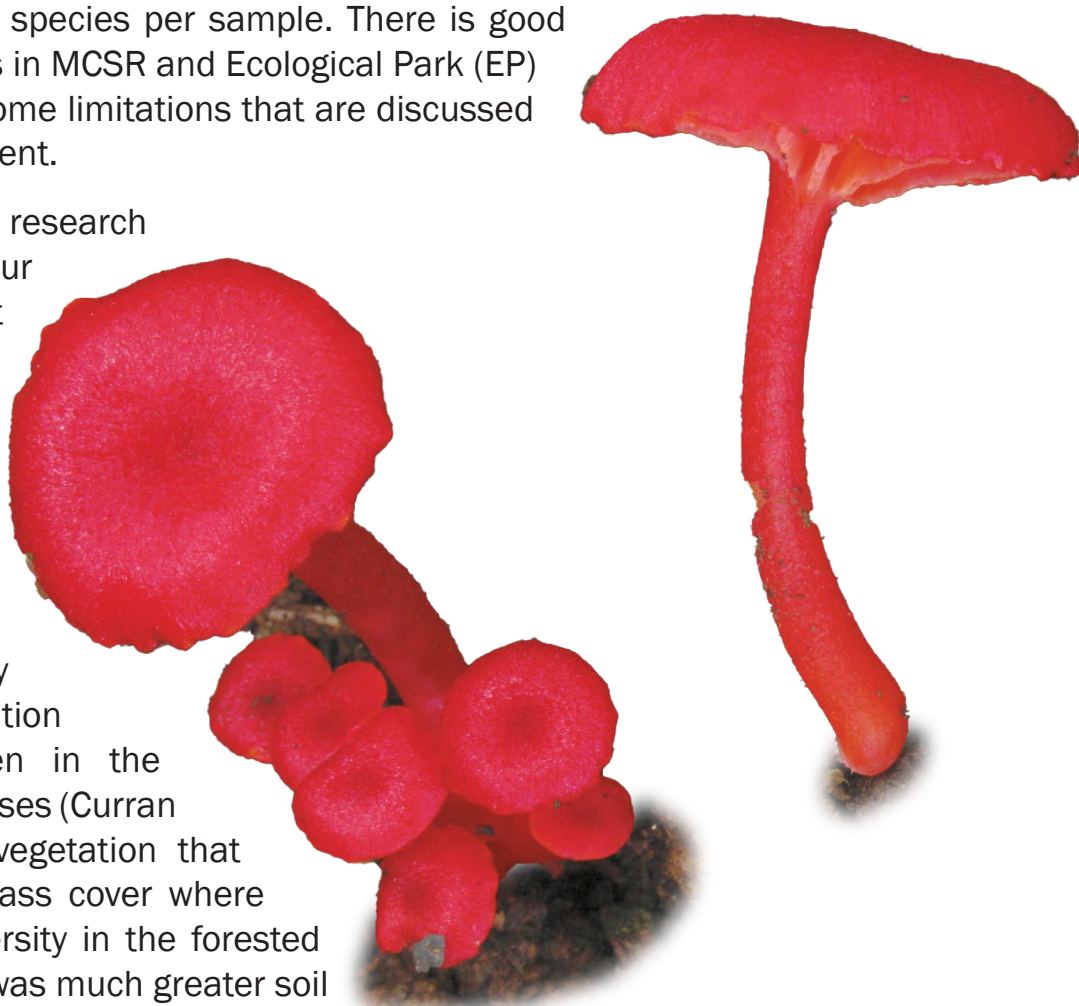


Fig. 3: Waxcap Hygrocybe bolensis is only know from MCSR & three sites in central NSW.

We look forward to reading the results of the current mycological research that is being undertaken at MCSR by Frances Guard, looking at how *Marasmius* and other rhizoid

producing fungi are creating “litter traps”. We hope once the study has been completed that the data is shared in as complete a manner as possible, including plant and fungi hosts as well as any environmental data. Ecological studies are rarely repeated, so it is important to retain data as well as the synthesis from published and unpublished documents. New methodologies may come along that allow reanalysis or meta-analysis from existing data. MCSR as a research hub should help retain and curate such studies.

3.1 WORKING TOGETHER TO BUILD FUNGAL KNOWLEDGE

Fungi make a valuable contribution to the biodiversity, ecological function and tourism value of the site. Investment should be made to increase mycological literacy, firstly among staff and volunteers at MCSR.



For the community to be able to engage with fungi they firstly need to know what they are, what roles they play in ecosystems and conservation issues they face. Building fungal literacy will take time but hopefully the integrative nature will find synergies with other biodiversity elements giving better understanding to the health of the whole site.

Fungi are a whole kingdom so to successfully understand the breadth of the different fungi groups, a range of experts will be required. A “Connector Mycologist” will oversee the various projects and engage the appropriate expert mycologists.

Experts in different fungal groups will:

- 🍄 Report to stakeholders on existing knowledge of the site,
- 🍄 Train SCC staff and volunteers,
- 🍄 Survey stakeholders throughout the project,
- 🍄 Report findings to stakeholders regularly.

We will accommodate differing skill and commitment levels among staff and volunteers to ensure participation at all levels. Photographers, philosophers, conservationists - we will provide engagement options for all.

Regularly repeating beginner training will accommodate turnover, ensuring local volunteers

understand the importance of fungi and the research. Additional training and education from the different subject experts will give volunteers opportunities to improve their skills and understanding.

3.1.1 ROLES OF PROPOSED RESEARCH PROGRAM

Connector Mycologist

A mycologist and project manager with general knowledge across subjects.

The Connector will engage subject matter experts as needed, provide introductory training to staff and volunteers and provide ongoing support for citizen science projects.

The Connector will track the different data sets being produced and make sure that consistent systems evolve for sampling methodologies. They will maintain standards and repeatability of data collection (e.g., assigning unique codes for each sample). This mycologist would be responsible for curating MCSR's fungi collections, sequencing and lodging the molecular barcodes.

Finally, the Connector will develop new projects as areas of interest expand.

Subject Experts

Mycologists and other specialists on organisms interacting with fungi.

Meetings to discuss collaborative data collection will be needed across the different biological groups if fungi data collection is to be integrated in some instances. For example, if analysis of animal scats are to be collected then the mycology experts need to work with the MSCR animal researchers.

Any unusual fungi should be collected even if they are unknown because it is now possible to use molecular sequencing to help with identification. Collecting fungi is a skill and improves with practice and interaction with different experts who are able to share the important characteristics of the different groups.

Some subject experts may also be volunteers; the local group the Queensland Mycological Society has a number of experts in different groups of fungi. Some of these members have already been involved in collecting fungal data over the years. For example, Dr Frances Guard has been helping staff (S Reif) work with volunteers to gather both observational records and reference fungal collections.

A list of likely subject experts are included in the Resources section. Depending on the timing of research, there may be other subject experts that should be considered for collaboration.

Site Experts

For site data and local species identification.

Visiting scientists are not likely to be able to identify local plants or animals to species level, so they will need to rely on local knowledge.

Volunteers

Community engagement and support.

The project will engage volunteers with many different interests and varying levels of complexity and commitment. Volunteers' knowledge of the site and their observation skills are valuable. Some volunteers may focus on increasing fungal knowledge through photography. Others may help with fungal collection or data management and others still may become experts of particular groups of fungi.

We recognise that there are many long-serving and committed volunteers who have supported MCSR over the decades.

Data Support & Bioinformatics

Sort, manage and interpret complex data.

Biodiversity data, particularly fungal data, can get complex quickly as fungi are likely to interact with a number of different species. Being able to capture species interactions requires the collection of specific details or 'rich data'. Expert support and advice on data management is critical.

Similarly, molecular data produced by even relatively simple projects give a large amount of data. This data can be used for ongoing analysis if the meta-data and the raw data are curated so that it is easily available. Ecological analyses also need to be carefully worked out based on the questions behind the research.

Bioinformaticians help plan projects, provide molecular pipelines and help with analysis and data storage. For example, bioinformaticians should be consulted when setting up the molecular database so that it can easily be linked to bioinformatic pipelines for metabarcoding.

3.1.2 MATERIALS & EQUIPMENT

Any scientific endeavour requires some sort of knowledge base to work from. To be able to monitor local fungi a library of reference material, a database and identification aids need to be developed. For these components to be successfully set up and maintained some of

these roles would need to be filled by staff and facilities.

Reference Library

To begin, reference material is likely to include field guides and more recent taxonomic literature. Some of this data can be compiled using guides in iNaturalist.

The literature is often technical but can be made more accessible by having an identification aid database which is continually developed and improved.

Reference Collections

Used to build the molecular library, facilitate new taxonomic treatments and to recognise rare species.

Digital Asset Management

Existing application iNaturalist will be used to capture observational records. iNaturalist stores digital images and records information, including photographer and copyright.

For other projects there will be other data collected that needs to be stored so that analyses over time are possible. There will need to be systems developed to manage the data collected and results. Collaborators are likely to also keep records themselves but it is important that copies of all the data produced are kept so that it can be used for future research.

Identification Aids

Identification is critical for working with biodiversity. Subject experts will develop identification aids for common and easily recognisable species. Once species have been identified once and the appropriate literature acquired it should be easier to identify and check identifications in the future.

There are already a number of resources available for the project included later in this document. Resources developed prior to 2020 should be updated so that consistent names are used wherever possible. To this end, the *Macrofungi of Mary Cairncross Scenic Reserve 2010* (McMullan-Fisher 2010b) should be updated. This should be carried out at the same time as building an online resource and iNaturalist Guide to help keep track of the full list of fungi that have been recorded at the site. Updating names and adding functional role information for the macrofungi including gilled fungi and some examples from other groups should be done first. Other groups like polypores need to be done by an expert in this area. Consultation with volunteers and staff will identify the most useful aids. More technical resources are likely required for more difficult to recognise fungi.

Laboratory Equipment

Building up knowledge about fungi is a scientific process so access to technical equipment is needed. For some of the ecological projects that consider change over time there will be samples that will need to be stored safely for some time.

The basic equipment needed to study macrofungi have already been purchased for the first education and collection training workshop in May 2023. As projects develop other materials and equipment may be needed.

3.1.3 FUNGAL EDUCATION & TRAINING

A fungal education and training program began on 1 June 2023 to teach interested volunteers and staff the basics about fungi and how to make an observational record in iNaturalist. This included searching on the site and discussing the fungi seen, the condition of the various specimens and a review of the different stages of development of common fungal reproductive structures.

After lunch there was a more focused session that introduced the basics of making voucher collections, including:

-  Taking photographs that focus on detailing important characteristics in the field;
-  Collecting rich data about the substrate and habitat, and laboratory-style photographs; and
-  Taking DNA samples and a short description.

These steps are summarised in the booklet *Barcoding Maleny Fungi Collections* (McMullan-Fisher 2023). Making herbarium vouchers of the macrofungi follows the procedures set out for the Queensland Herbarium (Prance and Fechner 2017).

Staff and volunteers since this training have been going out together once a month to look for fungi with Dr Frances Guard. We suggest the system be improved by having the Connector Mycologist follow up surveys with an online meeting during the fungi season.

Future training should upskill volunteers to be able to write longer descriptions and know what characteristics need to be recorded for different focus groups. The introductory training should be repeated so that eventually most volunteers have at least some fungal understanding.

3.2 DATA GAPS & BIAS

The macrofungi found at Mary Cairncross are probably better known than most other reserves on the Sunshine Coast. Currently data is biased towards the macrofungi so fungi that produce large reproductive structures are more easily seen. Even amongst this group there are large knowledge gaps particularly amongst the ascomycetes, polypores, mycorrhizal fungi and lichens.

The knowledge of other important groups of fungi are lagging, but the opportunities for utilising molecular technologies are well supported. This has the potential to fill the knowledge gaps. New benchmark data for vegetation, soil and decomposition will help discover the multitude of interactions fungi have with closely associated animals, plants and other organisms.

The baseline data will also increase understanding of the basal ecological processes that fungi are interacting with. Fungi have many interactions with microbes that includes protozoan and microfauna, for example. Waterbears' (tardigrades) diet includes bacteria, detritus, algae, fungi, mosses, slime moulds and micrometazoans such as nematodes and rotifers. Understanding food webs is useful, particularly if changing climatic factors cause some of these ecological processes that we rely on to fail.

To be able to get a full picture of the base of food webs, we need to look across biological groups and their interactions rather than focussing on singular groups. Ecological questions need to look at the interactions of animals, fungi, plants and microbes.

As part of understanding food webs and ecological processes, improving our understanding of the nutrient cycles is also important. For example, to get an understanding of the nitrogen cycle in the MCSR rainforests we can research the interactions between leguminous plants and their bacterial and fungal partners. Cyanolichens are also likely contributors of natural sources of nitrogen in the rainforest.

It would be wise to integrate important biosecurity and conservation actions into the *Environmental Reserves Network Management Plan 2021-2027*, particularly Volume III. The manual includes templates, restoration guidelines, links to other key documents and best practice manuals and provides the current legislative framework for restoration and natural area management.

Given all the above, it would be best if fungi could be integrated into all future Sunshine Coast environmental management documents.

3.3 POTENTIAL PROJECTS FOCUSED ON FUNGI

We know the most about the macrofungi at MCSR. These are also the group of fungi that along with lichens are the most easily seen by people with all skill levels of observation. This makes macrofungi the easiest group to involve people who are new to fungi. It also makes them an excellent group to use as a focus for education.

Some potential projects:

-  Monitor macrofungi at the site, starting with the most recognisable species;
-  Create new identification guides to improve mycological literacy of staff, volunteers and visitors;
-  Flag conservation species for monitoring;
-  Flag potentially problematic or weedy taxa;
-  Curate a macrofungal collection and create vouchers to enable molecular barcoding;
-  Increase understanding of less well-known fungal groups such as lichens and truffles; and
-  Update identification resources and create new ones as mycological literacy increases.

4. PRIORITY B: FUNGAL LIBRARY

NT Waxcap.

Humidicutis arcohastata.





EMERGING

molecular techniques utilising DNA are rapidly and radically increasing our understanding of ecological processes. In the past, natural history observation and research was reliant on being able to see organisms. This meant that fungi were easily overlooked as they are often only obvious when they are reproducing (e.g., when mushroom caps appear). The majority of many fungi's life cycles are unseen. Now with molecular technology we can take environmental samples like soil, leaf or animal scats and discover what fungi are present.

Modern molecular techniques for sampling of fungi involve sequencing environmental DNA, or eDNA. Other techniques include RNA sequencing or microsatellite analyses to carry out population studies. These new techniques are particularly useful as they can show what and where fungi are found, including inside hosts like plants or animals or in substrates like logs or scats. Specific analyses may look at what these fungi are doing or focus on getting population data that can be particularly important for conservation management of threatened species. Molecular data in the context of species interactions and ecology can help inform management.

To accurately use molecular tools in a local context some efforts need to go into building a molecular library to enable environmental analyses and rapid survey.

4.1 HISTORIC SAMPLING ISSUES

Before molecular techniques became cost effective, the traditional survey method for fungi was using visible reproductive structures such as mushrooms and puffballs. However, such methods do not give a full picture of the diversity and distribution of species because:

- 🍄 not all species produce visible or conspicuous reproductive structures (Vogt *et al.* 1992; Tedersoo and Nara 2010);
- 🍄 not all species produce reproductive structures every year (O'Brien *et al.* 2005; Tedersoo and Nara 2010); and
- 🍄 reproductive structure counts do not correspond to mycelial abundance below ground (Gardes and Bruns 1996).

It takes 3 – 8 years to develop a reasonable inventory of species that produce visible reproductive structures at a site. Nonetheless, many of these methods have some

application today and the results of historical research gave us some understanding of the fungi. Past and future collections are a valuable resource for building molecular libraries of local Australian species.

4.2 MOLECULAR SEQUENCING TECHNOLOGIES & DNA BARCODES

High-throughput sequencing (HTS) tools such as Illumina and Nanopore allow very large amounts of DNA to be sequenced rapidly and at increasingly low costs. These can be used to sequence whole genomes, barcodes or eDNA from environmental samples. However, the cost of sequencing a whole genome is still relatively high, so most databases utilise DNA barcodes. These are small regions of DNA that are highly conserved within a taxonomic group, but exhibit enough variation between species or genera that they can be used to distinguish between them.

Molecular data, including DNA barcoding, greatly improves our capacity to catalogue biodiversity and has helped to extend our understanding of species' distributions and activities. However, considerable effort is still needed to establish a DNA barcode reference library for all species. Currently, collaborative frameworks are working towards a Barcode of Life Data System (Centre for Biodiversity Genomics, University of Guelph 2021). It is worth noting that there are also local and global initiatives focussing on full genomic sequencing of species. It is yet to be seen if full genomic sequencing will completely replace barcoding systems or work as complimentary data.

An important consideration when developing a barcode library, as well as when designing eDNA studies, is choice of primers. Primers are used to select and amplify specific regions of DNA such as barcode regions for sequencing. Fungi are a large and diverse group so although there are some generally used primers that cover most fungi, some fungi like arbuscular mycorrhizas need group-specific primers to be used. More details on primers and other considerations like cost-effective batch sizes are detailed later in this report.

4.3 ENVIRONMENTAL DNA & RNA

Environmental DNA (eDNA) is the residual DNA that is left by organisms in environmental samples such as soil, scats, water or even air. There are a wide variety of techniques that can utilise eDNA to identify which species are present in an environment. Some of these techniques, such as species-specific primers, can identify the presence or absence of individual species or genera. Other techniques such as metabarcoding can simultaneously identify all or most of the species in a certain taxonomic group such as bacteria, fungi or animals.

Most of these techniques can, with some modifications, also utilise RNA. DNA and RNA are collectively known as nucleic acids. While DNA is the code of life, RNA produces the proteins

that cells are built from. The main difference between DNA and RNA in terms of environmental samples is that RNA breaks down much faster, which has scientific and logistical implications.

4.4 METABARCODING

Metabarcoding utilises eDNA to identify all or most of the species present in an environment. This is done through sequencing of nucleic acids that have been extracted from a substrate such as soil, leaf litter, scats or water. The sample is then amplified using primers that select for a certain type of organism. This is then sequenced and the results compared with databases in order to identify the organisms that are present. These databases include both global public access databases of fungi and any local reference molecular library that is built.

This type of sequencing yields a large amount of useful data but requires the development of a locally relevant fungal species molecular library. Without a library with local data as a reference, identification will not match to local Australian species. Thus, the long-term aim would be to voucher and sequence each species known from the reserve to create a complete reference set for all fungi at MCSR. This is particularly important if understanding of soil fungi is to be realised. However, in the short term it would be beneficial to highlight priority species, such as rare species, or species that are implicated in threatening processes.

There are a variety of sequencing technologies that can be used for metabarcoding. The two platforms recommended by this report are Illumina and Nanopore.

Metabarcoding can be carried out using RNA rather than DNA. The main difference between these methods is that RNA breaks down faster in the environment. This means that if RNA is used for metabarcoding then it can be used to develop a picture of what is currently living in an environmental sample. This tends to be used for projects that are focussed on specific species and what they are doing. However, using RNA for metabarcoding poses further challenges in sample storage, extraction and amplification.

Contrasting with DNA lasts longer in the environment, so if DNA alone is used then the metabarcoding will pick up organisms that may no longer be alive. This can be problematic if your interpretation needs to know if organisms are alive. These are often questions that are asked about species of conservation importance. Specific projects to focus on answering population questions should use appropriate techniques.

4.5 LOCAL MOLECULAR REFERENCE BARCODE LIBRARY

A database of local fungi that ideally links herbarium specimens to their sequenced DNA will

The sequencing platform traditionally used for building these kinds of libraries is Sanger. However, Nanopore sequencing may also be a good option. The main consideration when choosing which technology is preferable for building a molecular library is batch size. If a large number of samples can be batched together then Nanopore sequencing is preferable, otherwise Sanger is preferable. If Nanopore sequencing is chosen it is recommended that Hoosier Mushroom Society in the US be consulted, as they have used this technique successfully on thousands of herbarium specimens.

Any data collected for the local database should also be added to national and global databases including Genbank and UNITE so that it can be used by other researchers. We recommend that samples that are used for the library are either already in an herbarium or that they be deposited in an herbarium. Adding DNA sequences to the databases would be part of the process.

We recommend funding the development of a local molecular reference barcode library. This project would review the collections available from MCSR and potentially other collections that are found from the Blackall range. These collections are most likely to come from two herbaria (BRI and MEL) and possibly private collections amongst local researchers and members of the Queensland Mycological Society. If private collections are to be included for sequencing barcodes, we recommend these be submitted to one of the herbaria so that the reference collection is available to facilitate future research including taxonomy.

This project would require expertise in sampling and deciding what specimens are worth sampling. It would be expected that some of the samples will fail either to be sequenced at all or give poor data. Older and poorly preserved samples are likely to have a higher failure rate. Samples would also be assessed on the quality of the collection data available; samples with no images or notes are not recommended in most cases as these do not represent good reference samples on which to base local species concepts.

Considering the Australian Virtual Herbarium and other data, we estimate there are likely to be 200-360 available samples, including samples now being collected with mycological support by staff and volunteers.

*Fig. 4: Fan-shaped mushroom *Crepidotus innuopurpureus* has not been recorded at MCSR since 1996. This small recycler fan is a priority to record (MD Barrett).*

As well as processing samples to build the local library, data will need to be checked to see if there are already quality sequences available in molecular databases that have a supporting voucher specimen. As part of this, collections that were made from other sites on the Blackall range that had similar habitats would expand the number of collections that could be used to start the reference barcode library.

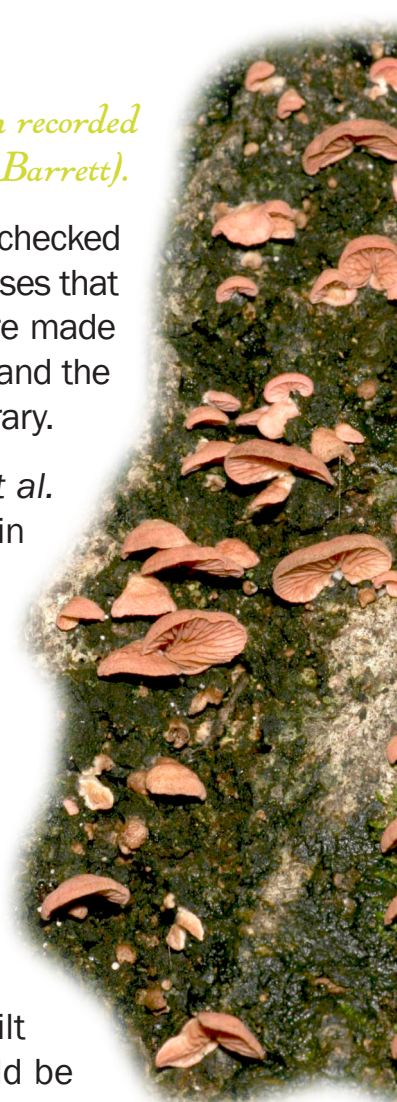
For example the recently named *Crepidotus innuopurpureus* (Crous *et al.* 2021) specimen came from Mary Cairncross Scenic Reserve, found in the subtropical rainforest, on dead wood of leafy trees, 27 Feb. 1996. This type specimen is preserved at National Herbarium of Victoria and has ITS and LSU DNA sequences already published and available (T.R. Lohmeyer, holotype MEL 2503290; ITS and LSU sequences MZ870345 and MZ870347, MycoBank MB 840921).

Currently this small fan shaped mushroom has only been recorded once. Raising the awareness that this fungus probably occurs on the site increases the chances of new sightings. For conservation efforts, having data on species over time is one way to gauge health of populations.

As part of this work we suggest a guide to the fungi of Maleny be built using iNaturalist (<https://www.inaturalist.org/guides>). This tool should be kept up to date and whenever possible linked to local voucher specimens. We recommend that when the historical collections are processed, they are added to this iNaturalist project (<https://www.inaturalist.org/projects/barcoding-maleny-fungi-collections>).

Building of the local library is a key step in integrating fungi into understanding the biodiversity of MCSR and the adjoining Ecological Park. As 2024 is likely to be a drier El Niño year, starting to build the library now is preferable as there are likely to be fewer quality collections found on site during the drier weather.

Building the library will likely take a decade or more. In the longer term it would be best to make good quality samples for species that appear opportunistically to add to the molecular library. If volunteers and staff are trained and supported by mycological expertise across different fungal groups, the library will be both a resource for knowing the species that occur on the site and supporting data analysis in environmental sampling.

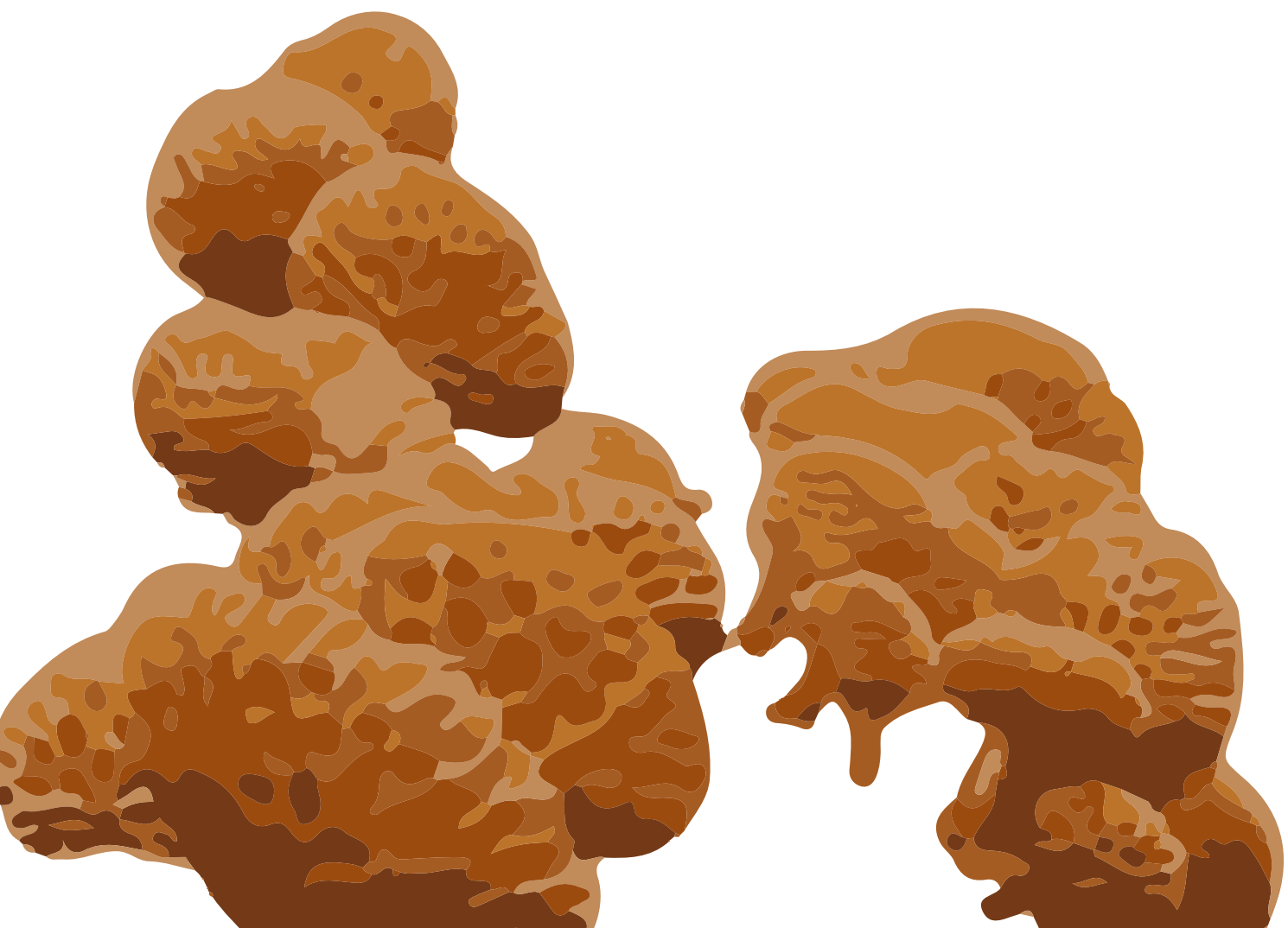




5. PRIORITY C: KNOWN THREATS

Orange Pore Fungus.

Favolaschia claudopus.





THE large-scale and systematic modification across landscapes for agriculture, forestry and urbanisation has caused the highest extinction rates ever recorded. These impacts have decreased ecosystem function across the globe and have contributed to catastrophic global warming. Fungi are also threatened by these processes although their extinction rates are less well recorded (Bidartondo *et al.* 2018; Cannon *et al.* 2018; Antonelli *et al.* 2020). The different groups of fungi are affected by local circumstances, historical damage, extent of modification of the environment and extent of recovery and/or restoration, if any. Fungi have rarely been included in climatic modelling, but we know that many of the combined issues of human-induced climatic warming and large-scale habitat modification have negative consequences.

5.1 LAND CLEARING & HABITAT MODIFICATION

Ecosystems function well when there is rich diversity across biological groups, and amongst this biological community there are at least some species that perform all of the necessary functions. In strong, resilient systems, there will be some functional redundancy (i.e., the same jobs will get carried out by a variety of organisms,) allowing for flexibility in the ecosystem to changing environmental conditions.

Land clearing and human modification causes simplification and often introduces exotic species so that surviving remnants are simplified and isolated. Overall loss or modification of habitat generally results in reduction of health and function of fungi. Specifically, most biotrophic fungi that depend directly on animal and plant hosts are lost along with their hosts. For example, without plant hosts, obligate mycorrhizas, endophytes and parasites cannot survive. And even where hosts survive but are unhealthy, the associated fungi will be negatively impacted. The smaller and more isolated remnant bushlands are, the fewer species remain and lower function of surviving fungi and their hosts. Such patches also have a greatly increased risk of localised extinctions.

Fungi that depend on animal dispersal, such as truffle-like mycorrhizal fungi, have decreased dispersal (Reddell *et al.* 1997) where these animals have become locally or entirely extinct. There are negative health and survival consequences for all three linked

groups: animals, mycorrhizal fungi, and plants.

Pollution and biocidal chemical use are shown to decrease the number of fungal groups, particularly lichens (Conti and Cecchetti 2001) and ectomycorrhizal fungi (Newbound *et al.* 2010). Increased nutrients and damage from industrial fertilisers have also been shown to damage soil fungi, including mycorrhizas and lichens.

5.2 CLIMATE CHANGE

In Australia, current and projected changes resulting from Anthropogenic climatic warming have been outlined in the State of the Climate Report (CSIRO and Bureau of Meteorology 2018). Little data exists on how increased temperatures will affect Australian fungal communities, and how this will contribute to soil carbon feedback cycles. Given fungi play a major role in soil processes including soil carbon storage, we need to include fungi in data collection. Better modelling of climate change scenarios will be dependent on increasing our understanding of how different types of fungi respond to increased temperatures, heatwaves and drought.

Increased frequency and severity of drought, decreasing rainfall, decreasing numbers of wet days combined with increasing intensity of rainfall events affect fungal communities in a variety of ways:

-  slower growth rates for most fungi, including lichens;
-  changes in the composition of communities as species that survive drier conditions likely become dominant;
-  decreased health of mycorrhizal partner trees due to water stress; and
-  changes in the reproduction patterns of fungi.

Drought is a major climatic driver of the assembly of soil microbial communities (Ochoa-Hueso *et al.* 2018). Global studies, including Australian sites, have shown that increasing aridity, as predicted by current climate change models, reduces both the diversity and abundance of soil fungi and other microbes (Maestre *et al.* 2015, 2016)

One of the few reviews that include Australian decomposition rates (Mackensen *et al.* 2003) highlights the lack of data across the diversity of Australian habitats and their dominant trees. Without data on decomposition, we are unable to monitor

South-eastern rainforest communities have been shown to harbour their own distinctive suites of species (Dell *et al.* 2020) and the loss of these non-fire adapted species from ecosystems is likely to result in a myriad of fungal species extinctions. We do not have similar data for fungi from south-east Queensland. Fungi need to be considered as changes in post-fire soil chemistry and plant community structure influence suitability of habitat for different species. Large, decayed logs are often consumed in intense bushfires (Hollis *et al.* 2011), which provide unique habitats for polypores that decompose logs and recycle the nutrients to living trees (Gates, Mohammed, Wardlaw, Davidson, *et al.* 2011; Gates, Mohammed, Wardlaw, Ratkowsky, *et al.* 2011). This group is important in decomposition processes that are critical for rainforests.

5.3 PROBLEMATIC & WEEDY FUNGI

As climate has warmed in the past few decades an unprecedented number of fungal and fungal-like diseases have recently caused some of the most severe die-offs and extinctions ever witnessed in wild animals and plants (Fisher *et al.* 2012). Globally, fungal infections causing widespread population declines are known from trees, crops, bats and frogs. The true numbers of extinctions and population declines caused by fungi and oomycetes are likely to be greater than we know. For example, the oomycete *Phytophthora* causes dieback across many Australian landscapes.

Considering the existing species list from the reserve, the following are likely problematic fungal species that are known to occur or are likely to arrive at MCSR.

Serious problem fungi:

-  Myrtle Rust (*Austropuccinia psidii*)
-  *Pyrrhoderma noxium*
-  *Phytophthora* spp.
-  Chytrid fungi

These are known to cause poor health and in some cases animal and tree death. These fungi are a high priority for collection and/or sequencing. These should be actively monitored and managed to prevent further declines in ecosystem fitness.

Myrtle Rust (*Austropuccinia psidii*) is an exotic rust that became naturalised in Queensland in 2011 and has affected 382 (17%) of the 2253 native myrtaceous taxa (Makinson *et al.* 2020). The impacts of Myrtle Rust are most pronounced in Australia's east coast rainforest

Species at the site that are highly susceptible include Native Guava (*Rhodomyrtus psidioides*), Thready-bark Myrtle (*Gossia inopholia*), Silky Myrtle (*Decaspermum humile*) and Silver Myrtle (*Rhodamnia argentea*). It is likely that the more highly susceptible species will need management interventions to help mature species survive at the site and supplementary planting may be needed. These may need to be timed during drier periods when the effects of Myrtle Rust are lower. Unfortunately, this is likely to be when seedlings are less likely to survive without interventions like watering. New double-stranded RNA (dsRNA) techniques against myrtle rust disease (Degnan et al. 2023) should be trialled both preventively and curatively to improve the health of susceptible plants.

Pyrrhoderma noxium (Zhou et al. 2018), previously known as *Phellinus noxius* and *Fomes noxius*, causes tree mortality in over 100 species. It is **one of six species in a complex that are known to be serious tree pathogens** (Garfinkel et al. 2021). This species has been the subject of ongoing monitoring and management including mapping. As this species is present on the site it should be a priority to determine how best to manage infected trees and ideally prevent the spread.

Preventing the arrival of pathogenic *Phytophthora* spp. will help protect sensitive plants, particularly dieback in Bunya Pines.

Chytrid fungi is an animal pathogen that affects amphibians, and potentially Platypus (*Ornithorhynchus anatinus*). It is important to prevent, particularly as another pest species, the Cane Toad (*Rhinella marina*), may carry and spread this disease-causing fungus. Ongoing monitoring is encouraged, including the use of molecular techniques like LAMP if the usefulness is proven.

Potential problem fungi:

- 🍄 Mushrooms: *Armillaria* spp., *Ganoderma* spp., *Gymnopilus* spp., and recently recorded *Cyclocybe parasitica*;
- 🍄 Polypores: Hymenochaetaceae family, including *Phellinus* spp., and *Hymenochaete setipora*;

🍄 Weedy fan: *Favolashia claudopus*; and

🍄 Weedy exotic stinkhorns: *Phallus rubicundus* and *Clathrus ruber*.

These are fungi that are either already present on site or occur nearby and are likely to arrive.

5.3.1 IMPORTANCE OF ACTIVE BIOSECURITY & HYGIENE

All regular visitors to the site including staff, volunteers, and contractors and researchers should be aware of biosecurity and hygiene protocols. Comprehensive biosecurity and hygiene protocols for accessing and leaving the site have the benefit of protecting from existing and emerging threatening species like fire ants.

Protocols should cover not just people who visit the site but also everything that is brought on site including equipment, particularly materials like potting mix and mulch that are more likely to contain living organisms.

Already, visitors who walk through the site are encouraged to wash down their shoes. This will help reduce soil borne fungi. Unfortunately, this will have no effect on airborne problem fungi. All visitors should be encouraged to come with clean clothing and equipment including bags and hats.

There are several biosecurity management tools that help the development of integrated management plans from problem species. Mapping and the use of zones to manage access, particularly at high-risk times, helps mitigate risk (Massenbauer 2018). We recommend using these tools to develop an integrated management plan for all problem species as many of the risk mitigation measures will also work for other groups.

Monitoring for potential problem fungi should be undertaken periodically and recommended protocols for hygiene of survey equipment should be implemented. Staff, volunteers and visiting researchers should know areas to avoid or to ensure decontamination after visiting areas containing problem species.

Potential projects to investigate the impacts of these problem species on reserve health are suggested. For example, Weedy *Favolaschia* could be studied by a number of techniques including LAMP which, if integrated, could allow the use of environment samples to map distributions. Once LAMP is shown to be useful, it could be adapted for other problem fungi or fungi of conservation importance.

Management of problem species has a number of stages: prevention, eradication, and then harm mitigation measures, particularly preventing the further spread. Once

Washing and drying of clothes and equipment does the bulk of hygiene work. Use of cleaning chemicals like Phytoclean and methylated spirits may assist in high-risk situations or where several locations must be visited in succession. Visits to successive locations should be avoided.

The Weedy *Favolaschia*, or Orange Pore Fungus (*F. claudopus*), previously Orange Ping Pong Bats Fungus (*Favolaschia calocera*), is a weedy invasive fan fungus that has spread around Australia, New Zealand and other countries in the last 50 years. Like many weeds, warnings to prevent the spread went unheeded. At MCSR there is at least one species of native *Favolaschia*, *Favolaschia* aff. *pustulosa*, was recorded during the 2010 surveys.

In 2021, management of MCSR became aware that Weedy *Favolaschia* was present on site but a subsequent call for historical fungal images show that it was present on the site from at least 2017. Although this has naturalised and eradication would be impossible, learning more about this problem species could still be very useful. Particularly as there is little data to support our understanding of the negative consequences to other decomposer fungi. Anecdotal evidence and some preliminary research suggest that like many weeds, it reduces diversity on the site.

There are a number of potential projects that could get more information about this fungal weed. This includes potential volunteer citizen science projects utilising professional mycologist support. There are also some potential molecular survey techniques, called LAMP that utilise species specific primers. These primers produce a simple colour change.

MCSR staff and volunteers could help increase the understanding of this weed by using

iNaturalist to monitor different types of woody substrate over time.

Researchers will monitor known substrates 1-3 times per week in fungi season to see how many fans are produced and how long they last. They will simultaneously record the diversity and abundance of other decomposer fungi. Understanding how competitive this species is against local saprotrophic fungi would be useful.

It is likely that if other substrates like logs are being recorded, at some stage the Weedy *Favolaschia* will appear. This is critical information to capture as it may help identify any resistant substrates.

By monitoring a diversity of substrates with other wood rot fungi, the data could be used to compare wood of different sizes with the Weedy *Favolaschia*.

Once patterns emerge from the morphological monitoring, then ideally some molecular testing could be done to gauge the true number of fungi in each substrate. This would include those that may not or rarely produce reproductive structures.

These projects are contingent on professional mycologist support. There would need to be initial in-person training to get started and then ongoing online support. These online support meetings should be more frequent during peak fungal reproduction periods.

5.3.2.2 PILOT STUDY LAMP TEST FOR WEEDY FAVOLASCHIA & OTHER PROJECTS

LAMP is a method that can detect the presence of the DNA of a species in an environmental sample such as soil or water. This means we are not reliant on finding mushrooms of the target fungus to monitor its spread. This is particularly important when it comes to monitoring and controlling invasive fungi, as once mushrooms or other reproductive structures are produced and spores have been released it is much harder to prevent further spread.

This method is also much faster, cheaper and requires less technical expertise and equipment than most other methods using environmental DNA. It is also possible to do the tests on site, providing immediate results.

If this use of LAMP technology is successful in field trials, it could be used at the site. If this is a proven tool, the method could be applied to other fungal species of interest including problem fungi-like organisms like bunya dieback and species of conservation importance like Waxcaps, particularly *Hygrocybe bolensis*.

Likely problem fungi LAMP tests would be particularly useful for pretesting materials to be used in restoration. For example, scat could be tested to see if using scats for nearby restoration would move problems into the new area. New areas could also be tested to see if problem fungi were already present to inform the importance of using clean materials.

5.4 ISSUES OF FUNGI SURVIVAL, ISOLATION & DISPERSAL

The threats to fungi from land clearing, habitat modification, exotic species, drought, increased temperature and the intensification of these threats due to climate warming have already been considered. For life to be self-sustaining, species need not only to be able to survive but also successfully reproduce and disperse.

Many fungi are able to reproduce asexually, which allows increased dispersal potential. This is commonly seen in some ascomycetes. Many fungi are also able to reproduce sexually and the associated recombination of genes in the progeny are important to allow adaption to change. One of the problems of isolation and reduced dispersal ability is that some species may no longer have compatible partners for sexual reproduction. So although the species may remain working in the ecosystem, they are less fit and more likely to go locally extinct.

Many fungi have dispersal distances of less than 100 m (Dahlberg and Mueller 2011). Those that historically dispersed greater distances via mammal partners suffer from the extinction of these mammals. Fragmentation affects the ability of fungi to colonise disconnected or isolated patches of bushland. The negative environmental impacts that have affected the other reserve diversity are likely to also be affecting fungi.

There is a paucity of data on the dispersal of Australian fungal species, and limited data on fungal dispersal globally. Without species - specific knowledge pertaining to dispersal, establishment and ecology, determining the appropriate distances for connectivity of landscape patches or scale of management actions is speculative (Komonen and Müller 2018). We also have very little understanding of spore bank and other propagules of fungi, including their response to desiccation, ultraviolet light, and spore longevity.

The upcoming predicted El Niño will likely result in longer drier periods. This is likely to

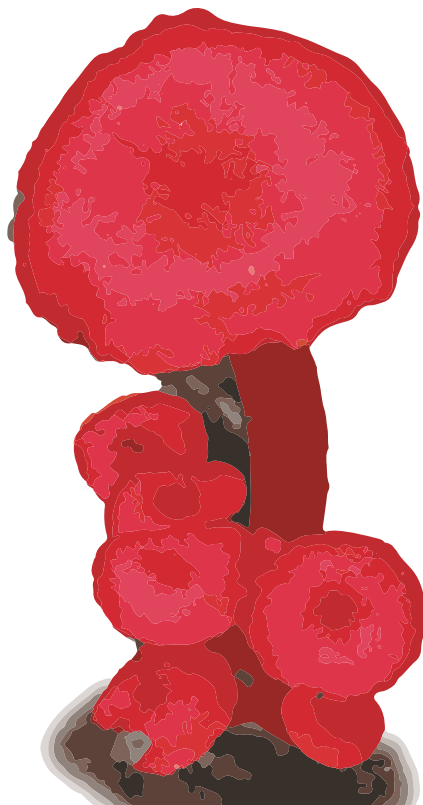
FUNGI FACT

QUEENSLAND CONSERVATION LISTS
DO NOT CURRENTLY CONTAIN ANY
FUNGI

6. PRIORITY D: ECOLOGICAL PROJECTS

Waxcap.

Hygrocybe bolensis.





A variety of ecological projects examining fungi could be carried out within the site, from scientist-supported citizen science projects to university student projects, to projects by professional mycologists with volunteer support.

Ecological projects are usually a combination of collecting environmental data and molecular data on the fungi present to answer specific questions. Most will depend to some degree on the availability of a local species to build a local molecular reference barcode library. Ecological questions are best answered in context, so site characteristics need to be considered as well as what methods are to be used. Two key areas where ecological function significantly depends on healthy communities of fungi are in soil and in decomposition.

6.1 SITE DESIGN CONSIDERATIONS

MCSR and future adjoining lands like the Ecological Park will likely be integrated for management. If the survey grid that covers MCSR is expanded, it will be useful for longer term monitoring. Working within the existing monitoring system will facilitate understanding of the different management areas. The restored areas in the Ecological Park are likely to undergo rapid change in the next 1-4 years and then some change in the next 5-15 years until the canopy is fully developed and steadily matures. The good level of connectivity of restoration areas to mature MCSR vegetation means that many of the dispersal problems facing fungi and other biota are likely to have less impact. This is likely to hasten restoration of the adjoining Ecological Park.

The high level of species richness, particularly for plants, means that we expect high levels of site heterogeneity for fungi as well. To successfully capture this diversity of fungi, sufficient replication is needed to answer ecological and conservation questions.

There are already central grid pegs that are spaced across the MCSR area, but this has only been minimally extended into the Ecological Park. These are useful as a basic sampling unit. Although the number for each regional ecosystem is unbalanced for design purposes, with most in the Complex Notophyll Vine Forest (CNVF). However, there are a

The Invertebrate study used a design that considered variation across the site, but with insufficient replication (Burwell *et al.* 2023), minimum of five replicates are needed:

- 🍄 Complex Notophyll Vine Forest (peg D3)
- 🍄 Piccabeen Palm Gallery Forest (peg C3)
- 🍄 Small *Eucalyptus grandis* ecotone between CNVF and Piccabeen Palm Gallery Forest (peg D6)
- 🍄 *Eucalyptus robusta* ecotone (peg E5)
- 🍄 Revegetation areas in the Ecological Park to the north of MCSR

The restoration areas in the Ecological Park have had various management including clearing and conversion to pasture to support stock, and the northern area has historically been used as a commercial palm nursery. Some natural recruitment has begun with weeding and some plantings.

For long-term integration it would be good to allocate core sites in association with their likely design implication, so it is possible to specifically consider various impacts with at least some replication, including edge effects.

Benchmarking for at least the most common regional ecosystems is important. This data will be useful for not only understanding the fungi at MCSR and how they are restored in the Ecological Park, but in a wider context too. MCSR being the largest remnant in good condition, the benchmark data could help other restoration efforts gauge relative success in restoring diversity.

When to take samples is an important consideration when planning biodiversity surveys. Some sampling considerations will need to consider the animal or plant hosts as well.

To be able to build a reference library, samples should be taken when the fungi are at their most obvious, which is when they are reproducing, typically the wetter months and seasons. Rainforest sites like MCSR often have more obvious fungal reproduction, with months that have more than 80 mm of rainfall likely to have at least some fungi reproduction. In

Maleny, this means the most likely months for fungal production are January – July and October– December. There may be considerable variability during La Niña, including the last three years when fungi were reproducing much of the year. Conversely, there are significantly drier periods during El Niño. Survey budgets should be increased in wetter years and ideally engage priority experts.

6.2 KEEPING TRACK OF DATA & KEEPING IT RICH

At the moment there is no single system that stores and tracks data, reports, identification and procedures like the booklet *Barcoding Maleny Fungi Collections*. The staff at Sunshine Coast Council do the best they can and have been very helpful in supplying information that is kept reasonably up to date.

Available data will increase as fungi are surveyed and sequenced into a reference library. There is no need to retain collections, rather samples will be submitted regularly to the herbarium for storage and become part of the international herbaria network. These collections will then become part of the herbarium databases that feed into the Global Biodiversity Information Facility (GBIF).

Molecular data should be re-analysed over time so that ecological and conservation trends for different fungi become clear.

Ideally, a bioinformatician or digital asset management expert should be engaged to organise all biodiversity data in the same or complimentary systems that facilitate being able to find information about associated organisms. Capturing all the associated host and habitat data is crucial. Information about the state of the organism is important to capture clearly; for example, is the plant host dead or alive?

To capture ecologically important interactions, single species-focussed data collection methodologies are not sufficient. Rich data keeps important cross biological and environmental information that explicitly connects species. Historically most biodiversity systems had a singular focus of supporting taxonomy. The emergence of molecular tools has increased the capacity to explore the details of inter-species interactions. At the moment there are no standard systems tracking data that facilitate the understanding of interactions. Collecting data of fungi often already requires additional information to be captured. For example, for decomposer fungi details of the substrate have been generically recorded as “hardwood log”. Ideally information about the plant species and the condition of the log would also be captured. This richer data will also be important to capture for biotrophic species that are intimately connected to other

Developing categories and systems for defining these richer data are important. These should be defined to suit each project. For example, in citizen science observation records it is reasonable to record substrates simply as categories like logs, litter or soil. For more ecologically important research, the sample design needs to account for greater detail.

6.3 COLLECTING FUNGI

Fungi are a whole kingdom with a broad range of ecological roles. Collecting fungi to support identification and the scientific process of characterising and naming species, the science of taxonomy as well as building a local molecular reference barcode library. The number of named fungi is expanding in part driven by the need to know what are being found in environmental samples. There are efforts to barcode all the diversity on earth in a Barcode of Life Data System (Centre for Biodiversity Genomics, University of Guelph 2021). Collecting good samples of fungi will contribute important local data.

It will take many years to build a full picture of the fungi found at the site. This process can be accelerated by supporting experts on different groups of fungi to train the pool of volunteers and staff. To begin with macrofungi are likely to be a focus for collection as these are the most easily recognised.

Capturing quality data for specific and public uses has become much easier with use of the internet and mobile phones.

We recommend the use of iNaturalist as a base system for gathering data for observational records and collections. As information about individual records or collections are advanced these can be easily updated.

iNaturalist is a useful centralised online location to keep track of collections, storing geographic data, photographer and copyright info, notes, identification, and a unique code including the DNA sample taken for barcoding. Additional fields in the project are used to track when samples are processed, etc. Collections and DNA samples are currently stored on site. These should be regularly submitted to the herbaria, ideally at the end of each fungi season. This backs up the data in a separate national system.

Currently, data is spread across multiple locations, including spreadsheets, iNaturalist and Herbaria. Keeping track of all the sources of data and that this data is kept up to date takes considerable organisation and time.

6.4 MOLECULAR METHODS

While building the molecular library, it should be noted that though metabarcoding is suitable for finding the different species in an environment, studies at a population level within a species will require different methods such as microsatellite analysis.

The stages of the metabarcoding process generally involve survey design, sample collection and storage, DNA extraction and amplification, DNA sequencing, bioinformatics and data analysis. How these stages are carried out, and the results obtained, depend on a variety of factors.

High Throughput Sequencing (HTS) are newer molecular sequencing technologies that allow very large amounts of DNA or RNA to be sequenced in a short time and at a much lower cost than earlier sequencing technologies such as the original Sanger sequencing. There are a variety of different platforms available that have different advantages and disadvantages that affect what purposes they are best suited to. Metabarcoding relies on HTS, and it may also be used for building the molecular library.

6.4.1 DNA VS RNA

Metabarcoding can be carried out on DNA or RNA. The main practical difference between these is that DNA can last a long time in the environment, while RNA is rapidly broken down. This means that RNA gives a better picture of what is currently living in an environmental sample, while DNA will also show organisms that may not currently be living in the sample. For example, DNA will pick up inactive spores, while RNA will only show living mycelium. However, if RNA is used then the samples need to be frozen right away using dry ice and may need to be stored at -80C. Using RNA also complicates the extraction process as it needs to take place rapidly and on ice so that the RNA is not degraded. Logistically, DNA is easier to work with.

6.4.2 CHOICE OF BARCODE REGIONS & PRIMERS

For either metabarcoding or building the molecular library, appropriate barcode regions are required. These will differ depending on the type of organism being studied.

For fungi, the most commonly used regions are ITS1, ITS2, LSU, SSU, and TEF. Different

How long a region can be targeted also depends on the sequencing platform. If a Sanger sequencing is used to build the molecular library, then multiple barcode regions may need to be amplified and sequenced separately. If Nanopore sequencing is used, then multiple regions may be amplified and sequenced together.

Primers can also be designed to amplify the DNA of a specific species or genera. These primers can detect presence or absence of the target DNA or RNA in a substrate. The LAMP method is one example of this, however there are many similar methods that vary in both sensitivity and the amount of specialised equipment needed.

There are a variety of molecular sequencing platforms available.

Sanger

Sanger sequencing is the earliest form of DNA sequencing that was developed. This method is very standardised, reliable and accurate. However, it can only sequence a small amount of DNA at a time. For this reason, it cannot be used for metabarcoding or whole genome sequencing. It will also only sequence short regions of about 300-1000 base pairs. Sanger sequencing is also relatively expensive but has the advantage that a single specimen can be sent off for sequencing relatively cheaply. However, there are cheaper rates for larger batches of samples.

Illumina

A high-throughput sequencing platform, Illumina can be used for metabarcoding or whole genome sequencing. It is very standardised and accurate. It sequences shorter reads (strings of DNA or RNA) of around 150-200 base pairs. The disadvantage of this is that it provides less information that could be used to distinguish each species in the sample. There are, however, bioinformatics pipelines for metabarcoding of fungi available

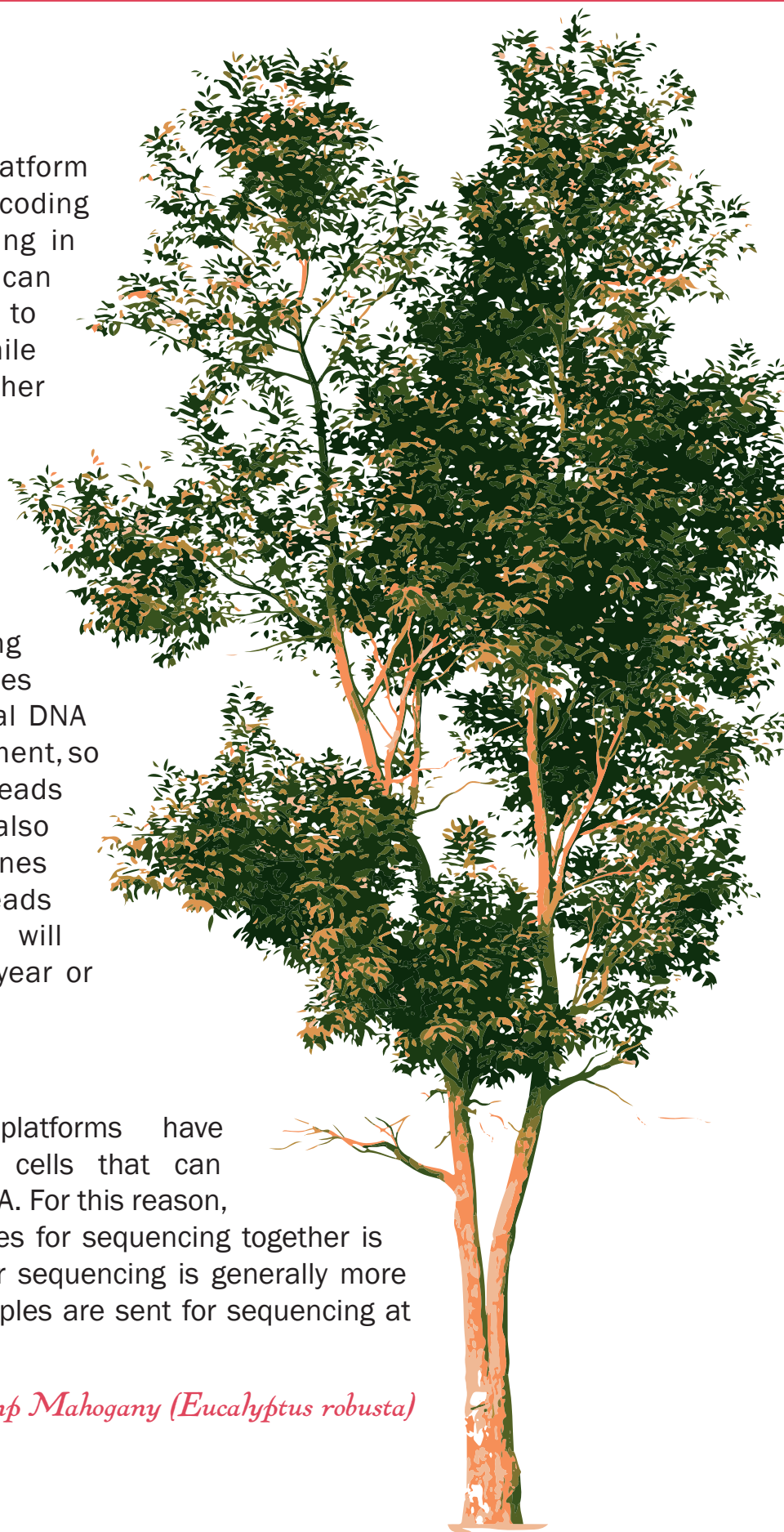
Nanopore

A high-throughput sequencing platform that can be used for metabarcoding or for whole genome sequencing in conjunction with Illumina. It can produce longer reads of up to thousands of base pairs. While Nanopore sequencing has a higher error rate than Illumina, this is being rapidly overcome by improvements in the algorithms that interpret the data. The longer reads produced by this platform could be used in metabarcoding to better identify each species present; however, environmental DNA is often broken up in the environment, so it is yet to be seen what length reads are useful. This technology is also newer, so bioinformatics pipelines for fungi using these longer reads are still being developed, but will likely be available in the next year or two.

6.4.4 BATCH SIZES

High-throughput sequencing platforms have consumable parts called flow cells that can sequence a certain amount of DNA. For this reason, sending larger batches of samples for sequencing together is more cost effective. Even Sanger sequencing is generally more cost effective when multiple samples are sent for sequencing at the same time.

Fig. 6: Swamp Mahogany (Eucalyptus robusta)



As samples for most platforms are often sequenced in batches of 96, with 2 samples as laboratory controls, designing projects in multiples of 94 samples would make sense for logistical and financial reasons. Sometimes larger batches of several hundred samples may be sequenced together to reduce costs.

When comparing sequencing of individual fungal specimens for the molecular library, a minimum of around 200 samples would be needed for Nanopore to be as cost effective as Sanger. However, the cost per sample falls dramatically after this, with costs of sequencing 10,000 samples roughly the same as the cost of sequencing 200 samples.

6.4.5 BIOINFORMATICS & DATA ANALYSIS

The computational process that sorts these results and aligns them with known species in a database is called a bioinformatics pipeline. This process requires high-powered computing. Some sequencing facilities offer this as a service. However, these services are not always designed by specialists in the specific type of organism (e.g., fungi). A custom pipeline for fungi is likely to produce better results. It will also be necessary to develop a custom pipeline to link the reads to the local molecular library. It will be necessary to hire a bioinformatician to develop this. We recommend consulting with a mycologist, bioinformatician and the lead researcher on a given project when deciding how to proceed.

FunGuild is a tool used to group fungi discovered in molecular sequencing and allocate functional groups to them. Using this tool will facilitate deeper ecological analyses and understanding than just the species names or other taxonomic groupings.

Molecular technologies are an area of rapid knowledge growth as global and local databases are being created, added to and improved. Any metabarcoding data gathered should be added to appropriate national and global databases.

It is also important to bear in mind that as the local database and global databases grow, any metabarcoding data can be reanalysed yielding more detailed results. This means that data collected now can be used to develop a baseline later on, even if not all species can currently be identified. It also means that it is very important that all raw sequencing data is kept and stored so that it can be accessed later for re-analysis.

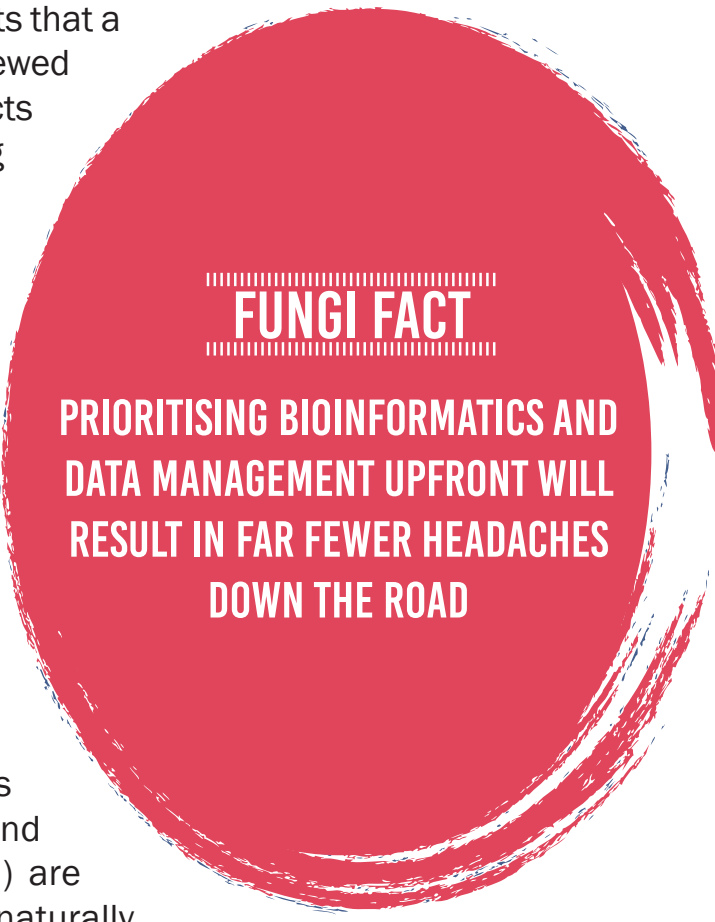
6.5 POTENTIAL ECOLOGICAL PROJECTS

The two main ecological focus areas that molecular technologies will enable are the roles of fungi in soil and decomposition processes.

We recommend that for most of these projects that a pilot project be carried out, analysed, and reviewed before tackling larger projects. Pilot projects help inform the practicalities of collecting data and reviewing these projects' logistical issues before scaling creates statistically powerful data and analyses that are designed to inform management.

Investigating the different mycological diversity and functional groups within different Regional Ecosystems (REs) is a key project. Note that samples and observation records need to explicitly include habitat so that ecotone data doesn't confuse our understanding.

Matching new restoration areas to their appropriate regional ecosystems or ecotones and then testing to see if beneficial fungi and their functions (e.g., beneficial mycorrhizal) are present. If important beneficial fungi do not naturally disperse into restoration within the first 5-10 years, then management interventions of returning beneficial fungi should be considered.



FUNGI FACT

**PRIORITISING BIOINFORMATICS AND
DATA MANAGEMENT UPFRONT WILL
RESULT IN FAR FEWER HEADACHES
DOWN THE ROAD**

7. UNDERSTANDING SOIL

Inkcap.

Coprinus truncorum.





SOIL is a critical interface for most terrestrial ecosystems. This is where plants, the primary producers, get their nutrients. Soil is an entire microcosm made up of food webs and interactions between massively diverse fungi, amoebae, archaea, bacteria, arthropods, roundworms, earthworms, algae, cyanobacteria, ‘protists’ and much more (Ruggiero *et al.* 2015).

Fungi play a central role in this system as saprotrophs, decomposing organic matter, cycling nutrients and contributing to soil structure. Fungi also interact with most plants and vegetation as mycorrhiza or root endophytes. They help plants gain nutrients and protect against parasites. Fungi also form the basis of many food chains, providing nutrients for plants or food for bacteria, animals particularly insects.

Bacteria are also extremely important in these systems. They also contribute to decomposition and are a source of nutrients for fungi, slime moulds, plants and insects. They also form complex partnerships with plants and fungi and play important roles in cycling nutrients such as nitrogen.

Developing a baseline understanding of soil communities at the site and how they change over time is necessary for understanding the overall site ecology. Soil structure along with community composition of at least some microorganisms should be studied. This should also include some data about the environmental, physical and chemical aspects of the soil.

Fungal community composition is strongly influenced by the aboveground plant community. Fungal species’ richness increases with structural complexity of vegetation, along with associated complexity of litter and soil, as the complexity of these systems creates more ecological niches. For example, a fallen tree may create a microclimate with extra species that only survive in more open conditions. Data about soil microbes should be connected with data about vegetation and leaf litter.

Historical methods for understanding fungi in the soil have included soil culturing, but these miss many soil fungi that are not culturable (Mueller, G.M. *et al.* 2004; Handelsman 2004), including mycorrhizas. Importantly, much of the diversity of fungi found in soil do not produce easily visible reproductive structures.

Rapid assessment of microbial diversity in the soil is now possible due to newer DNA sequencing technologies, but these techniques still need to be adapted for use. Emerging technologies involving high-throughput DNA sequencing have provided insights into diversity and structure of fungal communities. Working out which are the best protocols to follow for the collection of environmental soil samples needs to be informed by the ecological questions being asked, as well as taking the site specifics into consideration. Results have been shown to vary with sample size, sample distribution, soil nutrients, soil horizon, vegetation and dominant ground cover.

7.1 SOIL & FUNGI

Factors influencing community structure of fungi, including ectomycorrhizal fungi, are poorly understood (Erland and Taylor 2002). There is some preliminary research that has been undertaken looking at biotic and abiotic factors (Taylor et al. 2014) and how they influence community composition.

Some research has been done examining correlations of species of fungi with different soil nutrient statuses, including nitrogen, phosphorus, pH, calcium and sodium chloride; however, this requires further investigation in the Australian context. High-throughput sequencing technologies are enabling the quantification of fungal biodiversity in the soil.

Soil is complex to study as there are physical, chemical and biological aspects that interact. Data on the physical and chemical aspects of soil helps inform biological analysis. For example, fungal distribution has been demonstrated to be strongly related to soil pH (Taylor et al. 2014; Tedersoo et al. 2014).

We already know that geology and soil are important factors in understanding vegetation, as this is the basis for regional ecosystem mapping. At the finer scales, the suite of fungi that are found under the same species of tree are more likely to be similar than the suite of fungi found under other trees or dominant plants. Similarly, there is typically a great diversity of fungi in the soil of older plants. Unfortunately, in rainforests like those found at MCSR there are not obvious tree dominants. However, understanding the dominant fungi of the Piccabeen Palm Gallery Forest should be straight forward.

Fungal community composition is also likely to vary across soil depth. Where deep soils exist, this needs to be considered when deciding on sample collection techniques. However, Australian soils tend to be shallower than the overseas soils which many collection protocols are based on, so these will need to be adapted to suit the site.

The upper organic soil horizon fungal communities strongly correlated with vegetation variables, including tree density, proportions of seedlings to tree and the percentage of graminoid species. The organic layers typically have the highest species diversity as this is where much of the soil and decomposition processes are most active.

Alternatively, the lower mineral horizon fungal community structure was correlated with soil moisture, and related variables including soil carbon, organic soil depth, cation exchange capacity and bulk density. Vegetation factors that most strongly related to mineral soil communities included tree size and density (Taylor et al. 2014).

Fungi utilise a broad spectrum of organic compounds, from simple sugars, organic acids, and amino acids. They also break down more complex molecules using extracellular enzymes including cellulose, lignin, pectin, lignocellulose, chitin, starch, hydrocarbons and pesticides (Gadd 2007), hence fungi are also likely to influence soil chemistry and decomposition processes.

Finally, the time of year that samples are collected is important. If data is to be compared over time it is preferable to collect the samples at the same time of year. Certain fungi may be more prevalent even in molecular samples at certain times of year. Similarly, if there are significant weather pattern variations these are likely to influence the soil fungi.

To gauge maximum fungal activity it is recommended that soil samples be taken mid- to late-wet season as there is likely good diversity. It may also be worth sampling in the mid- to late-dry season, in order to understand seasonal shifts in communities. Soil samplings should also be taken in wetter, normal and drier years to inform managers of changes in the community. Once volunteers and staff are trained it may be possible for them to take extra soil collections throughout the year for future analysis.

7.1.1 LESSONS FROM CURRAN 2022 SOIL PROJECT

The Curran 2022 honours project was a useful pilot for studying soil fungi using metabarcoding. However, there were some issues with the project which should be addressed in future work. While there was some replication of samples, there was no replication of overall effects between different vegetation types or stages. In future there also needs to be replication at the transect level.

The study used the ITS2 region of the genome. However, this region is not suitable for studying arbuscular mycorrhizal (AM) fungi. AM are important plant partners in grasslands and rainforests, so not including this group significantly limits our understanding gained

from this study. This gap likely led to an under representation of these fungi in the results.

While the primer pair used in the DNA amplification was not specified, it is likely that the pair used was ITS3 and ITS4. There are more suitable primer pairs for future studies. It may also be beneficial to run the data through a bioinformatics pipeline specifically designed for fungi, as it is currently unclear which pipeline was used.

The study also only focussed on fungi. It would also be more useful to concurrently gather some information about other microorganisms such as bacteria and slime moulds. Getting data on the interactions between different types of microbes in soil will help inform important ecological and conservation questions.

The data from this study should be properly archived with better meta-data; for example, clarification of the specific primers used. This data should be reanalysed alongside future sample collections as a snapshot in time. As global databases improve with time and as a local molecular database is built up, this data can yield better results each time it is reanalysed. Future designs should take this study into consideration.

7.1.2 SPUN – SOCIETY FOR PROTECTING UNDERGROUND NETWORKS

There is an international project focussed on mapping mycorrhizas which is worth considering for collaboration. This project aims to generate global maps of mycorrhizal fungi, lobby for the protection of fungi and drive innovation in climate and biodiversity science. The project has been mapping mycorrhizal fungi on a global database and matching this to data about vegetation on soil, which is then being analysed through machine learning.

This project has released sample collection and analysis protocols that are useful when designing experiments. It is worth working within these protocols where possible, so that local data can be added to their database. (<https://www.spun.earth>)

7.2 SOIL SURVEY DESIGN CONSIDERATIONS

Detailed survey and collection methods will need to be designed based on the ecological question that is being asked. However, surveying and collecting soil samples in the near future will allow change in microbial communities over time to be monitored. These samples could also be used to investigate other organisms in future; for example, metabarcoding of arthropods. Therefore, the survey design needs to collect enough samples and associated information so that it can be used to answer different questions as they arise or when resources are available.

Samples need to be collected in a consistent manner from across the site. As there is already a grid system onsite, this could be used as the basis for a survey design. Taking many samples from across the site and storing them will allow them to be used in future research. However, if pressed for time or resources, it may be necessary to target high priority areas such as the remnant sections.

Minimum numbers of samples or replicates may be needed for future data analysis. It is preferable to take too many samples than too few. Survey points should be at least 10m apart to avoid pseudo-replication. Grid points in smaller high priority areas may need to be closer together than in larger lower priority areas in order to obtain enough samples for data analysis. Technical considerations such as batch size for sequencing should also be taken into account.

Fungal community composition is likely to vary with factors at the microsite level such as:

-  basic topographical position (e.g., distance from creek);
-  soil characteristics including depth and moisture;
-  nearby plants, their identity, relative size and age, relative available light;
-  presence of organic matter like logs, litter, etc.;
-  animal features like brush turkey nests, high density of worm or possibly spider holes;
-  nearby buildings, paths, potential edge effects, etc.; and
-  other factors that may have a strong influence on soil like recent gap creation or period of drought.

Many of these features may be at the scale of subsamples. For this reason, rich microsite data should be recorded for each subsample. This can be analysed for the whole quadrat as frequency data. This microsite data should include information such as photos, the presence of different tree species and their ages, ground cover, litter type and depth, canopy cover, etc. Keeping good records of this rich data will also allow future studies to be undertaken on subsamples.

It is recommended that local plant experts are included on the team so that this data can be collected. In the longer term, rich data would be sent into a field data collection device. Any sensitive areas like the swamp may need to be done by smaller teams that are fully skilled.

7.3 SOIL SAMPLE COLLECTION & STORAGE

Sample collection can be carried out by volunteers once they are trained in techniques, but will need to be supervised by professional scientists and staff onsite.

A quadrat should be created at each surveying point, possibly offset so that trampling or other surveys’ finding pegs do not impact them. We recommend using 30 m quadrats as this gives adequate room to ensure that you are not sampling the same fungi in each sample. Each quadrat is then divided into a grid of 9 points and subsamples of soil collected at each. This is the same as in the SPUN sampling protocol and is shown in the image below. This grid is also useful to record environmental data utilising 1x1 m sub-quadrates.

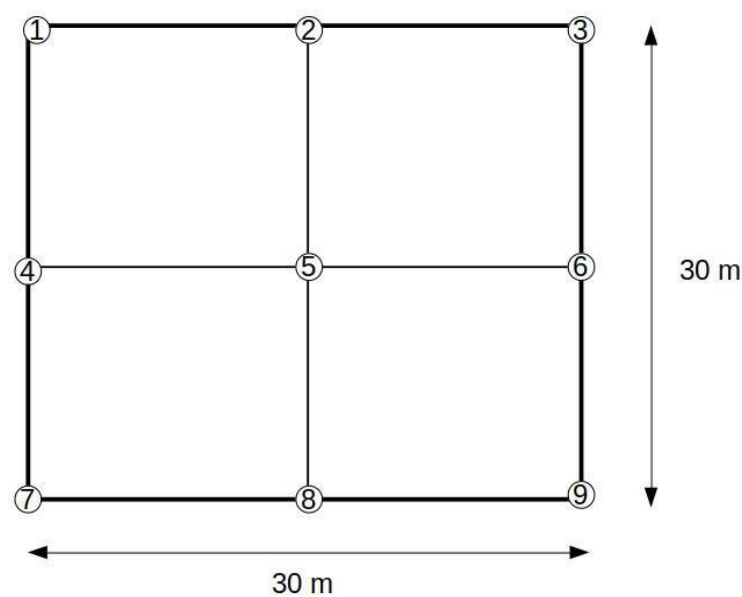


Fig. 7: SPUN sampling protocol showing nine subsampling locations for each 30-metre quadrat

These samples should be collected and stored in separate bags. While the SPUN protocol calls for mixing the 9 samples together right away, we recommend keeping them separate, so that they can be kept with their rich data about the details of the subsample. The samples can be mixed together later on to reduce the amount of sequencing, however keeping them separate allows for more analysis options later.

Environmental factors should be recorded for the microsite of each subsample (e.g., nearby large debris, diseased trees, distance from walkways, buildings, etc.) There may not be sufficient replication for some factors to be analysed, but by recording this information the door is kept open for as much future analysis as possible.

For shallow Australian soils a hand trowel is generally sufficient for collection, but to sample deeper soils a small auger may be needed. The soil samples are then placed in separate new zip-lock plastic bags and hand mixed in the bag so that DNA is distributed throughout the substrate.

It is important that the samples be collected and stored without cross-contamination. In order to avoid contamination, new nitrile gloves should be used for each new sample. Instruments such as troughs will need to be washed and sterilised with alcohol (preferably methylated spirits) between each sample collection.

Samples need to be placed on ice as soon as possible in order to prevent degradation. If RNA rather than DNA is being investigated, then freezing the samples in the field using dry ice may be necessary.

Samples will need to be kept frozen. A large freezer will be necessary for ongoing storage. While soil samples are small (around a tablespoon in size), there needs to be space to store large numbers of samples.

Soil samples will also need to be sent to a laboratory for further processing via cold shipping.

7.3.1 DNA EXTRACTION, AMPLIFICATION & SEQUENCING

Soil samples will need to be sent to a laboratory for extraction, amplification and sequencing. Samples from each survey point are pooled before extraction. Extracted DNA samples can also be saved frozen for future use. DNA extraction from soil is standardised and there are soil extraction kits available or a lab may have its own process. Choice of sequencing technology such as Illumina or Nanopore will determine which laboratory the samples need to be sent to.

The DNA amplification step can target different groups of fungi, so it is important to determine which groups are of interest before samples are processed. We recommend targeting arbuscular mycorrhizas (AM), ectomycorrhizas (ECM), saprotrophic fungi and other biotic groups such as endophytes and entomopathogens at this stage of the project. This will allow the samples to be benchmarked for different types of fungi. If it is found that one type is dominant or of more interest than the others, then future studies may consider using methods that target these groups specifically.

Isolating and amplifying the region of DNA that is of interest is done with primers. The primer pairs most frequently used for fungi with Illumina sequencing are ITS1 and ITS2 or

ITS3 and ITS4. However, these will not pick up all types of fungi, in particular they are not good for AM fungi. As this reserve is likely to have a high variety of both AM and ECM fungi, it will be necessary to use multiple primer sets. WANDA and AML2 are suitable primers for AM fungi and are being used in the SPUN sequencing methodology (Society for the protection of underground networks 2020) so results can also tie in with their project. It is recommended that at least two sets of primers are used so that all major groups of fungi are covered. However, this may shift as technology changes and new sets of primers are designed.

7.3.2 BIOINFORMATICS & DATA ANALYSIS

A bioinformatics pipeline will need to be chosen in consultation with a mycologist and/or bioinformatician. This pipeline will need to be linked to the local molecular library as well as global databases. Global databases that are usually used include UNITE and Genbank.

Once a species list for each sample is obtained then further statistical analysis can be done by a researcher. This should show how much species diversity there is in different sites, the types of species that are common and their ecological roles (e.g., decomposers vs mycorrhizas), and how fungal communities change over time. FunGuild should be used so that functional groups as well as species can be analysed.

The results of this analysis will also show whether the rainforest is dominated by AM fungi, or if there is a mix of AM and ECM fungi. This will also affect future methodology, including choice of primers. It will also be valuable to compare the different ecosystems on the site and see how fungal communities differ across them. In particular, how fungal communities differ between remnant and revegetated sites and how these change as revegetation proceeds. Long term this data will also provide information about the effects of disturbances and climate change. This information will also inform management of fungi on site.

A bioinformatician should be consulted when setting up the molecular database in order that it can easily be linked to bioinformatic pipelines for metabarcoding. They will also need to develop or adapt a pipeline to link raw sequencing data from metabarcoding to the local molecular and global databases. If a newer sequencing platform such as Nanopore is chosen, there may also need to be some further work adapting the pipeline and investigating the effects of issues such as read length and error rates on the output.

7.4 POTENTIAL SOIL PROJECTS

There are many potential soil projects. Some of these can be started now, but samples may be kept for a number of years until changes over time can be sampled. Thus, it is recommended to take samples soon before the worst of the predicted El Niño season comes into effect.

The first priority would be to properly benchmark remnant types so that the general pattern of soil fungi, other key microbial groups and basic physical and chemical information are established. Planning these projects should involve specialist mycological, design and bioinformatic consultation.

Priorities for soils are:

-  Benchmarking regional ecosystems for soil fungi and other key microbial groups via:
 -  Collected samples from remnant sites, and
 -  Collected samples from restoration sites
-  Resampling benchmark locations in 3-5 years to:
 -  Gauge trajectories and health of restoration areas,
 -  Determine if predicted El Niño has negative impacts, and
 -  Adapt management based on data and analyses as needed.

Future projects that are informed by benchmarking analyses could include:

-  Assessment of soil fungal diversity between remnant and restoration sites for each regional ecosystem where appropriate,
-  Assessment of habitat partitioning of soil within a site (what fungi live where and with what),
-  Longitudinal studies looking at biodiversity at the same given points over time, and
-  Combined studies that consider soil and important decomposition elements.

8. UNDERSTANDING DECOMPOSITION

Pink Oyster.

Pleurotus djamor.



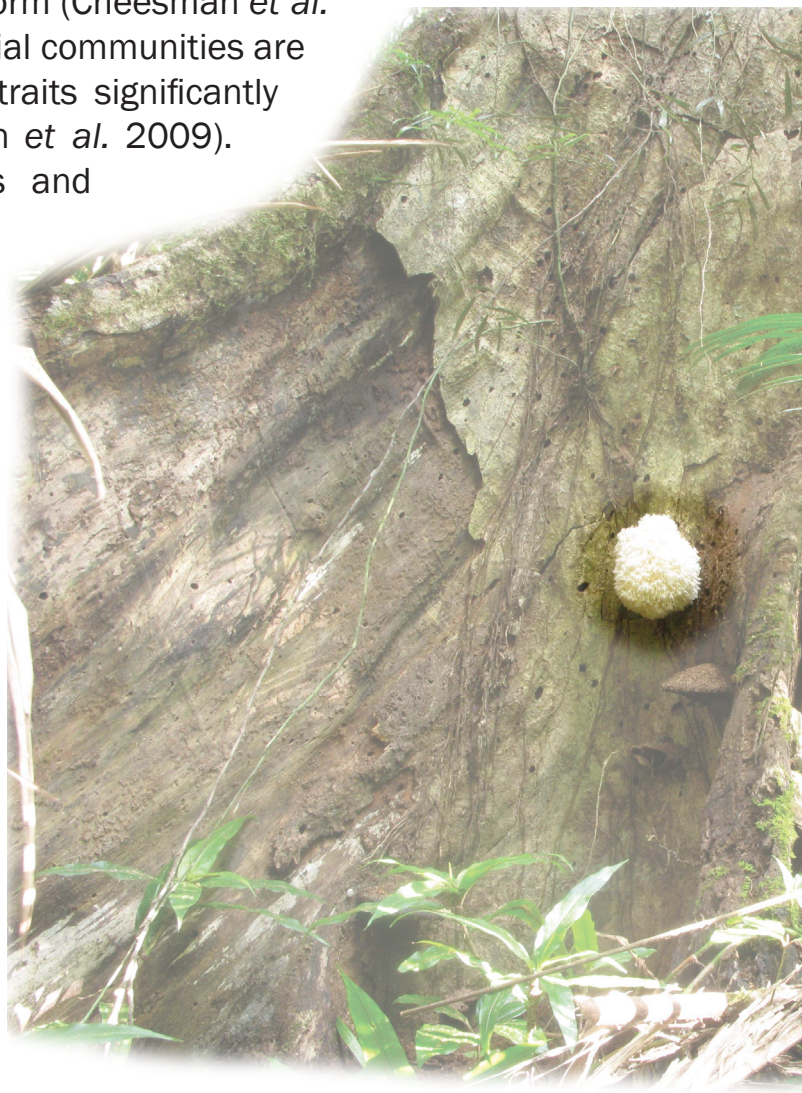
DECOMPOSITION

in the moist subtropics at MCSR is likely faster than in cooler and/or drier locations. The weather extremes that are predicted with climatic warming increase the urgency of understanding decomposition in these systems. Decomposition is a critical ecological process for soil fertility, nutrient cycling and food chains.

The lack of data on Australian habitats, their dominant trees and decomposition rates (Mackensen et al. 2003) has not significantly improved since this article. Some data suggests that in tropical Australian rainforests and savannahs termites have less impact than the global norm (Cheesman et al. 2018; Law et al. 2023). In Australia, microbial communities are more important and plant species' wood traits significantly contribute to decomposition rates (Weedon et al. 2009). Unexpectedly, it seems that wood traits and environmental factors have a greater effect on decay rates than endophytic fungi (Lee et al. 2022) that have a competitive advantage.

However, fungi are an important part of the decay community and with new molecular methods and functional trait-based analyses (Pietsch et al. 2014; Zanne, Abarenkov, et al. 2020; Zanne, Powell, et al. 2020) it is now possible to get a better understanding of how fungi contribute to decomposition cycles in Australia.

Fig. 8: Cascading Icicles (Hericium novae-zealandiae) is a weak tree pathogen that was observed reproducing on the dead buttress root of a Yellow Carabeen (Sloanea woollsii).



8.1 COLLECTION OF RICH DATA FOR DECOMPOSITION

The high diversity of plants in rainforests presents a research challenge. Part of the strength and resilience of rainforest systems is the high diversity of plant life and associated availability of food year round. For fungi, the high diversity of plant materials for decomposition are important. Different suites of decomposer fungi favour different substrates like logs, litter etc. Rich data includes the finer details of substrates like the original host plant's age, size and relative decomposition state.

Collecting rich data will enable future analysis and understanding of the links between plants and fungi. We suggest monitoring plant species, particularly trees, nearby tracks, plant death, timber fall, etc.

We know that the following likely have accelerated decomposition rates:

- 🍄 Litter of varying types including leaves or bark or commonly shed parts of plants;
- 🍄 Fine woody debris including twigs, woody materials less than 10 cm in diameter;
- 🍄 Coarse woody debris larger than 10 cm in diameter including stags, stumps and logs; and
- 🍄 Animal nests, such as that of the Australian Brush Turkey.

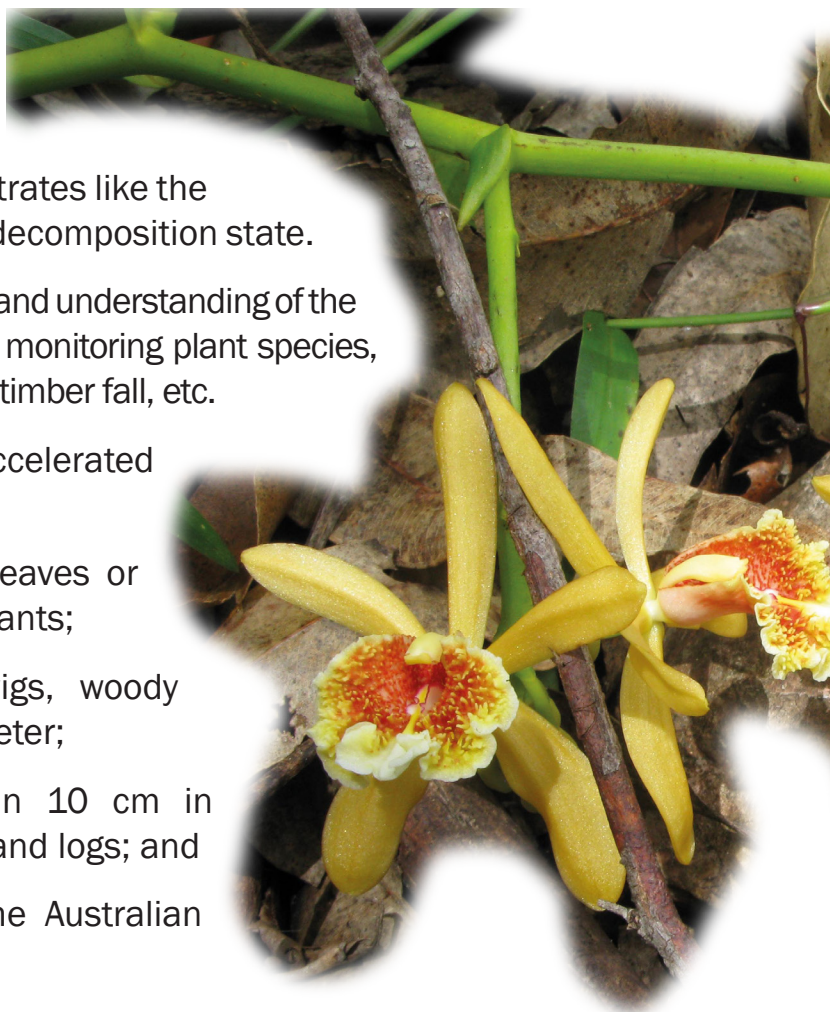


Fig. 9: Giant Climbing Orchid (Pseudovanilla foliata) is a mycoheterotrophic orchid that depends on wood decay fungi.

Additional rich data may include dominant species in the area, or substrate decay class. This is particularly important for coarse woody debris. This will help align future research with management priorities. For example, if a management plan addresses leaf litter, we can analyse leaf litter samples to inform the plan and avoid unintended consequences.

Although understudied, the role of canopy decomposition could be important to consider.

In some rainforests, the fungal communities that make canopy soil communities (Rousk and Nadkarni 2009; Orlovich *et al.* 2013; Looby *et al.* 2020) are sometimes closely similar to local soil communities. It is possible that canopy lichens, cryptogams and epiphytes may represent interesting niches that are not common in other vegetation types. Decomposition processes in the canopy are critical for the ongoing formation of tree hollows that contribute to the fitness of many hollow dependent animals like birds.



8.1.1 LESSONS FROM OSWALD LOG PROJECT

In 2014, MCSR staff and volunteers designed a small project with professional mycologists. This project observed four logs felled by Tropical Cyclone Oswald in 2013. These logs were left near the path so volunteers could easily monitor them and reduce disturbance.

The four logs were measured and photographed at project start, and some were tracked until 2016 to monitor the decay process. The logs were: White Booyong (*Argyrodendron trifoliolatum*, site/log 1), Sour Cherry (*Syzygium corynanthum*, site/log 2), Black Bean (*Castanospermum australe*, site/log 3), and Black Apple (*Planchonella australis*, site/log 4).

Volunteers recorded fungi including *Auricularia delicata* complex, Trooping crumble cap (*Coprinellus disseminatus*), Luminous Porecap (*Filoboletus manipularis*), Australian Shiitake (*Lentinula lateritia*), *Morganella purpurascens*, *Mycena yirukensis*, *Microporus affinis* and False Turkey-tail (*Stereum ostrea*). There were many fungi reproductive structures that were not easily recognised including polypores, corticoids and xylariales.

Unfortunately, the results of this volunteer project were not fully realised, although Dr McMullan-Fisher has some of the data in question. The Black Apple log still survives at the time of this report, but the other three have decomposed completely.

Subject matter experts could likely design a way to update existing recording sheets to be utilised in future research.

Since this project, new technologies resolve many of the difficulties encountered.

8.2 POTENTIAL DECOMPOSITION PROJECTS

Community engagement can likely support data and specimen collection in our proposed projects. Some of these projects can be designed to accommodate staff and volunteer capacity, adding opportunistic collection to existing routines.

Where capacity and funding allows, these decomposition projects should explicitly include slime moulds. Although they are not true fungi, slime moulds are important as they feed on bacteria and fungi.

Similarly studies will help researchers understand the interactions between nematodes and nematode trapping fungi in the context of nitrogen nutrient cycles.

8.2.1 OPPORTUNISTIC MONITORING DIFFERENT SUBSTRATES

Mycological expert support will help volunteers and staff collect good data during the course of normal activities. They could start to monitor stags, stumps, logs or other debris as these become available and there is capacity. This rich data set will help discover the different fungi that contribute to decomposition and what plant types and decay stages they favour. The collections made during such opportunistic monitoring would feed into the expansion of the molecular reference library.



Fig. 10: Mushrooms Armillaria fumosa log with Giant Climbing Orchid in the background

Linking the important areas of soil and decomposition is crucial. Research may show, for example, that fungal community patterns vary depending on the organic matter going into the soil.

8.2.1.1 WOODY DEBRIS MONITORING

Woody debris monitoring will likely provide greater knowledge on decomposition and a source of fungal collections to barcode, but also on other aspects of the ecology of the site.

This should include opportunistic monitoring of and fungi collection from woody debris substrates by volunteers with mycological expert support. This will help build the list of fungi found on the site and contribute to the local molecular reference barcode library.

Specifically, we recommend monitoring of the different fungi such as *Armillaria* found on the woody debris in the immediate vicinity of mycoheterotrophic plants like the Giant Climbing Orchid (*Pseudovanilla foliate*).

Some woody debris focus studies could also monitor the impact of Weedy *Favolaschia*.

8.2.1.2 OTHER SUBSTRATE MONITORING

The canopies of rainforests are rich in bryophytes, epiphytes, and lichens. When branches or other debris fall, this creates opportunities for investigating canopy diversity. Leaves and the bark of some trees are regularly shed and contribute diversity of litter that covers the ground.

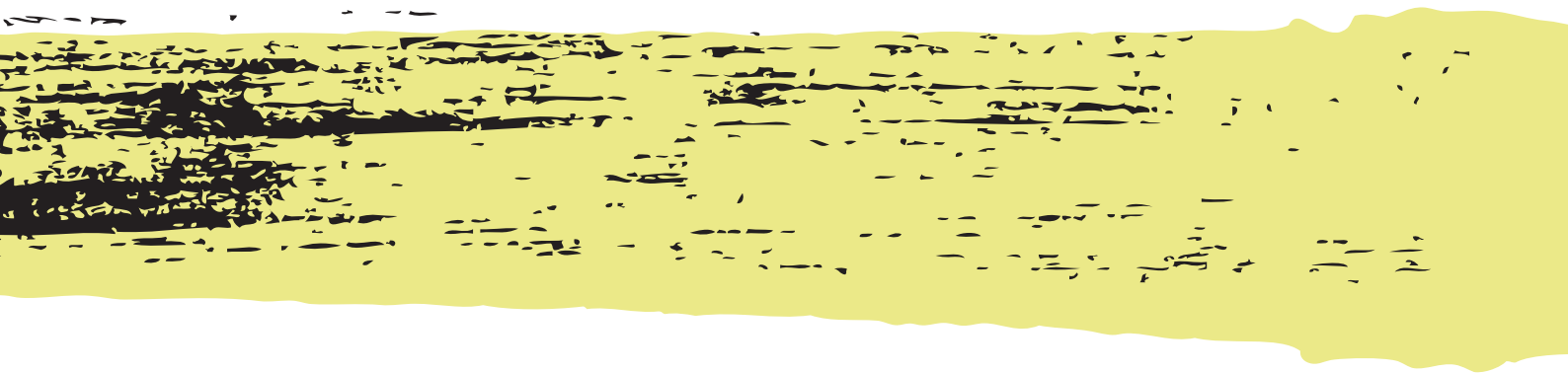
-  Canopy decomposition (e.g. Dr Frances Guard's litter trap experiments)
-  Canopy biodiversity including lichens
-  Litter decomposition:
 -  Associated with brush turkey mounds
 -  Associated with giant earthworms (family Megascolecidae)

9. INTEGRATION WITH PLANTS

Flower Pot Parasol.

Leucocoprinus birnbaumii.





INTEGRATING

Integrating fungal knowledge with existing plant knowledge will enhance our understanding of both groups and the site ecology. Different plants will have different suites of fungi that are associated with them. There are many fungi that live in and on living plants. Some of these fungi are beneficial, such as arbuscular mycorrhizas and ectomycorrhizas, but there are also parasitic fungi like rusts and smuts.

Other fungi such as polypores may be both parasitic and saprotrophic. There are also some endophytes, like the colourful disc fungus Burgundy Cups (*Phillipsia subpurpurea*), that are hidden in living plants but become visible on decomposing wood.

Saprotrophic fungi are diverse as are their preferences for different wood types and life stages. We suggest gathering this rich data when carrying out surveys for soil or substrate classes like logs. Similarly, if the preliminary benchmarking of soil fungi includes the dominant plants for samples, the preliminary analysis will give insights with close plant associations. We expect that mycorrhizas, endophytes and some parasites will have close associations with plants.

The high species richness of the MCSR and adjoining Ecological Park means that plant coverage will likely be limited due to costs, although we do recommend recording associated plant species whenever possible during collection. This rich data can be captured by recording details like whether the plant is alive, if the soil is sampled nearby, or if parts fall off and become debris.

One of the key questions to answer when it comes to plant restoration is whether nursery-grown plants have similar fungal relationships, particularly mycorrhizas and endophytes, as the wild seedlings and younger plants. If there are less or no fungal relationships with these nursery-raised plants, they likely have reduced fitness. Thus, it is important to gain an understanding of the extent of the loss of partners and how quickly rewilded plants develop these relationships. It is likely that plantings that are close to remnant patches will develop fungal relationships.

Similarly the potential beneficial fungal relationships with conservation-listed plants could improve at-risk plant populations' health and resilience. Sampling priorities for mycorrhizal and endophytic relationships should include: Birdwing Butterfly Vine (*Pararistolochia praevenosa*),

Large-flowered Silkpod (*Parsonsia largiflorens*), Romnaldia (*Romnaldia strobilacea*), Thready-barked Myrtle (*Gossia inophloia*), *Lenwebbia* sp. 'Blackall Range' (formerly *Austromyrtus* sp. Blackall Range), Native Guava (*Rhodomyrtus psidioides*) and Red Lilly Pilly (*Syzygium hodgkinsoniae*). Likely non-mycorrhizal, potentially endophyte samples include Queensland Nut (*Macadamia integrifolia*) and Maroochy Nut (*Macadamia ternifolia*).

The different plants found at MCSR have been supplied as an appendix. This data has been enriched where specific associations with different fungal groups, including mycorrhizas, are known. Recording and retaining the data about specific associated plant species will improve understanding of the specific associations between fungi and plants.

Understanding associations with the different fungi groups will help to understand the interactions between species at the site. The priority of different groups may increase if there is another dependent species that is under threat. For example, Australian Bush Rat populations are likely to be partially dependent on eating truffle-like fungi. These truffle-like fungi are likely to include both ectomycorrhizal fungi and arbuscular mycorrhizas. There is little Australian data on the dominant areas of these fungi to base assumptions on. Preliminary soil analyses will hopefully guide focused research on these questions.

9.1 PLANTS THAT FORM MYCORRHIZAS

Mycorrhizal fungi are important plant partners (Brundrett 2004, 2017a), that help with access to nutrients and water. Plants that have a diversity of partners are more resilient. Rainforest plants are thought to predominantly rely on arbuscular mycorrhizas, but many Australian plants are co-dominant with other mycorrhizal types including ectomycorrhizal mycorrhizas (Brundrett 2017b). Thus, we need data from the site to understand the details of these relationships.

Similarly most herbs, grasses, ferns and palms have roots colonised by arbuscular mycorrhizal fungi (Brundrett 2002; Dreyer et al. 2010; Lehnert and Kessler 2016). In the case of palms, arbuscular mycorrhizal plants have better salt and drought tolerance than those without (Meddich et al. 2015; Ait-El-Mokhtar et al. 2019).

Some of the important families at the site that are likely to have significant mycorrhizal relationships are Myrtaceae, Fabaceae (legumes) and Orchidaceae.

For example, Myrtaceae trees and shrubs are thought to be predominantly ectomycorrhizal. This is likely a priority research group as many are truffle-like fungi that feed fungivorous ground dwelling marsupials like the declining population of Australian Bush Rats. Many Australian plants often have co-dominant mycorrhizal groups. Assumptions that mostly

ectomycorrhizal plants include Flooded Gum (*Eucalyptus grandis*), Swamp Mahogany (*E. robusta*), Lemon-scented Tea Tree (*Leptospermum petersonii*), Brush Box (*Lophostemon confertus*) and White Bottlebrush (*Melaleuca salicina*). In the McMullan-Fisher 2010 report, one ectomycorrhizal reproductive structure of *Laccaria lateritia* was observed in Regional Ecosystem 12.3.4, which is where ectomycorrhizal tree species *Eucalyptus robusta*, *E. grandis* and *Melaleuca salicina* occur. It is likely most ectomycorrhizal fungi will be found in the vicinity of these tree hosts.

Most other MCSR myrtles are likely to partner with both ectomycorrhizal and arbuscular mycorrhizal fungi, including Red Apple (*Acmena ingens*), Lilly Pilly (*A. smithii*), Midyim (*Austromyrtus dulcis*), Grey Myrtle (*Backhousia myrtifolia*), Silky Myrtle (*Decaspermum humile*), Thready-barked Myrtle (*Gossia inophloia*), Sunshine Coast Myrtle (*Lenwebbia* sp. Blackall Range), Plum Myrtle (*Pilidiostigma glabrum*), Small-leaved Plum Myrtle (*P. rhytispermum*), Silver Myrtle (*Rhodamnia argentea*), Native Guava (*Rhodomyrtus psidioides*), Scrub Cherry (*Syzygium australe*), Sour Cherry (*S. corynanthum*), Purple Cherry (*S. crebrinerve*), Giant Water Gum (*S. francisii*) and Red Lilly Pilly (*S. hodgkinsoniae*). Many of these softer-leaved myrtles are sensitive to Myrtle Rust, so management needs to understand what fungi these are reliant upon. It is possible some of these sensitive plants' health and reproductive output could be improved by increasing the diversity of their partner fungi.

Another important family of plants that contribute to the nitrogen budget of the ecosystem are plants in the Fabaceae (legume) family including peas and wattles. At the site these include Blackwood (*Acacia melanoxylon*), Pink Lace Flower (*Archidendron grandiflorum*), Blood Vine (*Austrosteenisia blackii*), Giant Blood Vine (*Austrosteenisia glabristyla*), Large Prickle Vine (*Caesalpinia scortechinii*), Native Wisteria (*Callerya megasperma*), Black Bean (*Castanospermum australe*), Derris Vine (*Derris involute*), Twining Glycine (*Glycine clandestina*) and Snow Wood (*Pararchidendron pruinosum*).

These plants typically have symbiotic partners on their roots, such as nitrogen-fixing bacteria (*Rhizobium* spp.), some of which induce formation of root nodules. These plants are also able to partner with mycorrhizal fungi and ectomycorrhizas. These multiple symbioses give the plant additional benefits. Thus, these plants and their partners may be important in nutrient cycles, particularly as nitrogen that can be used by plants is often limited.

Analyses of these relationships would benefit from also gathering data on the bacteria that are commonly associated with these leguminous host plants. At other locations on the Sunshine Coast, species in the Casuarinaceae family partner with nitrogen-fixing bacteria (*Frankia* spp.) and mycorrhizas.

Orchids (family Orchidaceae) are highly evolved plants that often have complex dependencies on fungal partners and pollinators. They are sensitive to changes and are often on conservation lists.

Orchids, particularly their seeds, are mycoheterotrophic, meaning they are dependent on nutrition from fungi. Some develop green tissues so are able to create some of their own nutrients through photosynthesis. However, most orchids stay partially dependent on their supporting fungi for their whole life and achlorophyllous orchids that have no chlorophyll so are wholly dependent.

The fungi that support orchids include different types of mycorrhizas (Batty *et al.* 2004; Rasmussen and Rasmussen 2009; Dearnaley *et al.* 2012). Many orchids depend on orchid-specific mycorrhizas in the families Serendipitaceae, Ceratobasidiaceae, Tulasnellaceae and Sebacinaceae.

Other orchids may opportunistically live off ectomycorrhizal fungi like Russulas (Bougoure and Dearnaley 2005). So-called mycoheterotrophic orchids live off saprotrophic and parasitic fungi, as can be observed in the Giant Climbing Orchid (*Pseudovanilla foliata*).

MCSR has many epiphytic orchids in the canopies and on tree trunks, including Tiger Orchid (*Dendrobium gracilicaule*), Lily of the Valley Orchid (*D. monophyllum*), Spider Orchid (*D. tetragonum*), Rats Tail Orchid (*Dockrillia bowmanii*), Cascade Orchid (*Oberonia titania*), Hill's Orchid (*Sarcochilus hillii*), Pencil Orchids (*Dockrillia shoenina* and *D. teretifolia*), Orange Blossom Orchid (*Sarcochilus falcatus*), Shepherd's Bulbophyllum (*Bulbophyllum shepherdii*, *B. exiguum*, and *B. schillerianum*), *Cymbidium madidum*, *Cymbidium suave* and *Plectorrhiza tridentata*.

There are two ground orchids: Christmas Orchid (*Calanthe triplicate*) and the previously mentioned Giant Climbing Orchid. Many terrestrial orchids partner with mycorrhizas in Serendipitaceae (Oktalira 2021). The links between soil-dwelling orchids and fungi may also depend on soil and decomposition factors.

There are some other older mycorrhizal lineages that might be present at the site like liverworts (Rimington *et al.* 2018). Mosses may be biotrophic but are not typically mycorrhizal. Mosses and other bryophytes are important for maintaining moist microclimates as they hold moisture. As such these are critical plants to help with resilience to drying climates.

Non-mycorrhizal plants include Azollaceae, Proteaceae and Urticaceae. Other plants may be non-mycorrhizal or periodically or facultatively mycorrhizal, including some reeds, rushes, sedges, cycads and restiads.

9.2 PLANT FUNGAL ENDOPHYTES

Most naturally occurring plants contain fungal endophytes (Saikkonen *et al.* 1998; Rodriguez *et al.* 2009). Early researchers often assumed these would at some point become problematic, so these have previously been called ‘latent pathogens’. Some of this early research considered there were two groups of plant endophytes, those that were associated with grasses, so-called Clavicipitaceous endophytic fungi, and a mixed bag of unrelated fungi called Non-Clavicipitaceous endophytic fungi. As there are few grasses in the MCSR area these grass endophytes are not likely to be common; however, in the grassy areas of the adjoining Ecological Park, they may be present.

Fungal endophytes are now typically grouped into four classes (Rodriguez *et al.* 2009), with the original Clavicipitaceous endophytic fungi associated with grasses and now three classes of Non-Clavicipitaceous endophytic fungi. Non-Clavicipitaceous plant endophytes often live complicated lives and some may facultatively live as saprotrophs when their plant host dies. Many are shown to establish mutualisms with plants, conferring fitness benefits such as biotic and abiotic stress tolerance, nutrient acquisition and increased growth and yields.

The unusual life stages of some of these fungi means that they are increasingly considered for bioprospecting as they are rich in novel compounds (Suryanarayanan *et al.* 2009; Aly *et al.* 2011; Bhardwaj and Agrawal 2014). There is some work utilising endophytic microorganisms (including bacteria and fungi) in plant bioremediation and carbon sequestration (Deng and Cao 2017). These two areas are not considered further in this report.

The high plant richness and remnant status of the site means this is likely to be a highly diverse group of fungi. Depending on the circumstances and life histories of the fungi they are likely to significantly contribute to plant health. Thus, we suggest that a pilot study be scoped with technical mycological support. Possibly a pilot that included one representative of each priority plant group, as budget allows. A better understanding of the roles of these drought-tolerance boosting fungi is particularly important to maintain plant resilience in the face of warming climate.

9.2.1 ENTOMOPATHOGENS AS PLANT ENDOPHYTES

Entomopathogenic fungi, sometimes called zombie fungi, are found living on many arthropods including insects, spiders (Vega 2008). Rainforests are often rich in entomopathogenic fungi and the Australian Wet Tropics is a known hot spot. Subtropical rainforests are another place to look for these fungi. There are a few records from

Cordycipitaceae on the Sunshine Coast, including from Maleny.

It has recently been discovered that a number of entomopathogenic fungi also live part of their life cycle as beneficial plant endophytes. For example, *Ophiocordyceps sinensis* is an entomopathogenic fungus that parasitises and kills moth larvae. In a recent study carried out in China, *O. sinensis* was found living as an endophyte in the leaves and roots of the majority of local plants tested (Wang et al., 2020).



*Fig. 11: Zombie
or entomopathogenic fungus
infecting a cicada (M Honey)*

The study strongly suggests that caterpillars are infected with the fungus when they eat the infected plant roots. Recent studies have found that a number of other entomopathogenic fungi also live part of their life cycles as plant endophytes (González-Mas et al, 2021; Qiao et al., 2023). This suggests that these fungi are not only beneficial to their plant hosts as endophytes that can protect plants and increase growth, but also in controlling herbivorous insect populations.

However, no studies have been carried out on similar entomopathogenic fungi in Australia. As there are a number of entomopathogenic fungi found on the site, metabarcoding and/

or species-specific primers could be used to find out if any of these fungi are also growing within the roots or leaves of plants.

9.3 POTENTIAL PROJECTS INTEGRATED WITH PLANTS

The site is an important rainforest remnant with a high diversity of plants. This makes MCSR an ideal site to become a benchmark site for revealing the diversity of fungi that have close associations with plants. There are several data priorities, including plants with conservation implications.

There are only ten conservation listed plants at the site. All of these are priorities for gaining a better understanding of fungi dependence. We already know that some are threatened by Myrtle Rust, but there are also other fungi that may threaten plants at the site. It would be good to improve regular plant monitoring to explicitly consider plants that are likely to be affected by problem fungi.

Priority plants to survey for fungal mycorrhiza and endophytes:

-  Conservation listed plants;
-  Plants that are likely to be sensitive to problem fungi;
-  Myrtaceae trees and shrubs, leguminous plants, orchids and bryophytes;
-  Piccabeen Palms (*Archontophoenix cunninghamiana*); and
-  Nursery seedlings and recent plantings.

10. INTEGRATION WITH ANIMALS

Golden-scruffy Collybia.

Cyptotrampa asprata.





ANIMALS

that eat fungi, called fungivorous (Potapov *et al.* 2022) or mycophagous, disperse fungal spores in their scats.

Data on fungivorous animals at MCSR comes from the animal species list provided by SCC, as well as in reports and existing literature. This includes interactions of rare or threatened animals where data is available. Many animals are important in interactions with ecological processes like the food webs that drive many decomposition and soil processes.

Fauna surveys at MCSR:

- 🍄 Burnett, S., Bright, T., 2009. A Fauna Inventory of the Mary Cairncross Scenic Reserve, Maleny (Report to the Mary Cairncross Reserve Steering Committee).
- 🍄 Burnett, S., Nugent, D., Hemmings, M., Kernot, R., Bright, T., 2014. A baseline fauna monitoring study of Mary Cairncross Scenic Reserve, Maleny. University of the Sunshine Coast, Sippy Downs.
- 🍄 Burnett, S., Hemmings, M., Foster, N., Kernot, R., 2016. A baseline fauna monitoring study of Mary Cairncross Scenic Reserve, Maleny - Winter 2015 sampling. University of the Sunshine Coast, Sippy Downs.
- 🍄 Burnett, S., Kernot, R., 2022. Mary Cairncross Scenic Reserve fauna monitoring - Winter 2021.
- 🍄 Burnett, S., Zwar, A., Kernot, R., 2022. Mary Cairncross Scenic Reserve Fauna monitoring - Summer 2019/2020 (Unpublished report to Sunshine Coast Council).
- 🍄 Burwell, C., Rix, M., Lambkin, C., Ebert, K., 2023. Mary Cairncross Invertebrate Survey Report (Surveys Oct 2021). Queensland Museum.

Surveys for different groups of animals require different techniques as seen across these reports above. Vertebrates (amphibians, birds, fish, mammals & reptiles) are probably the best understood group of organisms in the MCSR. Since the two baseline vertebrate surveys in 2014-2015, efforts have been made to regularly survey groups so that data is comparable across two main local seasons - summer

(usually wet season) and winter (often dry season). To date, a single baseline survey was undertaken in 2021 for some groups of invertebrates, mainly focussed on some insects and spiders. The survey suggested several potential species for monitoring, as well as a single site in the revegetation area on the northern boundary of the proposed Ecological Park.

Although data is collected seasonally, we recommend that each animal's population health periodically be considered in its entirety to prioritise conservation urgency. Prioritisation will help inform adaptive management as circumstances change.

10.1 FUNGI & VERTEBRATES

Despite the higher number of vertebrate surveys, these only found about half of the animals known to be at the reserve due to rarity, seasonality and some taxa being of low detectability (Burnett and Kernot 2022). To discover trends, at least two data sets are required, ideally with similar data collection techniques to help with comparison. Opportunistic data collection continues to be important across biological groups and tools like iNaturalist can help collect and centralise images and audio files for verification.

Various survey methods captured different groups of vertebrates including camera trapping, acoustic monitoring, baiting and spotlight surveys. Vertebrate surveys focused on birds, insectivorous bats, small mammals, medium-sized nocturnal mammals, reptiles and incidentally recorded amphibians. Some focus was placed on feral animals, including from opportunistic data, for species like the Cane Toad, and more specific surveys from specific baited scavenger community surveys. Keeping feral species low is important for maintaining indigenous animal populations.

None of the vertebrate small mammal surveys explicitly considered fungi, although we note that there has unfortunately been a dramatic decline from 2015 to 2021 of two small mammal species populations: the Buff-footed antechinus (*Antechinus mysticus*) and Australian Bush Rat (*Rattus fuscipes*). The Buff-footed Antechinus is carnivorous and insectivorous, while the Australian Bush Rat is omnivorous and generally fungivorous. Understanding these animals' food sources will help the reasons for declining populations as well as conservation opportunities.

Fungi are an important food source for various animals and the amounts of fungi that they eat varies across species and season. The declining population of the Australian Bush Rat is concerning as this is a specialist fungivorous mammal at MCSR, along with other fungivorous specialists Long-nosed Bandicoot (*Perameles nasuta*) and Northern

There are other more opportunistically fungi eaters at MCSR. These fungivorous generalists include: Swamp Wallaby (*Wallabia bicolor*), Red-necked Pademelon (*Thylogale thetis*), Red-legged Pademelon (*Thylogale stigmatica*), Black-striped Wallaby (*Notamacropus dorsalis*), Red-necked Wallaby (*Notamacropus rufogriseus*), Mountain Brushtail Possum (*Trichosurus cunninghami*), Short-beaked Echidna (*Tachyglossus aculeatus*), Australian Brush Turkey (*Alectura lathami*) and Murray's Skink (*Karria murrayi*). The Land Mullet (*Bellatorias major*) is now locally extinct at MCSR. Blue-tongued Skinks (*Tiliqua*) have not been recorded from the site. It is worth noting that females who eat more fungi seem to come into breeding condition faster (Tony Bright personal communication). Such fungivorous generalists are also likely to be important for fungal dispersal, particularly those that travel greater distances during fungal reproductive periods. Theoretically, animals that eat fungivorous animals (e.g. birds and bats), may also disperse fungi through their faeces.

Land Mullet is one of the largest members of the skink family (Scincidae). These are listed as potential rewilding target fauna species in the Sunshine Coast Council's 2023 Back to Nature plan. Successful reintroductions will need to consider the diet of these animals and protect against the inadvertent movement of problem organisms, including fungi.

Brush turkey are connected to fungi in two ways. They are likely opportunistically fungivorous (Elliott and Vernes 2019; Gomez *et al.* 2022) and their nesting activities move significant amounts of litter including leaves, twigs and fine woody debris. Concerns were raised in a 2009 fauna report about excessive brush turkey population sizes due to overfeeding from the picnic ground (Burnett and Bright 2009). However, this issue was not directly addressed until 2014. Although occupancy of brush turkeys was calculated to be 82% of the reserve (Burnett *et al.* 2014) and appeared stable at this figure in winter 2015 (Burnett *et al.* 2016). Neither specific diet studies nor other research has been carried out.

For fungi, the activities of brush turkey may be significant as vectors for distributing fungal spores. Additionally, nesting activities may favour some decomposition fungi over others. Thus, we suggest research on these animals or other linked processes. Likely there are connections between decomposition rates, brush turkey and the

activities of worms.

Finally important for conservation management of amphibians are the problem fungi chytrids (Retallick *et al.* 2004; O'Hanlon *et al.* 2018). We stress the importance of good hygiene and biosecurity across all activities, including animal surveys.

10.2 FUNGI & INVERTEBRATES

'Invertebrates' is an umbrella term to describe animals that neither develop nor maintain a vertebral column, or spine. They vary in size (microfauna, mesofauna and macrofauna) and habitats. Invertebrates include arthropods like springtails (Collembola); insects; terrestrial and freshwater molluscs (slugs and snails); and worms, including earthworms and roundworms (nematodes). Different survey techniques favour the capture of different groups.

Invertebrates are a mega-diverse group that are important for many ecosystem functions like decomposition and food chains. Microfauna and mesofauna are food for larger animals like amphibians, birds, fish, lizards and mammals. Research on invertebrates should be integrated with fungi for animals that have close associations with fungi.

There has been one baseline survey that considered invertebrates at MCSR, particularly some insects and spiders (Burwell *et al.* 2023). This study included several of the vegetation, ecotone and management effects on invertebrates. Unfortunately, insufficient variables were replicated across locations, preventing statistical analysis.

This study of invertebrates, similar to the vertebrate surveys, also employed a large number of survey techniques depending on the focus animals in question. This included timed searches for ants during the day (ant collecting), searches for mygalomorph burrows during the day, timed hand searches of spiders and ants at night (night collecting), extraction of invertebrates from leaf litter on the ground (litter extracts), spraying living tree trunks with pyrethroid insecticide (bark sprays), unbaited pitfall traps, baited pitfall traps, Malaise traps, timed sweep netting of low vegetation, and coloured pans.

Some of the insect data linked habitat characteristics to vegetation, and some mentioned decay or were directly linked to litter both on the ground and in aerial traps. Occasionally, specific plants were also mentioned, and large trees were considered based on their trunk girth at breast height. Rich data like this improves understanding of fungi. Unfortunately, none of the animal surveys collected environmental nor substrate data explicitly.

entomopathogens, entomopathogenic fungi or zombie fungi. Many of these fungi are also found in living plant tissues. Oribatid mites are considered important dispersal vectors for parasitic fungi in soil like *Beauveria bassiana* (Renker et al. 2005). So any future studies on soil processes should consider these linkages and include some representatives in surveys.

There are many groups of arthropods that have close associations with fungi, such as beetles and springtails. Some of these are fungivorous and often inhabit the reproductive structures of macrofungi. Focused collecting on such groups should seek to identify fungi. It is possible longer distance dispersal of these fungi occurs with the movement of insectivorous birds. (Torbaty *et al.* 2016; Elliott *et al.* 2019; Caiafa *et al.* 2021).

This report makes some suggestions about potential indicator species with a focus on groups that are likely to increase or decrease with restoration. These include a number of ants, flies and spiders. We specifically recommend focusing on Friendly Flies (*Tapeigaster* spp.) as these are known to lay their eggs in macrofungi, and the males then protect them. So the larvae, at least, are fungivorous specialists.



Fig. 12: Friendly Fly (Tapeigaster) male on top of mushroom (W Boatwright)

10.2.1 INSECTS

Many beetles (Coleoptera), particularly Ambrosia, Bark, Dung and Minute Beetles, are fungivorous and often have adaptations to utilise and disperse associated fungi (Harrington 2005). Most beetles are important parts of ecosystems. For example, dung beetles facilitate nutrient recycling, secondary seed dispersal, parasite control, soil movement and dung decomposition. Some beetles are dispersal agents for fungi, including some problem fungi, thus data on potential dispersal agents would be useful to inform risk management.

Native Dung Beetles and four minute fungus beetles in a 2023 report (Burwell *et al.* 2023) were associated with White Crust Fungus and *Ganoderma* species. In the report one of these records was linked to a living plant host that is erroneously labelled 'from live turmeric tree' but actually was a tamarind (personal communication V Sandoval, Oct 2023).

It would have been possible to get more data out of this sampling of fungi reproductive structures if there had been some volunteer help in sorting the arthropods that hatched. Volunteers can be trained to do basic sorting and support, allowing scientists to spend their time on the much trickier identification.

Insect data from this report (Burwell *et al.* 2023) considered habitat characteristics and linked them to vegetation and decay, but did not provide any estimates of relative abundance of different arthropod data. If indicator species are used to inform monitoring priorities, abundance must be gauged.

Even apparently unrelated insects may spread some fungi. For example, butterflies in the Southern USA were shown to spread beneficial and detrimental fungi. Currently, it is unknown if any moths or butterflies have interactions at MCSR. There is a possibility of interactions with Richmond Birdwing Butterfly, as endophytes and mycorrhiza of host plants or possibly soil fungi.

10.2.2 OTHER INVERTEBRATES

Some mites were also opportunistically sampled from infected insects (Burwell *et al.* 2023). Mites are often moved by insects as recently seen in the uncontrolled spread of varroa mites in honeybee colonies. Antagonistic and mutually beneficial relationships between mites, insects and fungi (Hofstetter and Moser 2014) may be an important area for future research.

Giant earthworms (Megascolecidae) are common at the site. A pilot study would be needed to understand the relative importance of fungi and microbial decomposition. Litter decomposition research in collaboration with zoologists and microbiologists could be carried out to develop understanding of interactions and activities of the giant earthworms.

Similar to springtails and beetles, roundworms (nematodes) are highly diverse and common in natural areas (Stirling 2019). Several genera of fungi found at MCSR (McMullan-Fisher 2010c) may be trapping nematodes *Pleurotus*, *Hohenbuhelia* and possibly others like *Resupinatus* (Liu *et al.* 2009; Jiang *et al.* 2017). These are fungi that decompose woody materials. These fungi are thought to prey on nematodes and give access to nitrogen which is a limited nutrient in woody materials. There are also roundworms that have especially adapted mouthparts to enable them to specialise in eating fungi (Stirling 2019) including reproductive structures, hyphae, spores and smaller yeasts.

Lastly, there are a number of terrestrial and freshwater snails that feed on fungi. At least a third of some snails' diet periodically consists of macrofungi and lichens (Parkyn *et al.* 2015). One of the concerns about changing rainfall patterns is that it may reduce this food source, threatening the slugs and snails. Fraser's Banded Snail (*Sphaerospira fraseri*) and Pine Rivers Bristle Snail (*Squamagenia separanda*), Black-spotted Semi-slug (*Stanisicaron aquila*) and Red Triangle Slug (*Triboniophorus graeffei*) have been recorded at the site (Australian Living Atlas).

10.3 ANIMAL SCAT OPPORTUNITIES FOR DATA & RESTORATION

Future animal surveys should include data collected from fresh scats (animal faecal matter). Fresh scats provide an easy source for both molecular population analysis and identification of the animals' food. For marsupials there have been some studies (Tay *et al.* 2018; Treloar *et al.* 2023) using various molecular techniques. Scat DNA sampling can be more cost-effective for surveying species at low densities with large home ranges, because broad scale live-capture may be resource-intensive and a stressor for animals. Molecular sampling methods, however, are not infallible, and each animal species must be assessed via pilot studies.

Scat quality and freshness is particularly important, so expert mycologist support is required. Only the best scats should be used for molecular studies of both animal population data and dietary studies. Having a molecular reference library available will increase the accuracy of dietary studies.

Some animals may need to be trapped for a short period to procure optimally fresh samples (e.g. land snails).

Other scats may also be a potential source of inoculum for moving beneficial fungi like mycorrhiza (Tay *et al.* 2018) and other organisms for restoration and revegetation. Before any scats can be utilised for restoration work, the source of the scats would need to be screened for potential problem fungi and any other potentially problematic organisms.

Opportunistic scat collections may show how populations and diets potentially vary with disturbances.

Using scats for restoration may be particularly useful for the restoration of diversity in difficult to restore areas and/or sites isolated from common animal vectors. Further research could compare the diversity of fungi at sites with animal access and connectivity versus isolated bushland with little or no animals. The abundance of dispersal animals within MCSR means that this extra work in dispersing fungi and other beneficial organisms is not necessary, but MCSR may provide a source of scats for restoration elsewhere in the Blackall range.

Scat samples may also be a way of testing animals that are ready to be returned to the site (e.g., Land Mullet). Scat testing could help inform reintroduction locations based on availability of key foods.

If there are conservation concerns with fungivorous animals, we recommend including fungi in any preliminary dietary studies. Population genetic health and dietary studies on scats can be simultaneously carried out on the Australian Bush Rat (*Rattus fuscipes*), Long-nosed Bandicoot (*Perameles nasuta*) and Northern Brown Bandicoot (*Isodon macrourus*). Similarly, dietary and conservation genetics should be studied for populations being considered for rewilding, such as Land Mullet (*Bellatorias major*).

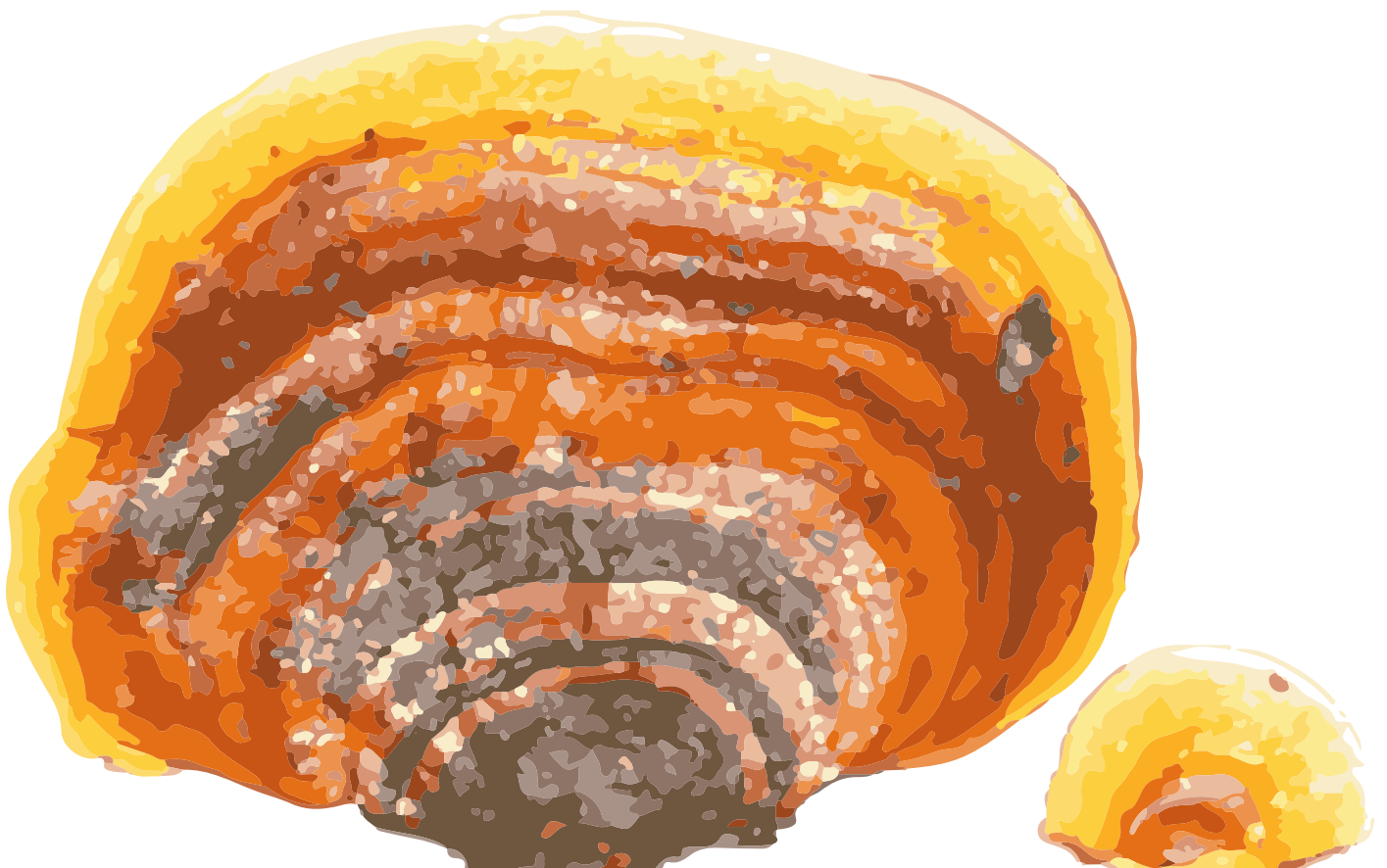
- 🍄 Fungivorous Vertebrates: Australian Bush Rat, Long-nosed Bandicoot and Northern Brown Bandicoot;
- 🍄 Fungi-eating Generalist Vertebrates: wallabies, pademelons, possums, echidnas, brush turkeys and skinks;
- 🍄 Arthropods: springtails (Collembola); mites, particularly Oribatid mites; beetles (Coleoptera), particularly ambrosia, bark, dung and minute; and Friendly Flies (*Tapeigaster* spp.);
- 🍄 Roundworms (nematodes); slugs and snails; and
- 🍄 Any fungi infected animals or ‘zombies’.

- 🍄 Brush turkey egg incubation mounds; and
- 🍄 Areas with low litter layers and high concentrations of giant earthworms.

11. PROJECT OUTCOMES

Golden Curtain Crust.

Stereum ostrea.





WE hope this report will be the first step in engaging the community, volunteers and MCSR as fungal stewards. With the use of molecular tools there are great opportunities for understanding fungi and their critical ecological roles in soil and decomposition. There are many opportunities to increase knowledge of the animals and fungi, plants and fungi, and fungi and other organisms at the site.

This report summarises what is currently known about the fungi at MCSR. These are mainly the macrofungi that were recorded in the 2010 report. The collections that were made to voucher this historic report now represent valuable specimens that can be used to start to build a molecular library.

This report highlights various knowledge gaps at MCSR, including incomplete data and understanding of fungi at the site, particularly missing data from:

-  Fungi that are likely to have threatened populations or be naturally rare;
-  Problematic and weedy fungi including fungi that cause animal and plant diseases;
-  Critical plant partners including mycorrhizas and endophytes, particularly for plants that are facing threats and suffering population declines;
-  Soil and decomposition fungi including yeasts;
-  Canopy dwelling fungi including Lichens particularly Cyanolichens;
-  Fungi that are food for animals;
-  Fungi that live on and in animals including beneficial and antagonistic fungi;
-  Fungi-like slime moulds and Oomycetes like Phytophthora; and
-  Other important biodiversity that contributes to ecological processes particularly nutrient cycling, decomposition and food webs: Bacteria, nematodes, meso- and microfauna, and other microbes like 'protozoa'.

This report outlines potential procedures that will need to be developed if projects from this report go ahead, primarily focussed on gathering rich data. One of the systems that

has already begun is the work/focus/system for making voucher collections to support the molecular barcoding for main macrofungal groups. Longer term this should be expanded to include all functional groups of fungi.

The large number of potential dispersal animals means that the fungi that are already reproducing at MCSR are likely to be well dispersed. The chances of new fungi being moved into the reserve will depend on the connectivity of the different species. The greater the type, intensity and frequency of disturbances at the site, the greater chances are that some species, including fungi, will go extinct at the site. Those species with lower reproductive effort, and those that are already rare and on conservation lists, are the most important for monitoring and potentially active conservation management.

Improving data collection in monitoring so that linkages between interdependent species become part of the focus of research is recommended. The following research opportunities are suggestions that will explicitly link various fungi groups. Opportunistic data collection continues to be important across biological groups and tools like the use of iNaturalist can help collect images and audio files for verification in one place.

As there are a number of animals that are likely to eat fungi, we suggest that pilot research should start with one or two animal species in main groups to develop the local techniques and allow time for building a local molecular reference barcode library.

As much as possible, survey effort budgets should be increased in wetter years and ideally engage priority experts for wetter periods. The upcoming drier El Niño period should be used to get started on a reference library of materials that have already been collected. Soil samples should also be collected prior to this period.

11.1 SOME MANAGEMENT CONSIDERATIONS

There are many different types of fungi that interact with various other species and together contribute to ecological health. We encourage a more holistic approach to biodiversity management. Research questions need to consider different groups of species that are involved in important processes simultaneously. Simply having species lists to manage from are not sufficient to understand the complex biological connections. To facilitate better management, richer biodiversity data needs to be recorded and current systems will need to be improved to capture explicit species and environmental interactions.

Management needs data to inform decisions and monitoring systems should consider at least some examples of the different ecological functional groups. Research and education

that help management understand the ecological processes that drive soil health could be a game changer for improving conservation outcomes.

Getting this data will take some time and investment in people, equipment, and the development of processes. Building a molecular reference library and utilising molecular tools like LAMP tests will enable the mapping of problem and conservation species alike.

Biosecurity, including monitoring and hygiene procedures, are important for managing problem species. There are some fungi that need to be kept out of the site, for example the *Phytophthora* spp. that causes dieback in native plants. Some problem species are already present on the site, including *Pyrrhoderma noxium*. Even though this may be an endemic fungus, preventing the spread of this tree-damaging fungus should be a priority. Getting an accurate identification of the variety that is present will help inform managers of the likely level of risk this presents.

Management plans should include efforts to conserve plants that are sensitive to Myrtle Rust. Some of these threatened populations could be improved by understanding their relationships with fungi.

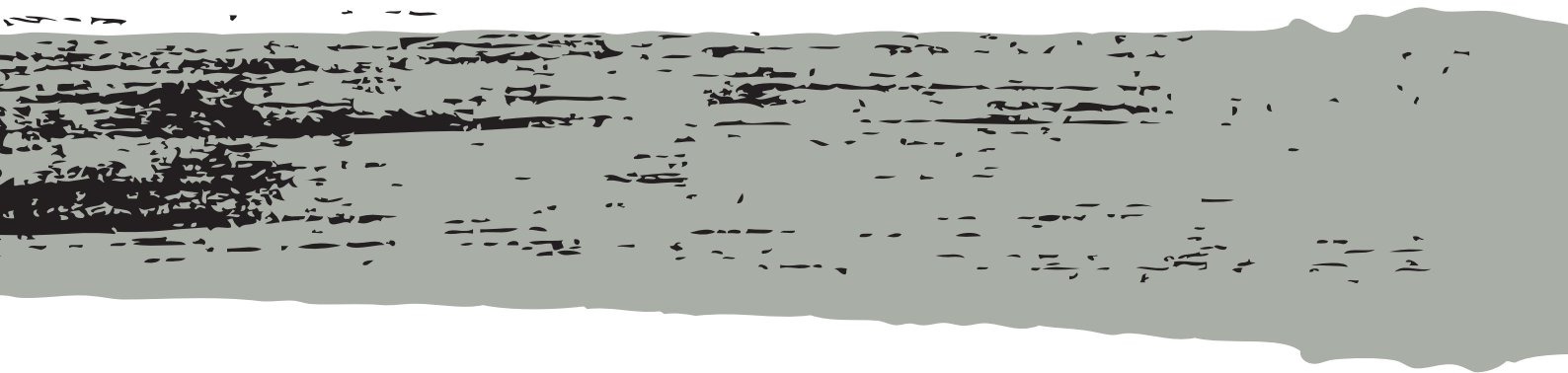
Land managers should avoid actions that simplify the fungal community. Management that is appropriate for areas of high human usage should not be carried over into areas of high conservation value. For example, the need to remove dangerous limbs and trees near buildings and the picnic area may not always apply to conservation areas. Similarly, mulching can overly simplify important habitats. Its use to suppress weeds and increase organic matter is useful in highly managed areas like car parks and around buildings; however mulch is a highly processed and simplified environment for fungi and may present a biosecurity risk. Most importantly, the sources of this mulch, the stags, stumps, logs, leaves, bark and other organic material should as much as possible be left for the local fungi, bacteria, meso- and micro-fauna, and other microbes to decompose in food webs. A diversity of substrate supports a diversity of life.

RESOURCES

Bridal Veil Stinkhorn.

Phallus indusiatus.





LOCAL RESOURCES

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GLOSSARY

iNaturalist

An application for identification and collection of data on living organisms.

ITS

Internal Transcribed Spacer. Molecular region of ribosomal DNA.

LAMP

Loop-mediated isothermal amplification. Molecular technique.

Lichens

Composite organism that arises from algae or cyanobacteria living among filaments of multiple fungi species in a mutualistic relationship.

LSU

Large SubUnit. Molecular region of ribosomal DNA.

Macrofungi

Fungi with reproductive structures larger than 2mm in diameter. Easily seen with the naked eye.

Metabarcoding

Molecular technique that identifies many taxa within the same sample.

Microfungi

Fungi with reproductive structures smaller than 2 mm in diameter. Not easily seen without magnification.

Mushroom

The above-ground reproductive body of many

macrofungi, that are usually fleshy and has gills as the spore-bearing structures.

Mutualistic Endophyte

Fungi that live inside plants and benefit plant health.

Mycoheterotrophy

A form of nutrition in which a plant depends on fungi as its primary or supplementary source of carbon/energy.

Nematode Trapping

Fungi that live in soil and decaying organic matter and trap and consume nematodes (roundworms).

Non-mycorrhizal

Plants that do not typically form mycorrhizas. Many have other adaptations to getting increased nutrition from the environment (e.g., cluster roots).

Orchid Mycorrhizas

Fungi that are usually soil saprotrophs but also partner with orchids. Assist orchid seed germination and growth.

OTU

Operational Taxonomic Unit. Species equivalents for phylogenetic and molecular analyses.

Parasitic

Organisms, including fungi, that live off other living organisms. May harm and/or benefit their hosts.

Group of organisms derived from more than one common evolutionary ancestor.

Morphogroup of fungi that have pored reproductive surfaces. Many also have tough textures (e.g. bracket fungi).

Polyphyletic group of micro-organisms that are important in soils and decomposition.

Organisms that break down organic material and by doing so recycle nutrients. Also called rotters, saprotrophs, decomposers, etc.

Organism that feeds on or derives nourishment from decaying organic matter.

Animal faeces.

South-east Queensland

In the kingdom Amoebozoa, these feed on bacteria, fungi and other protozoa and are important in food webs. Historically considered fungi.

Small SubUnit. Molecular region of ribosomal DNA.

Standing dead wood or trees.

Remaining woody base of trees and shrubs after trunk has been removed.

Plural taxa. Classifying group such as species, family or class.

Translation Elongation Factor. Molecular region of ribosomal DNA.

In the polyphyletic kingdom Chromista. These are often pathogenic, including the problematic group of water moulds in the genus *Phytophthora*.

Mushrooms in the family Hygrophoraceae.
Includes *Cuphophyllus* and *Hygrocybe*

Fungi that are able to decompose lignan as well as cellulose and hemicellulose. These give tissue a pale colour.

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APPENDIX I: MCSR FUNGI

Eyelash Fungus.

Scutellina aff. *subhirtella*.



Family	IUCN	Taxa	Common Name	Function	Seen
Ascomycete	—	<i>Entonaema</i> sp. 'yellow'	—	R	2010
Club	—	<i>Xylaria</i> aff. <i>apiculata</i>	—	R ?B	2010
Club	—	<i>Xylaria</i> aff. <i>hypoxylon</i>	—	R ?B	2010
Club	—	<i>Xylaria</i> <i>grammica</i>	—	R ?B	?2015
Club	—	<i>Xylaria</i> <i>polymorpha</i>	—	R ?B	2010
Club	—	<i>Xylaria</i> sp. 'skinny khaki'	—	R ?B	2010
Cup & Disc	—	<i>Chlorociboria</i> sp.	—	R ?B	2010
Cup & Disc	—	<i>Hymenoscyphus</i> sp.	—	R - ?ME	2010
Cup & Disc	—	<i>Phillipsia subpurpurea</i>	Burgundy Cups	R - ?ME	10,12,19,21,23
Cup & Disc	—	<i>Scutellinia</i> aff. <i>subhirtella</i> (previous <i>Scutellina</i> sp.)	Eyelash Fungus	R	2010
Coral	🔍	<i>Hericium novae-zealandiae</i> (<i>H. coralloides</i>)	Cascading Icicles	P - R	2010
Coral	🔍	<i>Pterula fascicularis</i> (<i>Deflexula fascicularis</i>)	—	R	2010
Earthstar	—	<i>Geastrum saccatum</i>	Rounded Earthstar	?	2021
Earthstar	—	<i>Geastrum triplex</i>	Collared Earthstar	?	2010

INTERNATIONAL UNION FOR CONSERVATION OF NATURE (IUCN) CONSERVATION STATUS

🔍 NEEDS ASSESSMENT 🌟 (NT) NEAR THREATENED

NEEDS ASSESSMENT **(NT) NEAR THREATENED**

(AM) ARBUSCULAR MYCORRHIZAL (B) BIOTROPHIC (BV) BACTERIOVORE (ECM) ECTOMYCORRHIZAL (L) LICHEN (ME) MUTUALISTIC ENDOPHYTE
(NT) NEMATODE TRAPPING (P) PARASITIC (R) RECYCLER (WR) WHITE ROT (?) UNVERIFIED

Family	IUCN	Taxa	Common Name	Function	Seen
Mushroom	 NT	<i>Humidicutis arcohastata</i>	—	?B, R	?
Mushroom		<i>Humidicutis mavis</i> (<i>Hygrocybe mavis</i>)	—	?B, R	2010
Mushroom		<i>Hygrocybe bolensis</i>	—	?B, R	2010
Mushroom		<i>Hygrocybe</i> sp. 'buff-yellow'	—	?B, R	2010
Mushroom	—	<i>Hypholoma fasciculare</i>	Sulphur Tuft	R	10,20
Mushroom	—	<i>Laccaria lateritia</i>	—	ECM	2010
Mushroom	—	<i>Lacrymaria asperospora</i> (<i>Psathyrella asperospora</i>)	—	R	2010
Mushroom	—	<i>Lentinellus ursinus</i>	Bear Lentinus	R	2020
Mushroom	—	<i>Lentinula lateritia</i>	Native Shiitake	R	21,23
Mushroom	—	<i>Lentinus sajor-caju</i>	Funnel Woodcap	R	2018
Mushroom	—	<i>Leratiomyces ceres</i>	Chip Cherries	R	10,19,20,23
Mushroom	—	<i>Leucocoprinus birnbaumii</i>	—	R	2010
Mushroom	—	<i>Leucocoprinus fragilissimus</i>	Fragile Dapperling	R	2019
Mushroom	—	<i>Macrolepiota</i> sp.	—	R	2010
Mushroom	—	<i>Marasmiellus</i> / <i>Setulipes</i> sp.	—	R	2010
Mushroom	—	<i>Marasmiellus</i> sp. 'decurrent'	—	R	2010
Mushroom	—	<i>Marasmius</i> aff. <i>elegans</i>	—	R	2010
Mushroom	—	<i>Marasmius</i> aff. <i>haematocephalus</i>	—	R	2010

INTERNATIONAL UNION FOR CONSERVATION OF NATURE (IUCN) CONSERVATION STATUS

NEEDS ASSESSMENT  **(NT) NEAR THREATENED**

(AM) ARBUSCULAR MYCORRHIZAL (B) BIOTROPHIC (BV) BACTERIOVORE (ECM) ECTOMYCORRHIZAL (L) LICHEN (ME) MUTUALISTIC ENDOPHYTE
(NT) NEMATODE TRAPPING (P) PARASITIC (R) RECYCLER (WR) WHITE ROT (?) UNVERIFIED

NEEDS ASSESSMENT **(NT) NEAR THREATENED**

(AM) ARBUSCULAR MYCORRHIZAL (B) BIOTROPHIC (BV) BACTERIOVORE (ECM) ECTOMYCORRHIZAL (L) LICHEN (ME) MUTUALISTIC ENDOPHYTE
(NT) NEMATODE TRAPPING (P) PARASITIC (R) RECYCLER (WR) WHITE ROT (?) UNVERIFIED

Family	IUCN	Taxa	Common Name	Function	Seen
Polypore	—	<i>Polypore</i> sp. bruises	—	R	2010
Polypore	—	<i>Polypore</i> sp. UK aff. <i>Coltriciella</i>	—	R	2010
Polypore	—	<i>Pycnoporus coccineus</i>	—	R (WR)	2010
Polypore	—	<i>Sanguinoderma</i> (<i>Amauroderma</i> sp.)	—	R, P	2010
Polypore	—	<i>Sanguinoderma rude</i>	Red-staining Stalked Polypore	R, P	10,19,21
Polypore	—	<i>Sanguinoderma rugosum</i>	—	R, P	2020
Polypore	—	<i>Trametes hirsuta</i>	Hairy Bracket	R	2010
Polypore	—	UK Bracket 'dark top, pale pores, no S'	—	R	2010
Polypore	—	UK Bracket 'karki yellow' sensu SMF2448	—	R	2010
Puffball	—	<i>Lycoperdon purpurascens</i> (<i>Morganella purpurascens</i>)	—	R	2010
Puffball	—	<i>Lycoperdon</i> sp. 'stem, brown with warts'	—	R	2010
Slime Mould	—	<i>Ceratiomyxa fruticulosa</i>	—	R, BV	2012
Slime Mould	—	<i>Fuligo septica</i>	Dog Vomit Slime Mold	R, BV	20,23
Slime Mould	—	<i>Lycogala epidendrum</i> complex	Wolf's Milk	R, BV	2010
Stinkhorn	—	<i>Phallus multicolor</i>	—	R	2019
Stinkhorn	—	<i>Pseudocolus garciae</i>	—	R	2020
Truffle-like	—	<i>Glomus</i> sp.	—	AM	2010

INTERNATIONAL UNION FOR CONSERVATION OF NATURE (IUCN) CONSERVATION STATUS

NEEDS ASSESSMENT  **(NT) NEAR THREATENED**

Fig. 12: The partially-fungivorous Northern Brown Bandicoot (Isodon macrourus) may be an important fungal distributor at Mary Cairncross Scenic Reserve.



FUNCTION

(AM) ARBUSCULAR MYCORRHIZAL (B) BIOTROPHIC (BV) BACTERIOVORE (ECM) ECTOMYCORRHIZAL (L) LICHEN (ME) MUTUALISTIC ENDOPHYTE
(NT) NEMATODE TRAPPING (P) PARASITIC (R) RECYCLER (WR) WHITE ROT (?) UNVERIFIED

APPENDIX II: MCSR PLANTS

Collared Earthstar.

Geastrum triplex.



Family	Taxa	Common Name	Fungi Function	NCR	EPBC	Habit
Acanthaceae	<i>Pseuderanthemum variabile</i>	Love Flower	NM	—	—	Herb/Groundcover
Agavaceae	<i>Cordyline rubra</i>	Red fruited Palmlily	AM	—	—	Shrub
Akaniaceae	<i>Akania bidwillii</i>	Turnipwood	?	—	—	Small Tree
Amaranthaceae	<i>Alternanthera denticulata</i>	Lesser Joyweed	AM, NM	—	—	Herb/Groundcover
Amaranthaceae	<i>Deeringia amaranthoides</i>	Shrubby Deeringia	AM, NM	—	—	Herb/Groundcover
Anacardiaceae	<i>Euroschinus falcatus</i>	Ribbonwood	AM	—	—	Tree
Anacardiaceae	<i>Rhodosphaera rhodanthema</i>	Deep Yellowwood	AM	—	—	Small Tree
Annonaceae	<i>Melodorum leichhardtii</i>	Zig-zag vine	AM	—	—	Vine
Apiaceae	<i>Hydrocotyle acutiloba</i>	Pennywort	AM	—	—	Herb/Groundcover
Apiaceae	<i>Hydrocotyle laxiflora</i>	Stinking Pennywort	AM	—	—	Herb/Groundcover
Apiaceae	<i>Hydrocotyle pedicellosa</i>	Pennywort	AM	—	—	Herb/Groundcover
Apiaceae	<i>Hydrocotyle tripartita</i>	Pennywort	AM	—	—	Herb/Groundcover
Apocynaceae	<i>Marsdenia rostrata</i>	Common Milk Vine	AM	—	—	Vine
Apocynaceae	<i>Melodinus acutiflorus</i>	Hairy Melodinus	AM	—	—	Vine

2006 QLD NATURE CONSERVATION REGULATION (NCR) & ENVIRONMENTAL PROTECTION BIODIVERSITY CONSERVATION ACT (EPBC) STATUS

⚠ (EN) ENDANGERED 🌱 (NT) NEAR THREATENED ♀ (VU) VULNERABLE

 (EN) ENDANGERED (NT) NEAR THREATENED (VU) VULNERABLE

Family	Taxa	Common Name	Fungi Function	NCR	EPBC	Habit
Aristolochiaceae	<i>Pararistolochia praevenosa</i>	Birdwing Butterfly Vine	?	NT	—	Vine
Asclepiadaceae	<i>Tylophora paniculata</i>	—	AM	—	—	Vine
Aspleniaceae	<i>Asplenium australasicum</i>	Birds Nest Fern	AM, NM	—	—	Fern
Aspleniaceae	<i>Asplenium polyodon</i>	Mare's Tail Fern	AM, NM	—	—	Fern
Asteraceae	<i>Sigesbeckia orientalis</i>	Indian Weed	AM, ?ECM	—	—	Herb/Groundcover
Athyriaceae	<i>Diplazium assimile</i>	—	AM	—	—	Fern
Azollaceae	<i>Azolla pinnata</i>	Ferny Azolla	?BNF	—	—	Aquatic
Bignoniaceae	<i>Pandorea jasminoides</i>	Bower Vine	AM	—	—	Vine
Bignoniaceae	<i>Pandorea pandorana</i>	Wonga Vine	AM	—	—	Vine
Blechnaceae	<i>Blechnum camfieldii</i>	—	AM	—	—	Fern
Blechnaceae	<i>Blechnum indicum</i>	Swamp Water Fern	AM	—	—	Fern
Blechnaceae	<i>Doodia aspera</i>	Prickly Rasp Fern	AM	—	—	Fern
Boraginaceae	<i>Austrocynoglossum latifolium</i>	Forest Hounds Tongue	AM, NM	—	—	Herb/Groundcover
Boraginaceae	<i>Ehretia acuminata</i>	Koda	AM, NM	—	—	Tree
Caesalpiniaceae	<i>Caesalpinia scortechinii</i>	Large Prickle Vine	AM	—	—	Vine
Campanulaceae	<i>Lobelia purpurescens</i>	White Root	AM	—	—	Herb/Groundcover
Capparaceae	<i>Capparis arborea</i>	Caper Bush	AM, NM	—	—	Small Tree
Celastraceae	<i>Celastrus australis</i>	Staff Vine	AM	—	—	Vine
Celastraceae	<i>Hedraianthera porphyropetala</i>	Hedrianthera	AM	—	—	Shrub

FUNCTION

(AM) ARBUSCULAR MYCORRHIZAL (BNF) BIOLOGICAL NITROGEN FIXATION (ECM) ECTOMYCORRHIZAL (EN) ENDOPHYTE (MH) MYCOHETEROTROPHIC
(NM) NON-MYCORRHIZAL (OM) ORCHID MYCORRHIZAL (?) UNVERIFIED

 (EN) ENDANGERED (NT) NEAR THREATENED (VU) VULNERABLE

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Family	Taxa	Common Name	Fungi Function	NCR	EPBC	Habit
Himantandraceae	<i>Galbulimima baccata</i>	Galbulimima, Northern Pigeonberry Ash	?	—	—	Tree
Hymenophyllaceae	<i>Crepidomanes vitiense</i>	—	AM	—	—	Fern
Icacinaceae	<i>Citronella moorei</i>	Churnwood	AM	—	—	Tree
Juncaceae	<i>Juncus usitatus</i>	Common Rush	AM, NM	—	—	Grass/Sedge
Lamiaceae	<i>Clerodendron floribundum</i>	Smooth Lollybush	AM	—	—	Small Tree
Lamiaceae	<i>Plectranthus parvifolius</i>	Plectranthus	AM	—	—	Herb/Groundcover
Lamiaceae	<i>Teucrium argutum</i>	—	AM	—	—	Herb/Groundcover
Lauraceae	<i>Beilschmiedia elliptica</i>	Grey Walnut	AM	—	—	Tree
Lauraceae	<i>Beilschmiedia obtusifolia</i>	Blush Walnut	AM	—	—	Tree
Lauraceae	<i>Cinnamomum oliverii</i>	Olivers Sassafras	AM	—	—	Tree
Lauraceae	<i>Cryptocarya erythroxylon</i>	Pigeonberry Ash	AM	—	—	Tree
Lauraceae	<i>Cryptocarya glaucescens</i>	Jackwood	AM	—	—	Tree
Lauraceae	<i>Cryptocarya macdonaldii</i>	Bill's Laurel	AM	—	—	Tree
Lauraceae	<i>Cryptocarya obovata</i>	Pepperberry	AM	—	—	Tree
Lauraceae	<i>Cryptocarya triplinervis</i>	Three-veined Cryptocarya	AM	—	—	Tree
Lauraceae	<i>Endiandra compressa</i>	White Bark	AM	—	—	Tree
Lauraceae	<i>Endiandra discolor</i>	Rose Walnut	AM	—	—	Tree
Lauraceae	<i>Endiandra pubens</i>	Hairy Walnut	AM	—	—	Small Tree

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Family	Taxa	Common Name	Fungi Function	NCR	EPBC	Habit
Meliaceae	<i>Dysoxylum rufum</i>	Hairy Rosewood	AM, ECM	—	—	Tree
Meliaceae	<i>Melia azedarach</i>	White Cedar	AM, ECM	—	—	Small Tree
Meliaceae	<i>Synoum glandulosum</i>	Scentless Rosewood	AM, ECM	—	—	Small Tree
Meliaceae	<i>Toona ciliata</i>	Red Cedar	AM, ECM	—	—	Tree
Menispermaceae	<i>Carronia multiseppalea</i>	Carronia	AM	—	—	Vine
Menispermaceae	<i>Hypserpa decumbens</i>	Southern Hyperspa	AM	—	—	Vine
Menispermaceae	<i>Legnephora moorei</i>	Round-leaved Vine	AM	—	—	Vine
Menispermaceae	<i>Stephania japonica var discolor</i>	Snake Vine	AM	—	—	Vine
Mimosaceae	<i>Acacia melanoxylon</i>	Blackwood	AM,ECM,BNF	—	—	Tree
Mimosaceae	<i>Archidendron grandiflorum</i>	Pink Lace Flower	AM, ECM	—	—	Small Tree
Mimosaceae	<i>Pararchidendron pruinosum</i>	Snow Wood	AM, ECM	—	—	Small Tree
Monimiaceae	<i>Doryphora sassafras</i>	Sassafras	NM	—	—	Tree
Monimiaceae	<i>Palmeria scandens</i>	Anchor Vine	NM	—	—	Vine
Monimiaceae	<i>Wilkiea huegeliana</i>	Veiny Wilkiea	NM	—	—	Shrub
Monimiaceae	<i>Wilkiea macrophylla</i>	Large-leaved Wilkea	NM	—	—	Shrub
Moraceae	<i>Ficus coronata</i>	Creek Sandpaper Fig	AM	—	—	Small Tree
Moraceae	<i>Ficus fraseri</i>	Sandpaper Fig	AM	—	—	Tree
Moraceae	<i>Ficus obliqua</i>	Small-leaved Fig	AM	—	—	Tree
Moraceae	<i>Ficus superba var. henneana</i>	Deciduous Fig	AM	—	—	Tree

FUNCTION

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 (EN) ENDANGERED (NT) NEAR THREATENED (VU) VULNERABLE

Family	Taxa	Common Name	Fungi Function	NCR	EPBC	Habit
Myrtaceae	<i>Leptospermum petersonii</i>	Lemon-scented Tea Tree	AM, ECM	—	—	Shrub
Myrtaceae	<i>Lophostemon confertus</i>	Brush Box	AM, ECM	—	—	Tree
Myrtaceae	<i>Melaleuca salicina</i>	White Bottlebrush	ECM, AM	—	—	Tree
Myrtaceae	<i>Pilidiostigma glabrum</i>	Plum Myrtle	AM	—	—	Shrub
Myrtaceae	<i>Pilidiostigma rhytispermum</i>	Small-leaved Plum Myrtle	AM	—	—	Shrub
Myrtaceae	<i>Rhodamnia argentea</i>	Silver Myrtle	AM	—	—	Tree
Myrtaceae	<i>Rhodomyrtus psidioides</i>	Native Guava	AM	⚡ EN	—	Small Tree
Myrtaceae	<i>Syzygium australe</i>	Scrub Cherry	AM	—	—	Small Tree
Myrtaceae	<i>Syzygium corynanthum</i>	Sour Cherry	AM	—	—	Tree
Myrtaceae	<i>Syzygium crebrinerve</i>	Purple Cherry	AM	—	—	Tree
Myrtaceae	<i>Syzygium francisii</i>	Giant Water Gum	AM	—	—	Tree
Myrtaceae	<i>Syzygium hodgkinsoniae</i>	Red Lilly Pilly	AM	♀ VU	♀ VU	Small Tree
Nephrolepidaceae	<i>Arthropteris tenella</i>	—	AM	—	—	Fern
Olaeaceae	<i>Jasminum singuliflorum</i>	Soft Jasmine	?	—	—	Vine
Olaeaceae	<i>Olea paniculata</i>	Native Olive	?	—	—	Tree
Onagraceae	<i>Ludwigia octovalvis</i>	Willow Primrose	AM	—	—	Shrub
Onagraceae	<i>Ludwigia peploides</i>	Water Primrose	AM	—	—	Herb/Groundcover
Orchidaceae	<i>Bulbophyllum exiguum</i>	—	OM	—	—	Epiphyte
Orchidaceae	<i>Bulbophyllum schillerianum</i>	—	OM	—	—	Epiphyte

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Family	Taxa	Common Name	Fungi Function	NCR	EPBC	Habit
Rosaceae	<i>Rubus moluccanus</i> var. <i>trilobus</i>	Large-leaved Raspberry	AM	—	—	Shrub
Rosaceae	<i>Rubus mooreii</i>	Silky Bramble Berry	AM	—	—	Shrub
Rosaceae	<i>Rubus probus</i>	Tall Rose Leaf Bramble	AM	—	—	Shrub
Rosaceae	<i>Rubus rosifolius</i>	Native Raspberry	AM	—	—	Shrub
Rubiaceae	<i>Atractocarpus chartaceus</i>	Narrow-leaved Gardenia	AM	—	—	Shrub
Rubiaceae	<i>Cyclophyllum longipetalum</i>	Brush Canthium	AM	—	—	Tree
Rubiaceae	<i>Galium leptogonium</i>	—	AM	—	—	Herb/Groundcover
Rubiaceae	<i>Hodgkinsonia ovatiflora</i>	Hodgkinsonia	AM	—	—	Tree
Rubiaceae	<i>Ixora beckleri</i>	Native Ixora	AM	—	—	Shrub
Rubiaceae	<i>Morinda jasminoides</i>	Sweet Morinda	AM	—	—	Vine
Rubiaceae	<i>Psychotria simmondsiana</i>	Small Psychotria	AM	—	—	Shrub
Rutaceae	<i>Acronychia oblongifolia</i>	Common Acronychia	AM	—	—	Tree
Rutaceae	<i>Acronychia pubescens</i>	Hairy Acronychia	AM	—	—	Small Tree
Rutaceae	<i>Argyrodendron trifoliolatum</i>	White Booyong	AM	—	—	Tree
Rutaceae	<i>Flindersia schottiana</i>	Bumpy Ash	AM	—	—	Tree
Rutaceae	<i>Halfordia kendack</i>	Saffron-Heart	AM	—	—	Tree
Rutaceae	<i>Melicope micrococca</i>	White Doughwood	AM	—	—	Tree
Rutaceae	<i>Melicope vitiflora</i>	Leatherwood	AM	—	—	Small Tree
Rutaceae	<i>Sarcomelicope simplicifolia</i>	Baurella	AM	—	—	Small Tree

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