What role do plant-fungal mutualisms play in restoration ecology? Assessing the impacts of coastal dune modification on mycorrhizae, and whether reconnecting mycorrhizal networks can facilitate restoration of dune vegetation.

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What role do plant-fungal mutualisms play in restoration ecology? Assessing the impacts of coastal dune modification on mycorrhizae, and whether reconnecting mycorrhizal networks can facilitate restoration of dune vegetation.

Abstract

Background - Anthropogenic landscape modification, through such processes as deforestation, agricultural and urban expansion, significantly threatens biodiversity and ecosystem function by disrupting species interactions, particularly mutualisms. Whilst the effects of landscape change on other mutualisms, such as pollination, have been well studied, relatively little is known about impacts on the mutualistic association between plants and mycorrhizal fungi within the soil. Plant-mycorrhizal associations occur in all terrestrial ecosystems, for approximately 80% of all known terrestrial plant species, and are fundamental to the ecological function and diversity of vegetation communities. Disruption of plant-mycorrhizal mutualisms could thus drive a reduction in biodiversity across modified landscapes, and prevent the recovery of plant communities in response to restoration intervention by land managers.

Aims - The first aim of this study was to determine whether the abundance and functional identity of fungi within native plant roots vary between reconstructed and remnant coastal dune habitats, using a comparative field-based study within the Illawarra region of southern New South Wales. The second aim was to assess whether the application of a mycorrhizal inoculate (obtained from remnant dunes) to nursery-grown plants prior to their introduction to reconstructed dunes facilitates their establishment and enhances vegetation recovery, through both field and mesocosm-based experiments.

Study system – Since European colonisation of the Illawarra region approximately 200 years ago, the landscape has been extensively modified through removal of coastal vegetation for agriculture and urbanisation. Since the 1980s and early 1990s, many of the coastal dunes were reconstructed by local land managers through the deposition of sand from nearby mines and reintroduction of native vegetation, in order to limit coastal erosion, protect urban assets from destructive storms and wave surges, and restore the native coastal ecosystems. The ecological function of these reconstructed dunes relative to those in which the native vegetation was not destroyed by European settlement is not known.

Results - For the field-based study I found that there were no significant differences in the abundance and composition of fungal structures between plants on reconstructed and remnant coastal dune habitats. Rates of mycorrhizal colonisation of plant roots vary substantially across the coastal landscapes, but was not influenced by the history of disturbance of the dune vegetation. In the mesocom experiment, there was a non-significant trend towards increased growth of native plant seedlings in response to mycorrhizal inoculation. However, in the field experiments, I detected significant positive effects of inoculate addition on survivorship of native seedlings, although this depended upon the identity of the plant species. Inoculation had no effect on Lomandra longifolia survival, with all plants surviving, whilst inoculation moderately improved survival rates of the grass Poa labillardieri.

Study outcomes and implications – My study has demonstrated that mycorrhizal associations between plants and their fungal mutualists may not always be adversely affected by habitat disturbance and subsequent reconstruction. Furthermore, inoculating seedlings with additional mycorrhizae is unlikely to significantly increase rates of vegetation restoration at reconstructed dunes in the short-term. It is probable that

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mycorrhizae were either not impacted by the original deforestation of the coastal dunes or were able to rapidly recolonise the dune when it was rehabilitated and reform functional networks with the reintroduced plants. I observed, however, that coastal plant communities are still highly fragmented and degraded by a variety of disturbance processes, including alien plant invasion, vandalism and attack by vertebrate pests, such as rabbits. It is suggested that future research investigate the incidence and magnitude of these disturbances between remnant and reconstructed dunes, what their impacts are on native vegetation restoration, and the mechanisms by which these impacts can be reduced.

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WHAT ROLE DO PLANT-FUNGAL MUTUALISMS PLAY IN RESTORATION ECOLOGY?

ASSESSING THE IMPACTS OF COASTAL DUNE MODIFICATION ON MYCORRHIZAE, AND WHETHER RECONNECTING MYCORRHIZAL NETWORKS CAN FACILITATE RESTORATION OF DUNE VEGETATION.

By

EILYSH R. THOMPSON

A research report submitted in partial fulfilment of the requirements for the award of the degree of

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Abstract

Background - Anthropogenic landscape modification, through such processes as deforestation, agricultural and urban expansion, significantly threatens biodiversity and ecosystem function by disrupting species interactions, particularly mutualisms. Whilst the effects of landscape change on other mutualisms, such as pollination, have been well studied, relatively little is known about impacts on the mutualistic association between plants and mycorrhizal fungi within the soil. Plant-mycorrhizal associations occur in all terrestrial ecosystems, for approximately 80% of all known terrestrial plant species, and are fundamental to the ecological function and diversity of vegetation communities. Disruption of plant-mycorrhizal mutualisms could thus drive a reduction in biodiversity across modified landscapes, and prevent the recovery of plant communities in response to restoration intervention by land managers.

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Chapter 1 – Introduction

1.1 Consequences of anthropogenic landscape modification for ecosystems and biodiversity

Biological invasions, climate change and anthropogenic landscape modification as a result of deforestation agriculture and urbanisation are the most prevalent forms of anthropogenic disturbance (Sala et al., 2000; Fahrig, 2003; Didham et al., 2007; Tylianakis et al., 2008). These disturbances result in altered vegetation communities, a reduction in native plant diversity, increases in alien plant species, altered nutrient cycles and disrupted ecological networks amongst resident biota (e.g. pollination). Such impacts are predicted to increase significantly with the rapid expansion of the human population (Sala et al., 2000).

Biological invasions pose a considerable threat to native biodiversity (Vilà et al., 2011), and occur when a species is introduced to, and spreads across, a novel range (Mack et al., 2000). Biological invasions are frequently associated with a reduction in the diversity and ecological function of invaded communities (Vilà et al., 2011). Such impacts can occur via several pathways: (1) intense direct competition between invaders and native species for limited resources, such as light and soil nutrients (Mack et al., 2000); (2) modification of disturbance regimes, such as fire (Mack & D’Antonio, 1998; Brooks et al., 2004), (3) modification of abiotic ecosystem processes, such as nutrient cycling (Ehrenfeld, 2003); and (4) modification of ecological interactions amongst resident native species (Stinson et al., 2006).

Climate change, an increasingly important driver of human-induced global environmental change, involves shifts in precipitation regimes, reduction in snow and ice extent and a decrease in diurnal temperature ranges associated with our warming
climate (Walther et al., 2002). Aside from biodiversity loss, there are a number of ecological consequences of global warming for vegetation communities, including: phenological shifts, with earlier onset of spring-time activities (e.g. flowering and fruiting) and delayed onset of autumn activities (e.g. loss of leaves) (Menzel & Estrella, 2001); geographical and altitudinal range shifts, particularly towards polar latitudes (Easterling et al., 2000); altered community compositions, as a result of range shifts and disrupted ecological networks, such as pollination (Cleland et al., 2007).

The most important and prevalent form of anthropogenic disturbance is landscape modification as a result of deforestation, and conversion of indigenous vegetation to agricultural and urban land. Anthropogenic landscape modification involves either the exploitation of ecosystem products (e.g. logging) or the conversion of natural habitat for human use (e.g. agricultural development) (Foley et al., 2005). Ultimately these land use changes are being driven by the need to provide food, water, clothing and shelter to a bourgeoning human population (Foley et al., 2005). Land use modification comprises agriculture, urban expansion, clear-felling forests, mineral and aggregate extraction (Foley et al., 2005). Through these practices we have managed to significantly alter the world in which we live. Agricultural land now occupies 40% of the lands surface, making it one of the planets largest terrestrial biomes (Ramankutty & Foley, 1999). Over the past 300 years we have seen a net loss 7-11 million km$^2$ of forest primarily for agricultural expansion and timber-extraction (Ramankutty & Foley, 1999). In the 2000’s tropical forests alone were being lost at a rate of 76,000 km$^2$ per year (Archard et al., 2014).

These forms of anthropogenic landscape modification all ultimately lead to destruction and fragmentation of natural habitat. Such modification can have
significant adverse effects on the functioning of natural ecosystems along with their constituent biota. A recent analysis of global threats to biodiversity found that anthropogenic landscape modification is currently the single greatest contributor to biodiversity loss, and is predicted to remain so for the next 100 years (Sala et al., 2000). Large vertebrate consumers are likely to be most at risk of landscape modification as their low abundance, high energy needs and large home range requirements make them particularly vulnerable (Duffy, 2003; Raffaelli, 2004). Biodiversity loss is expected to be greatest in areas that are currently being encroached upon by human activities, e.g. tropical forests where deforestation leads to the local extinction of most plant species and the associated animal species upon which they rely (Sala et al., 2000). And hot spots of diversity, including coastal land margins and riparian corridors, are expected to suffer large biodiversity losses (Sala et al., 2000).

Anthropogenic land use modification influences biodiversity and hence ecosystem functioning via a number of mechanisms. Habitat loss directly influences biodiversity by negatively affecting genetic diversity (Dixo et al., 2009), species richness (Finlay & Houlahan, 1997) and population size (Flather & Bevers, 2002). Genetic diversity may decline when habitat loss leads to reduced connectivity of populations (Dixo et al., 2009). Species richness is strongly correlated to habitat area and so a reduction in habitat size inevitably leads to species losses (Finlay & Houlahan, 1997). This is also the case with population size; for example, Flather and Bevers (2002) found that habitat size within the landscape accounted for >96% of the total variation in population abundances.

There are also a number of indirect mechanisms by which habitat loss negatively influences biodiversity. Habitat loss truncates food chain length, with
species from the higher trophic levels being lost first due their larger area requirements (Komonen et al., 2000; Dobson et al., 2006). Reduction of the terrestrial trophic chain length due to habitat loss has been compared to that of the marine ecosystem “fishing down of the food chain”, where fishing has focused on the removal of higher trophic chain species (Dobson et al., 2006, p. 1918). Habitat loss has also been linked with reductions in dispersal success (Cordeiro & Howe, 2003; Garcia & Chacoff, 2007). In the Atlantic Forest of North Eastern Brazil it has been predicted that 33.9% of tree species will become regionally extinct due to changes to seed dispersal (i.e. reductions in vertebrate dispersers) (da Silva & Tabarelli, 2000).

Habitat loss has also been shown to alter species interactions. A review by Tylianakis et al. (2008) found that landscape modification increased pathogenic infection of plants and animals (Yanoviak et al., 2006); positively impacts generalist predators (Rand et al., 2006); adversely affect the network of interactions encompassed by the decomposer food web (Wardle, 1995); cause shifts in competitive interactions between species (Autumm et al., 2006; Elzinga et al., 2007); and negatively affect mutualisms involving plants, such as pollination and mycorrhizae (Chacoff & Aizen, 2006; Aguilar et al., 2006).

Indirect mechanisms by which anthropogenic landscape modification affects biodiversity are poorly understood. For example, understanding how habitat destruction disrupts mutualism networks is particularly understudied. Mutualisms are interspecific interactions in which each species benefits from engagement with one another (Herre et al., 1999). Classic examples include plant-pollinator interactions and mycorrhizal associations of plants and endophytic root fungi. Plant-mycorrhizal associations occur in basically all ecosystems and involve approximately 80% of all terrestrial plant species (Read 1991; Van der Heijden et al., 1998). While habitat
destruction can disrupt mutualism networks, restoration efforts seek to rebuild functioning ecosystem including their mutualistic networks. Thus, understanding impacts of restoration activities on mutualisms forms is important, particularly as mutualisms may well facilitate restoration.

The aim of the remainder of this introductory chapter is to create a generalised framework for understanding the role of mutualisms in restoration ecology. First, I examine the effects of anthropogenic landscape modification on plant mutualisms and the mechanisms by which such changes are driven. Next, I focus on mycorrhizal associations and examine their role in regulating ecosystem processes and the consequences for plant communities of anthropogenically-disrupted mycorrhizal networks. This will lead into a review of the role that reconnecting mycorrhizal networks plays in plant community restoration. In the final section of my introduction I will outline the explicit aims of this thesis, along with its subsequent structure.

1.2 Patterns and mechanisms of disruption of plant mutualisms in response to anthropogenic landscape modification

On the whole, mutualisms involving plants are generally weakened as a result of anthropogenic landscape modification (Tylianakis et al., 2008). Such mutualisms include plant-pollinator, seed disperser and mycotrophic interactions (Tylianakis et al., 2008). Plant-pollinator mutualisms are almost always negatively affected by landscape modification (Aguilar et al., 2006). There are observable reductions in pollinator diversity, with flower-visiting fauna becoming more homogenous and less active with increasing distance from native habitat (Chacoff & Aizen, 2005). Habitat destruction also disrupts pollinator services, with the quality and quantity of pollen deposited on plant stigmas decreasing with increasing distance from natural
vegetation (Charcoff et al., 2008). However, Montero-Castaño and Vilà (2012) found that the response of pollinators to global change varied considerably between ecosystems and taxa. Visitation rates of vertebrate pollinators were the most negatively affected by landscape modification. It was suggested that this was due to vertebrates generally requiring larger foraging areas (Montero-Castaño and Vilà, 2012). Insect visitation rates became more negatively affected when landscape alteration was more extreme (<5% natural habitat remaining within a given area) (Montero-Castaño and Vilà, 2012). Furthermore, pollinator richness decreased more significantly in altered grasslands but not in altered forests, with the opposite being the case for pollinator abundance (Montero-Castaño and Vilà, 2012).

Seed dispersal is also negatively affected by anthropogenic landscape modification (Cordeiro & Howe, 2003; Garcia & Chacoff, 2007). Habitat fragmentation reduces the mobility of seed dispersers, impairing the dispersal of seeds between habitat fragments (Cordeiro & Howe, 2003; Garcia & Chacoff, 2007). Furthermore, seed predation has been shown to increase in fragmented habitats (Garcia & Chacoff, 2007). It has been suggested that the negative effects of landscape modification on seed dispersal and predation could further compound the effects of reduced pollination on plant reproduction (Tylianakis et al., 2008). A decline in seed dispersal and increase in predation negatively affect the opportunities for plant regeneration, leading to a further degradation of plant populations across modified landscapes.
1.2.1. Importance of arbuscular mycorrhizal fungal associations for plant communities

Arbuscular mycorrhizae are symbiotic associations between fungi and plants (Harris, 2009). In a mycorrhizal relationship, fungal hyphae colonize a plant host’s roots and spread out into the surrounding soil (Harris, 2009). These hyphae form branched structures called arbuscules within plant root cortical cells, and it is across these structures that the mycorrhizae transfer mineral nutrients that they have accessed from the surrounding soil to the host plant (Harris, 2009). In turn, the mycorrhizae receive carbohydrates as a function of photosynthesis from the host plant (Harris, 2009). These associations occur in all but a few terrestrial ecosystems (i.e. boreal forests) (Read, 1991) and in most plant families (~80% of all plant species) (Van der Heijden et al., 1998). The importance of mycorrhizal associations in terrestrial ecosystems is highlighted by the discovery of arbuscules and non-septate hyphae within fossilized root fragments from the early Devonian, suggesting that this association has in the very least been in existence for over 400 million years (Remy et al., 1994).

Arbuscular mycorrhizal fungal (AMF) associations play a fundamentally important role in the function of plants, communities and ecosystems (Rilling, 2004). At the organismal level they enhance the growth, reproduction and physiological health of plants by facilitating their access to limited nutrients (Harris, 2009). In some cases, mycorrhizae may supply plants with up to 90% of their phosphorous and 80% of their nitrogen requirements (Van der Heijden et al., 2008). They increase rates of nutrient uptake, by increasing the surface area over which the plant can absorb nutrients from the soil (Harris, 2009). The fine highly branched hyphae of mycorrhizas are much smaller in diameter than plant roots and are thus able to
explore greater volumes of soil. Hyphae secrete organic acids to enhance the chemical decomposition of organic detritus, which improves nutrient uptake and allows the plants to reallocate resources to growth and reproduction more so than nutrient acquisition (Harris, 2009). In this way mycorrhizae can mediate interactions amongst co-occurring native plants, by allowing host plants to reallocate resources to competitive strategies and reproductive output (Marler et al., 1999). Plant hosts with the highest level of connectivity with mycorrhizal fungi are competitively superior over neighbouring plants (Mora & Zobel, 1996; Marler et al., 1999; Hart et al., 2003; Reynolds et al., 2003; Scheublin et al., 2007).

Since mycorrhizal fungi can strongly influence competitive hierarchies of neighbouring plants, they can often play an important role in shaping and maintaining the composition of plant communities (Marler et al., 1999, Reynolds et al., 2003). For instance, the presence of mycorrhizae can facilitate the shift from early to later successional plant communities (Janos, 1980; Hart et al., 2003; Kardol et al., 2006). In early successional communities, mycorrhizal diversity is likely to be low and restricted to the patches of least disturbed soil (Hart et al., 2003). Such an environment favours primary successional plants, which are less dependent on mycorrhizae for growth and reproduction (Hart et al., 2003). As time passes, mycorrhizal diversity begins to increase as fungal spores are dispersed to the site (Hart et al., 2003). This environment now favours later successional plant species that are more dependent upon mycorrhizae for their growth (Hart et al., 2003). Arbuscular mycorrhizas can maintain the coexistence between neighbouring plants, and thus community diversity, by boosting the competitive ability of weakly competitive plants that would normally be excluded by superior competitors (Allen and Allen 1990, Moora and Zobel, 1996, Hart et al., 2003). Alternatively, they can
potentially reduce community diversity if they favour a superior competitor (Allen and Allen 1990, Moora and Zobel, 1996, Hart et al., 2003). Composition of AMF communities can influence the overall productivity and diversity of plant communities as they increase the range over which plants can exploit limiting resources (Van der Heijden et al., 1998; Klironomos et al., 2000; Van der Heijen et al., 2008). Van der Heijden et al. (1988) found that grassland microcosms inoculated with a mixture of four AMF species had plant diversity and productivity figures 105% and 45% higher, respectively, than microcosms where a single species AMF inoculate was applied.

Mycorrhizal associations may indirectly influence other biotic communities (Cahill et al., 2008). A study by Cahill et al., (2008) found that the suppression of AMF fungi could alter plant-pollinator mutualisms. After three years of fungal suppression they noted a shift in the type of floral visitors from large bodied bees to small-bodied bees and flies and a 67% reduction in the number of floral visits per stem. They suggested that these findings were a result of the disturbed AMF communities causing a shift in competitive interactions amongst the plant community leading to an altered patch-level floral display (Cahill et al., 2008). As AMF associations are so interlinked with plant community and ecosystem structure it is apparent that a decline in their diversity and abundance would lead to a decline in the diversity of plant communities and the function of ecosystems more broadly.

1.2.2. Effects of anthropogenic landscape modification on mycorrhizal associations

Like plant-pollinator and seed dispersal mutualisms, mycorrhizal mutualisms are most often negatively affected by anthropogenic landscape modification (Tylianakis et al., 2008). In agricultural systems anthropogenic landscape
modification has been shown to have an adverse effect on AMF associations via a number of pathways. Disturbance practices such as excessive irrigation, over-grazing and tillage have all been shown to reduce mycorrhizal abundance (Kabir, 2005), species richness (Jansa et al., 2003; Antunes et al., 2009) and spore numbers (Oel et al., 2003). Tillage disturbs the networks of AM hyphae within the topsoil, and dilutes AMF propagules by churning top and lower layers of soil (Kabir, 2005). This negatively impacts the survival of AMF propagules and reduces the level of plant root infection (Kabir, 2005). Such disturbance regimes have also been shown to negatively affect species richness and structure of the resident AM fungal communities, with faster growing, more infective species becoming more abundant (Jansa et al., 2003; Antunes et al., 2009). Declines in species diversity and abundance inevitably leads to declines in spore abundance (Oehl et al., 2003), with the viability of the remaining spores being further reduced by exposure to solar radiation and salinity via soil disturbance (Rotem et al., 1985). Oehl et al. (2003) found that AMF species diversity and spore numbers were highest in the undisturbed agricultural grasslands, and then gradually decreased as management practices intensified, becoming lowest with continuous maize mono-cropping. Other mechanisms of disturbance that have been shown to impact on mycorrhizal associations include nutrient enrichment (i.e. N and P deposition) and vegetation removal. Nitrogen deposition weakens mycorrhizal associations as it down-regulates the control that fungi hold over plants, by reducing the plants reliance on nitrogen supplied by fungi (Wei et al., 2013). Agricultural practices such herbicide cleaned mono-cropping systems, where pastures are vegetated for short periods of time, reduces fungal diversity by limiting the period over which root colonization and sporulation can occur (Oehl et al., 2003).

While the effect of anthropogenic landscape modification on AMF in
agricultural systems is well researched, very little is currently understood about its effects in natural areas. Allen et al. (1998) found that AMF communities shifted from diverse suites of fungi to ones dominated by Glomus spp. with large-scale conversion of tropical forest to grassland. Glomus spp. have also been shown to dominate fungal communities following burning of mature tropical forests (Allen et al., 2003). This trend of reduced species diversity with disturbance is not universal. Picone (2000) and Johnson & Wedin (1997) found that AMF species diversity and spore abundance did not significantly decline with the conversion of mature forest to grasslands in Costa Rica. Further studies comparing forested and deforested lands have also found this to be the case (Zhang et al., 2004; Stürmer & Siqueira, 2011). Differences in findings have been suggested as being a product of intensity of disturbance regimes, with fire causing greater diversity loss as AMF species are completely lost from the soil and thus must repopulate these areas via immigration (Allen et al., 2005). Aside from the research surmised above very little is known about the mechanisms behind these patterns.

1.3 Role of mycorrhizae in ecosystem restoration

As human exploitation of the environment increases so do the costs to biodiversity and ecosystems services (Sala et al., 2000; Tylianakis et al., 2008). The main strategy presently being used to regain these losses is ecological restoration (Hobbs & Norton, 1996; Bullock et al., 2011). Ecological restoration is currently defined as the process of assisting and accelerating the recovery of degraded ecosystems (SERI, 2004). The principal goal of restoration is to get the ecosystem to a point where it is functionally similar to a relatively non-degraded ecosystem (Hobbs & Norton, 1996; Bullock et al., 2011). Most commonly, restoration focuses on the reintroduction of a number of plant species that are dominant within the reference
ecosystem, with the hope that their establishment will consequently push the degraded ecosystem on a trajectory towards recovery from disturbance (Palmer et al., 1997).

A recent meta-analysis on restoration success found that current restoration practices are only partially successful, with restored systems having median response ratios for ecosystem services and biodiversity at 80% and 86% of those attributed to their reference ecosystems (Benayas et al., 2009). This limited success has been in part attributed to strongly structural approaches when planning and evaluating restoration projects (Forup et al., 2008). Essentially, when restoring ecosystems we tend to focus on the structural aspects of that community, such as species abundance and richness (Palmer et al., 1997; Forup et al., 2008). This approach is in many respects fundamentally flawed. First, restoration may be ineffective as the landscape properties (i.e. soil chemistry, microbial community) of the ecosystem to be restored may have significantly changed with degradation, meaning that it no longer has the ability to sustain that community (Palmer et al., 1997). It has thus been suggested that not only should we be considering structural components during restoration planning and evaluation but also functional elements, such as the ecological processes that maintain these communities like species interactions (Forup et al., 2008; Kardol & Wardle, 2010).

One such functional element of ecosystems that is beginning to gain support as a restoration tool is the reconnection of soil ecological mutualisms, such as plant-mycorrhizal associations (Kardol & Wardle, 2010). As previously established, the presence of mycorrhizae is strongly linked with plant diversity, productivity and ecosystem heterogeneity (van der Heijden, 1998; Klironomos et al., 2000; Van der Heijden et al., 2008). There is also emerging evidence that these mutualisms may have cascading effects on other biotic communities such as the aboveground invertebrates
Further we know that mycorrhizal communities are sensitive to a number of global environmental changes including landscape disturbance (Allen et al., 1998; Allen et al., 2003; Jansa et al., 2003; Oehl et al., 2003;). Therefore, if mycorrhizae are absent from disturbed habitats then restoration of native plant communities may be limited, and ecosystem recovery may be dependent upon the reestablishment of mycorrhizal associations, not just simply the replacement of native plants alone.

However, the role that mycorrhizae play in restoration of disturbed communities is poorly understood. My database search revealed only 28 studies that experimentally examined the effects of reintroducing mycorrhizal fungi on plant survival, growth and reproduction (Table 1). Of these studies, 82% showed an improvement with the addition of a fungal inoculum to disturbed soil. An improvement was regarded as either a significant increase in plant performance with inoculum application or a trend towards significance. Plant growth parameters where improvements were recorded included shoot and root biomass, nutrient uptake, stem diameter, inflorescence production and survival (Richter & Stutz, 2002; Caravaca et al., 2003; Zhang et al., 2011). For example Caravaca et al. (2003) found that shoot biomass of inoculated shrub species was up to 630% higher than that of their uninoculated counterparts, one year after planting. Of the 18% of studies where no improvements were recorded, there was generally a likely explanation given. In their study on the restoration of a semi-arid degraded steppe Maestre et al. (2002) suggested that the effect of inoculation on seedling survival in the field was most likely reduced by drought summer conditions, which increased rates of fungal mortality. Other explanations given for such a result included that the potential benefit of nursery inoculation was masked by the natural colonization of seedlings by
remnant AMF populations within the soil (Maestre et al., 2002; White et al., 2008; Cook et al., 2011), that the inoculum used was not adapted for the site-specific conditions, or that the inoculation procedure was not successful (Walker, 2003). In cases where the response of plants to different inoculum types was compared, indigenous inoculums (inoculum sourced from undisturbed reference ecosystems) generally outperformed commercial inoculums (inoculums containing a mix of fungi prepared commercially) or no significant differences were found between the two (Sylvia et al., 1993; Greipsson & El-Mayas, 2000; White et al., 2008). For example, in the restoration of coastal dunes, Sylvia et al. (1993) found that shoot biomass of plants grown in indigenous inoculated treatments was twice that of plants grown in commercial inoculated treatments, after one growing season.

Across the studies there were a wide range of ecosystem types represented, from tropical forests to steppe grasslands to coastal sand dunes. Despite the variety of ecosystem types, all but three of them were conducted within the Northern hemisphere, mainly in Western Europe and North America, demonstrating a significant geographical bias (Fig. 1). Further to this, of the studies conducted in the Northern hemisphere 44% were conducted in Spain, highlighting a regional bias. As of yet no such studies have been conducted within Australia on the effects of mycorrhizae on restoration.
Table 1 Summary of key mycorrhizal inoculation studies globally, including comparisons between the vegetation community, disturbance type, methodology and whether or not restoration potential was improved. Table is sorted by geographical location (northern and southern hemisphere) and then by vegetation type within each of these categories.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Region</th>
<th>Plant Community</th>
<th>Disturbance type</th>
<th>Innoculum type</th>
<th>Plant Species of concern</th>
<th>Inoculation method</th>
<th>Type of Study</th>
<th>Fungi</th>
<th>Response variable</th>
<th>Restoration potential</th>
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<td>Allen, M. F., Allen, E. B., &amp; Gómez-Pompa, A.</td>
<td>2005</td>
<td>Yucatan Peninsula</td>
<td>Tropical forest</td>
<td>Agriculture &amp; Fire</td>
<td>Indigenous</td>
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<td>Innoculation in nursery</td>
<td>Field experiment</td>
<td>AM</td>
<td>Height, Survival</td>
<td>Improved</td>
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<td>Field experiment</td>
<td>EM</td>
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<td>Field experiment</td>
<td>EM</td>
<td>Survival, height stem diameter, Foliar nutrient concentration</td>
<td>Not improved</td>
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<td>Authors</td>
<td>Year</td>
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<td>Field experiment</td>
<td>AM and EM</td>
<td>Height, diameter</td>
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<td>Indonesia</td>
<td>Tropical peat swamp forest</td>
<td>Logging and burning</td>
<td>Single species</td>
<td>Shorea balangeran and Dyera polyphylla</td>
<td>Inoculation in nursery</td>
<td>Field experiment</td>
<td>AM and EM</td>
<td>Survival, biomass, stem diameter, height, leaf number, Foliar N &amp; P</td>
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<td>Authors</td>
<td>Year</td>
<td>Region</td>
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<td>Innoculum type</td>
<td>Plant Species of concern</td>
<td>Inoculation method</td>
<td>Type of Study</td>
<td>Fungi</td>
<td>Response variable</td>
<td>Restoration potential</td>
</tr>
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</tbody>
</table>
Figure 1 Distribution of mycorrhizal inoculation studies globally. Apparent geographical bias of studies to Northern hemisphere, with the highest concentration of studies being in Spain (40%)
1.4 Study objectives

The overall objective of this study was to examine the effects of anthropogenic landscape modification and subsequent rehabilitation on native plant-fungal interactions, termed mycorrhizae. I tested these effects across an extensively urbanised and deforested coastal landscape, using a combination of field and experimental techniques. Specifically, my objectives consisted of two key questions:

(1) How does anthropogenic landscape modification disrupt the mycorrhizal interactions between native plants and their fungal mutualists?

Plant-mycorrhizal mutualisms are known to be sensitive to a number of global environmental changes including land use modification in the form of deforestation. It remains poorly understood whether plant-fungal mutualisms are disrupted by such disturbances, and the consequences of such disruption for remnant native plants. To answer this question I compared rates of colonisation of native plant roots by fungi between remnants dunes (i.e. those in which native vegetation was not removed during human land modification) and reconstructed dunes (i.e. those in which native vegetation and the sand substrate were removed as a result of anthropogenic landscape change during settlement by European immigrants, but which have subsequently been reconstructed through fabrication of the dune formation and reintroduction of nursery-grown native plant seedlings). I predicted that reconstructed dunes would have a lower abundance and different functional identity of fungi than their remnant counterparts.
(2) Can reconnecting disrupted plant-fungal mutualisms facilitate the restoration of modified landscapes?

I aimed to test whether the application of a native mycorrhizal inoculate to nursery-grown plant seedlings prior to revegetation facilitates their establishment and growth, and in turn facilitate the recovery of rehabilitated coastal dunes. This was achieved using a combination of field and mesocosm experiments for a variety of native plants that are commonly used in dune revegetation programmes.
Chapter 2 – Methods

The methods section is divided into three parts. First (section 2.1), I describe the biological and geophysical contexts of the study region and its history of landscape modification and rehabilitation. Second (section 2.2), I explain the methods used to evaluate differences in rates of fungal colonisation of plant roots between reconstructed and remnant coastal dunes. Third (section 2.3) I explain the methods used to experimentally test the hypothesis that inoculation of reconstructed dune soil with mycorrhizae from remnant dune soil enhances the restoration of coastal dune vegetation.

2.1 Study region and habitat

Both studies were conducted within the fore dune of a number of coastal dune complexes on the south coast of New South Wales (NSW), Australia, between 16\textsuperscript{th} March and 7\textsuperscript{th} October, 2015. All sites fell within the Sydney Basin Bioregion, one of 85 bioregions identified within Australia (Environment Australia, 2000). This region has a temperate climate, defined by moderate temperatures and uniform annual rainfall (Tozer \textit{et al.}, 2010). It experiences warm summers and cool winters with maximum temperatures in summer sitting at ~30°C and in winter at ~22°C and annual rainfall averages of 1083 -1253 mm/year (Australia Bureau of Meteorology, 2015).

The coast of the south coast region is characterised by a narrow and unbroken coastal plain (Tozer \textit{et al.}, 2010). This topographic feature stretches extensively east of Nowra across the Shoalhaven floodplain (Tozer \textit{et al.}, 2010). North of Nowra however, the coastal plain becomes restricted to small strips of low-lying land confined by headlands and rock shelves (Tozer \textit{et al.}, 2010). These areas are commonly associated with freshwater lagoons, tidal lakes and coastal embayments.
Along the eastern edge of the coastal plain there are a number of sand plains of marine origin as well as a couple of examples of perched aeolian dunes (Tozer et al., 2010). The sandy soils of these areas have a fairly low fertility, which declines with increasing proximity to the ocean and its salt-laden winds (Tozer et al., 2010). This study was undertaken on the edge of these sand plains, on the fore dunes of beaches, which act as a barrier between marine and terrestrial environments (Short, 2007).

Within the Sydney Bioregion there are two main vegetation communities that can be found within the fore dune complex: Coastal Sand Scrub and Beach Strand Grassland (Tozer et al., 2010). Coastal Sand Scrub is characterised by low, thick shrubs up to 3m in height and is restricted to fore dunes that directly border the coast (Tozer et al., 2010). As it is located in a highly exposed area with saline and nutrient poor soils, vegetation is dominated by hardy salt-tolerant species (Tozer et al., 2010). These species include Banksia integrifolia subsp. integrifolia, Westringia fruticosa, Lomandra longifolia and Acacia longifolia subsp. sophorae (Tozer et al., 2010). The other vegetation type Beach Strand Grassland is a very simple open grassland community located between the high-tide mark and Coastal Sand Scrub (Tozer et al., 2010). It is dominated by Spinifex sericeus, as harsh, saline conditions make it inhospitable to most other species (Tozer et al., 2010). Carpobrotus glaucescens may also be present along with a number of weed species including Cakile edentula and Hydrocotyle bonariensis (Tozer et al., 2010). Both vegetation communities cover an existing area of 3,100 ha, which is approximately 35-50% of their pre-colonial size (Tozer et al., 2010). Of this, 1,700 ha is currently located within conservation reserves (Tozer et al., 2010). Figure 2 shows the transition down the dune face, from Coastal Sand Scrub on the left side of the photograph to Beach Strand Grassland on the right.
Figure 2 dune profile at Puckey’s Estate, with Beach Strand Grassland in the foreground grading into Coastal Sand Scrub.

2.1.1 Landscape disturbance

As beaches play an integral part in the growth of coastal community economies, their management tends to focus more heavily on creating a positive recreational experience than conserving ecological processes. Quite often large swathes of beach dune vegetation are cleared for coastal development, particularly associated with tourism infrastructure (Nordstrom, 2000). Within the study region, Coastal Sand Scrub has undergone significant clearing, with at least half of it being cleared for development (Tozer et al., 2010). A large percentage of the remaining Coastal Sand Scrub is situated within reserves, but these too are vulnerable to developmental pressures, and recreational activities (Tozer et al., 2010). Disturbance to dune vegetation from development and agriculture (e.g. reduction or removal of native vegetation) has been linked to increased plant invasion (Kercher & Zedler, 2004; Silliman & Bertness, 2004; French, 2012; Lambert et al., 2014). These disturbances can alter ecosystem processes in a way that gives non-native species
competitive dominance over native species (Kercher & Zedler, 2004; Silliman & Bertness, 2004; French, 2012; Lambert et al., 2014). In the study region plant invasion has been identified as one of the most serious biological threats to native vegetation communities (Mason & French, 2007; Mason et al., 2007; Mason & French, 2008). Bitou bush (*Chrysanthemoides monilifera* subsp. *rotundata*) and *Lantana camara* are two of the most common weed species threatening coastal vegetation (Mason et al., 2007; Downey et al., 2010; Turner & Downey, 2010; French, 2012). They both out-compete and in many cases totally replace native flora within the dune system (Mason et al., 2007; Downey et al., 2010; Turner & Downey, 2010; French 2012).

Impacts caused directly by people who visit beaches recreationally are also emerging as potentially significant contributors to dune destruction (Schlacher et al., 2008). The mechanical impacts of trampling have been linked to dune vegetation degradation, through production of more uniform stands of vegetation (Liddle & Grieg-Smith, 1975). Beach Strand Grasslands subject to intense recreational use, particularly trampling, are known to have a reduced vegetative cover (Tozer et al., 2010). Another stressor for beachside ecosystems is sand mining (Andrés and Mateos, 2006). Specifically within my study area, the North side of Bellambi Lagoon, Seven Mile Beach and Perkins Beach have been mined extensively for sand (Table 2). This practice damages dunes by not only destroying habitat but also altering the sediment budget and thus hastening erosion (Thornton et al., 2006). Sand mining also has strong ties with plant invasion, with bitou being introduced into mined systems to rapidly stabilise dunes, before its weed status was recognised (Winkler et al., 2008). Other ongoing threats to dune systems include rubbish dumping, firewood collecting and small scale burning (Tozer et al., 2010).
2.1.2 Landscape development

Within our study system we know that beaches were vegetated with native plants up until the early 1900’s (Wollongong City Council, 2014). It was not until the 1940’s that aerial photographs revealed wide-spread clearing as a result of European colonisation and agricultural expansion across the region (Wollongong City Council, 2014). However, despite this widespread clearing, many dunes retained remnant patches of native vegetation. This clearing left the beaches highly susceptible to erosion by aeolian processes and storm surges, which was realised in a number of major storm events including those of 1964 and 1974 (Wollongong City Council, 2014). From the mid 1980’s management programmes were enacted by Council to reconstruct many of these cleared dune systems (Wollongong City Council, 2014). The fore dunes were first re-profiled, to a set of engineering specifications, whereby they were shaped so that they reached a height of 4.5m above AHD and had a maximum seaward dune face gradient of 1 in 4 (Wollongong City Council, 2014). Dunes were then fenced off to prevent further damage from recreational use and to limit sand loss (Wollongong City Council, 2014). After the re-profiling was complete, a staged programme of revegetation was undertaken (Wollongong City Council, 2014). At first the seaward dune face was planted with marram and spinifex grass, to stabilise the dune for further revegetation (Wollongong City Council, 2014). Once these grasses had established across the seaward face of the dune, the landward dune face was then stabilised by planting a number of native shrub and tree species, including Coastal Tea-tree (*Leptospermum laevisatum*) and Coastal Wattle (*Acacia longifolia subsp sophorae*) (Wollongong City Council, 2014). In the last stage of dune reconstruction, a variety of other species were planted at the rear of the dune, including Coastal banksia (*Banksia integrifolia*) (Wollongong City Council, 2014).
Since this initial work was done, ongoing maintenance has continued, with a focus on further planting to boost dune vegetation diversity and weed control (Wollongong City Council, 2014). The degree to which beaches were cleared and then revegetated can be seen in the examples presented in Figures 3 and 4. Both Fairy Meadow and Corrimal Beach were completely cleared by 1977 and then fully revegetated by 2011. Puckey’s Estate and Bellambi Lagoon on the other hand, maintained remnant pockets of vegetation from the 1940’s onwards.
Figure 3 comparison of aerial photographs between reconstructed and remnant habitat types. Early photographs show beaches post clearing pre-revegetation. Later photographs show beaches after extensive revegetation (Wollongong City Council, 2015)
Figure 4 comparison of aerial photographs between reconstructed and remnant habitat types. Early photographs show beaches post clearing pre-revegetation. Later photographs show beaches after extensive revegetation (Wollongong City Council, 2015)
2.2 Study 1 – Comparison of rates of fungal colonisation of native plant roots between reconstructed and remnant dune sites

The first study was undertaken at 16 coastal sites, from Bulli Beach (34°20’35”S, 150°55’25”E) in the north to Curraong Beach in the south (35°0’5”S, 150°47’42”E)(Fig. 5). These sites were divided into two habitat treatments: (1) those that were cleared and then subsequently reconstructed (hereafter termed ‘reconstructed’ dunes) and (2) those that have retained remnant vegetation since European colonisation (hereafter termed ‘remnant’ dunes). Reconstructed sites were defined as ones which had been cleared of vegetation in the 1940’s and then revegetated from the mid 1980’s onwards (e.g. Fairy Meadow Beach, Fig. 3 and Corrimal Beach, Fig. 4). Remnant sites were defined as ones that had maintained pockets of remnant native vegetation from the 1940’s onwards (Puckey’s Estate, Fig. 3 and Bellambi Lagoon Fig. 4). To ascertain whether or not dunes were remnant or reconstructed, aerial photographs that were taken from 1948 to 2011 were consulted along with Wollongong City Council’s Dune Management Strategy Reports (Table 2).
Figure 5 Map of New South Wales (N.S.W.) coastline showing the 16 study sites. White points = remnant sites, black points = reconstructed.
Table 2 List of sites used for both studies, including information on current and previous revegetation programs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Site</th>
<th>Location</th>
<th>Reconstructed/ remnant</th>
<th>Vegetation extent</th>
<th>Revegetated</th>
<th>Current revegetation program</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bulli Beach</td>
<td>34°20'35&quot;S, 150°55'25&quot;E</td>
<td>Reconstructed</td>
<td>No vegetation 1948-86, 1993 vegetation evident</td>
<td>Yes, around 1986</td>
<td>Yes</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>Woonona Beach</td>
<td>34°21'9&quot;S, 150°55'12&quot;E</td>
<td>Reconstructed</td>
<td>Sparse vegetation present from 1948-1986, 1993 established vegetation</td>
<td>Yes, around 1986</td>
<td>Yes</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>Bellambi Beach</td>
<td>34°21'49&quot;S, 150°55'14&quot;E</td>
<td>Reconstructed</td>
<td>Sparse vegetation present from 1948-1986, 1993 established vegetation</td>
<td>Yes, around 1986</td>
<td>Yes</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>Corrimal Beach</td>
<td>34°22'43&quot;S, 150°55'11&quot;E</td>
<td>Reconstructed</td>
<td>Sparse vegetation present from 1948-1984, 2001 established vegetation</td>
<td>Yes, shortly after 1984</td>
<td>Yes</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>Towradgi Beach</td>
<td>34°23'26&quot;S, 150°54'36&quot;E</td>
<td>Reconstructed</td>
<td>Appearance of dune vegetation 1984</td>
<td>Yes, shortly after 1984</td>
<td>Yes</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>Fairy Meadow Beach</td>
<td>34°23'46&quot;S, 15054'21&quot;E</td>
<td>Reconstructed</td>
<td>Sparse vegetation present from 1948-1986, 2001 established vegetation</td>
<td>Yes, around 1986</td>
<td>Yes</td>
</tr>
<tr>
<td>1</td>
<td>North Wollongong Beach</td>
<td>34°24'46&quot;S, 150°54'7&quot;E</td>
<td>Reconstructed</td>
<td>Vegetation present 1948, cleared by 1977 and re-established by 1993</td>
<td>Unknown</td>
<td>No</td>
</tr>
<tr>
<td>1 &amp; 2</td>
<td>City Beach</td>
<td>34°25'25&quot;S, 150°54'22&quot;E</td>
<td>Reconstructed</td>
<td>Sparse vegetation present from 1948-1987, 1993 established vegetation</td>
<td>Yes, around 1987</td>
<td>Yes</td>
</tr>
<tr>
<td>1</td>
<td>Bellambi Lagoon</td>
<td>34°22'31&quot;S, 150°55'24&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Study</td>
<td>Site</td>
<td>Location</td>
<td>Reconstructed/remnant</td>
<td>Vegetation extent</td>
<td>Revegetated</td>
<td>Current revegetation program</td>
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</tr>
<tr>
<td>1</td>
<td>Puckey's Estate</td>
<td>34°24'24&quot;S, 150°54'5&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>Yes</td>
</tr>
<tr>
<td>1</td>
<td>Perkins Beach</td>
<td>34°31'31&quot;S, 150°52'37&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>1</td>
<td>Killilea Beach</td>
<td>34°20'35&quot;S, 150°55'25&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>1</td>
<td>Minnamurra Beach</td>
<td>34°36'13&quot;S, 150°52'1&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>1</td>
<td>Seven Mile Beach</td>
<td>34°50'7&quot;S, 150°44'46&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>1</td>
<td>Comerong Island</td>
<td>34°51'55&quot;S, 150°44'54&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>1</td>
<td>Currarong Beach</td>
<td>35°0'5&quot;S, 150°47'42&quot;E</td>
<td>Remnant</td>
<td>Established vegetation present 1948</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>
2.2.1 Study species

My selection of the dune species for this part of the study was informed by vegetation surveys at each of the 16 sites. I surveyed sites for 30 minutes to record resident plant species. The two species that were selected for the study were present at all sites, and include the tufted graminoid *Lomandra longifolia* (family Lomandraceae) and the prostrate, succulent forb *Carpobrotus glaucescens* (family Aizoaceae). Both species are native to Australia, characteristic constituents of dune vegetation and play an important role in dune stabilisation (Tozer et al., 2010). Furthermore, they are widely used along the east coast of Australia for restoration of disturbed dunes and are known to form symbiotic relationships with mycorrhizal fungi (Logan et al., 1989; K. French unpub. data). Thus, if reconstructed dunes lack the suite of fungal mutualists that are required by these two species for establishment and growth, dune restoration may be significantly hampered.

*Lomandra longifolia* (common name spiny-headed mat-rush) is a large perennial dioecious, tufted graminoid that is common across a wide range of habitats in eastern Australia (Quirico, 1993) (Fig. 6). It has large strap-like leaves with toothed tips (Quirico, 1993). These leaves are generally around 50cm long, but can be up to 1m in length (Quirico, 1993). Its flowers are clustered upon spiky, largely branched inflorescences (Quirico, 1993). Male flowers produce pollen and are typically 3-3.5 mm long (Quirico, 1993). While the female flowers are approximately 4.5mm long and emit a heavy-smelling nectar, which attracts pollinators (Quirico, 1993). *Lomandra longifolia* is known to form a symbiotic relationship with mycorrhizal fungi (Logan et al., 1989; K. French unpub. data). Studies have found internal and external hyphae present along with arbuscules and vesicles (Logan et al., 1989).
Logan et al. (1989) found that plants collected from the NSW coast had an average VAM colonisation of 37%.

Figure 6 *Lomandra longifolia* at Puckey’s Estate

*Carpobrotus glaucescens* (common name pigface) is a creeping salt-tolerant succulent herb, which is restricted to coastal dunes of eastern Australia (Jacobs & Hight, 1984) (Fig. 7). It has succulent leaves, which have a triangular cross-section that develop a reddish colour with age (Jacobs & Hight, 1984). It produces numerous bright pink solitary flowers and a purple fruit (Jacobs & Hight, 1984). *C. glaucescens* is known to form mycorrhizal relationships, but the extent of root colonisation has varied between papers (Logan et al., 1989; K. French unpub. data). Logan et al., (1989) found *C. glaucescens* to have a VAM colonisation of 1%, while
more recent studies have found an average colonisation rate of 18±14.6% (K. French unpub. data).

2.2.2 Experimental design and sampling

To compare the rates of AMF colonisation between restored and remnant dune sites, root samples were collected from two dune plant species. For each plant species, 14 sites were sampled; seven reconstructed and seven remnant (Fig. 8). At each site, a 500 m transect was set up along the fore dune, parallel to the shoreline. Along this transect up to ten plants from each species were selected randomly. The length of the transect and the sampling style were chosen to ensure that spatial variation in mycorrhizal populations was adequately sampled across each dune site. Roots were sampled from within the first 30cm of soil at the base of the plant, which is where the
majority of mycorrhizal activity occurs (Kabir et al., 1998). For each plant, three separate root sub-samples were taken (approximately 100g of root mass each), to account for the spatial variability of mycorrhizae at the individual plant level. For each of the three sub-samples, the roots were traced back to their parent plant to ensure that no contamination between species occurred. Upon removal, roots were bagged together (i.e. all three sub-samples were pooled together to form one sample per plant) and refrigerated. Roots were then washed with distilled water and stored within a 70% ethanol solution until clearing (Utobo et al., 2011).
Figure 8 Sampling design used to examine the variation in fungal colonization between reconstructed and remnant dunes. Reconstructed sites: Corrimal (Co), Fairy Meadow (FM), Bulli (Bu), Bellambi (Be), Towradgi (T), City (Ci), North Wollongong (NW) and Woonona (W). Remnant sites: Seven Mile (SM), Bellambi Lagoon (BL), Comerong Island (CI), Killalea (K), perkins (Pe), Currarong (Cu), Minnamurra (M) and Puckeys (Pu). Species sampled were Lomandra longifolia (L) and Carpobrotus glaucesens (C). Up to ten plants were sampled at each site.
2.2.3 Assessment of rates of fungal colonisation of plant roots

In order for the abundance of root fungi to be determined, the roots were cleared and stained, following the methods outlined by Utobo et al. (2011). The initial clearing process targeted the removal of plant cellular contents, including the cell membrane and cytoplasm, whilst retaining plant cell walls and fungal structures (Utobo et al., 2011). First, the roots were removed from the ethanol preservative and thoroughly rinsed with distilled water in order to remove any fungal and soil contaminants stuck to the outside of the root epidermis. I then selected fine roots of < 2 mm in diameter and cut them into 1 cm sections. In order to clear the cell contents, these root sections were placed in small plastic vials filled with 10 % KOH, before being heated to 90 °C in a water bath for 60 minutes. After heating, the KOH solution was drained from the vials and the roots were again washed with distilled water. These rinsed roots were placed back in vials and covered with 1% HCl for approximately 18 hours.

Once the cellular contents of the roots were removed, the fungal structures were stained (Utobo et al. 2011). Roots were immersed in a staining solution of 2% Parker Quink permanent ink in 1% HCl. The roots were heated at 60°C for 30 minutes. After heating, the staining solution was removed and the roots were rinsed in distilled water. Washed roots were placed back in the vials with a destaining solution of 48% glycerol, 4% lactic acid and 48% distilled water, for a period of 2-days. Roots were removed from the destaining solution and 10 of the segments were placed on microscope slides for quantification. Remaining roots were stored in vials containing 50% glycerol.

After the root segments were mounted each microscope slide was scored for both mycorrhizal and non-mycorrhizal structures. Due to time constraints four slides
(out of a potential 10) were scored per species per site, 112 slides in total.

Mycorrhizas can be distinguished from non-mycorrhizal symbioses through a number of characteristics: mycorrhizal hyphae are aseptate, i.e. they do not have cell walls dividing the hyphal cells; the hyphae terminate in structures called arbuscles in the cortical cells; and may also terminate in structures called vesicles (Brundrett, 2009; Seerangan and Thangavelu, 2014; Majewska et al., 2015). In this study I identified three mycorrhizal structures: vesicles, arbuscles and aseptate hyphae (Table 3).

There are also a number of non-mycorrhizal fungal structures that inhabit plant roots. For this study I scored two non-mycorrhizal structures that were easily identifiable. These were dark septate endophytes, and chytrid spores (Table 3). Dark septate endophytes are currently classified as either conidial or sterile fungal endophytes, which form septate melanised inter- and intracellular hyphal and microsclerotia structures (Mandyam & Jumpponen, 2005; Rodriguez et al., 2011).
Table 3 Plates of fugal structures identified from microscope slides, along with their distinguishing features and ecological role.

<table>
<thead>
<tr>
<th>Fungal functional type</th>
<th>Fungal structure</th>
<th>Diagnostic feature</th>
<th>Ecological Role</th>
<th>Reference</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycorrhizal</td>
<td>Aseptate hyphae</td>
<td>Filamentous fungal structure, without segments</td>
<td>Propagate the association between host and fungi</td>
<td>Brundrett, 2004; Seerangan and Thangavelu, 2014; Majewska et al., 2015</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Vesicle</td>
<td>Spherical structure joined to the terminating end of the hyphae</td>
<td>Storage of host derived nutrients</td>
<td>Brundrett, 2004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arbuscle</td>
<td>Branched structure joined to the terminating end of a hyphae</td>
<td>Major site of mineral and nutrient exchange between host and fungi</td>
<td>Brundrett, 2004, Seerangan and Thangavelu, 2014; Majewska et al., 2015</td>
<td></td>
</tr>
<tr>
<td>Fungal functional type</td>
<td>Fungal structure</td>
<td>Diagnostic feature</td>
<td>Ecological Role</td>
<td>Reference</td>
<td>Example</td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
<tr>
<td>Non-mycorrhizal</td>
<td>Chytrid spores (e.g. genus Olpidium)</td>
<td>Heptagonal structures not connected to hyphae</td>
<td>Germinate into reproductive structures which may be parasitic or saprobic</td>
<td>James et al., 2006</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>Dark Spetate endophytes</td>
<td>Filamentous fungal structure, with segments</td>
<td>Currently unknown, but it is likely that there are both pathogenic and mutualistic species. Mutualistic species may have a role similar to that of mycorrhizal fungi</td>
<td></td>
<td>Mandyam &amp; Jumpponen, 2005; Rodriguez et al., 2011</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Very little is presently known about the function of dark septate endophytes, though their broad host range and high abundance suggests that it might be integral for ecosystem functioning (Mandyam & Jumpponen, 2005; Rodriguez et al., 2011). It has been proposed that they may increase host fitness by facilitating host mineral nutrient uptake, degrading complex organic material, improving host water uptake and drought/heat tolerance and providing protection from herbivores and pathogens (Mandyam & Jumpponen, 2005; Rodriguez et al., 2011). In contrast, some research suggests that they are parasitic or pathogenic, and decrease host plant fitness by altering resource allocation (Rodriguez et al., 2011). Chytrid spores, are the resting spores of the fungal phylum Chytridiomycota (Chytrids) (James et al., 2006). These spores will eventually germinate and form a new zoosporangium (reproductive structure) (James et al., 2006). Chytrids may be saprotrophic or parasitic (James et al., 2006).

Figure 9 Image of microscope slide with L. longifolia root segments. Each cross is an intersection. The slide is moved back and forth under the microscope until 75 intersections have been scored for fungal structures.
The presence of AMF was scored for each microscope slide using the magnified intersections method outlined by McGonigle et al. (1990). Roots were brought into focus under the microscope so that one line of the cross-hatch reticule in the eyepiece was perpendicular to the root samples (McGonigle et al., 1990). Prior to further examination the average width of each root sample was estimated and recorded. When the cross-hatch aligned with a root segment it was termed an intersection and the sample was scored for the presence of a number of fungal structures (McGonigle et al., 1990). The slide was then moved back and forth under the microscope in a zig-zag pattern until 75 intersections had been scored (Fig. 9) (McGonigle et al., 1990). Due to time constraints, I first aimed to optimise my sampling by working out the number of intersection required to reduce fluctuations in estimates of error variation. I did this by sampling 5 roots with evidently high variation in the spatial distribution of fungal structures across the roots, quantifying the abundance of fungal structures, and then calculating change in standard deviation with increasing sampling effort (Figure 10). My level of optimal sampling was chosen as the point at which the estimate of the standard deviation did not decrease with increasing sampling effort; i.e. at approximately 75 intersections.
Figure 10 Estimate of fungal colonization made using the magnified intersections method. Data is presented as cumulative average of % fungal colonization after each additional intersection is scored.

2.2.4 Data analyses

General linear mixed models were used to examine the variation in the percentage of root colonised by each of the fungal structures between the two habitat types (i.e. remnant and reconstructed dunes, considered to be fixed effects), as well as amongst the 14 sites (i.e. random effects). These analyses were performed using the statistical package JMP 11. Data were square root transformed as necessary to normalise the distribution of residuals and improve homogeneity of variance. Where significant effects were found, post hoc comparisons between means were conducted using the Tukey Honestly Significant Difference (HSD) multiple comparison test. Furthermore, regression analyses were performed to determine the relationship between percentage root colonisation of fungal structures and plant size.

The compositional differences in fungal structure assemblages between habitat types and sites were compared using a distance-based permutational multivariate analysis of variance (PERMANOVA), performed with the statistical package
PRIMER 7 (Clarke and Gorley, 2015). Estimates of compositional similarity between habitat types and amongst sites were determined using a Bray-Curtis Similarity matrix (Clarke, 1993; Clarke and Gorley, 2015). All analyses were completed using both presence/absence and percentage abundance data, to ensure that the contribution of less common fungal structures to the composition of the assemblage were detected. The compositional differences in fungal assemblages between habitat types and sites were also visualised using non-metric multidimensional scaling (nMDS) ordination plots (Clarke, 1993).

2.3 Study 2 – Testing the facilitative effects of mycorrhizal inoculation on dune restoration, using field and mesocosm experiments

The facilitative effects of mycorrhizae on native plant seedling establishment and growth in reconstructed dunes were tested using field and mesocosm-based experiments. Both experiments consisted of inoculating nursery-grown native plant seedlings with soil derived from remnant dunes (i.e. those containing remnant native vegetation), planting them into reconstructed dunes (i.e. those in which vegetation was cleared upon European colonisation but where vegetation has been replaced over the past one to two decades), and monitoring rates of seedling establishment and growth through time. Details on how each experiment was carried out are provided in sections 2.3.1 and 2.3.2 below.

2.3.1 Study species

*Lomandra longifolia* (refer to section 2.2.1)
*Poa labillardieri* (common name: common tussock grass) is a dense perennial grass, which is common in moist habitats in southern and eastern Australia (Jacobs *et al.*, 2008). It has very long coarse leaves, which are mostly basal and 80cm in length (Jacobs *et al.*, 2008). It produces terminal inflorescences and flowers most of the year (Jacobs *et al.*, 2008). *P. labillardieri* is known to form mycorrhizal relationships, but the average extent of root colonisation has not been noted in the literature (Hayes *et al.*, 2003).

Figure 11 *Poa labillardieri* at Fairy Meadow Beach
2.3.2 Field-based experiment

The field-based experiment was performed at six reconstructed fore dunes within the Illawarra region, which were being revegetated by local land managers and restoration practitioners at the time of the study. The experimental plants consisted of seedlings of *Lomandra longifolia* and the tufted grass *Poa labillardieri*. These two species both occur naturally along the coast and are commonly used by contractors in the restoration of coastal dune habitats of the Illawarra region (A Bearsdmore, 2015 pers. Comm.). They are also known to form AMF associations (Logan et al., 1989; K. French unpub data; Hayes et al., 2003) and may thus experience inhibited establishment and growth if their mycorrhizal mutualists are not present within the soil of reconstructed dunes.

For the field experiment, a total of 130 plants of each of the two plant species were obtained from the Wollongong Botanic Garden’s Greenplan Nursery (21st of April 2015), which supplies local land managers with plants with which to revegetate reconstructed dunes across the Illawarra (A Bearsdmore, 2015 pers. Comm.). Seedlings were propagated from locally-sourced seeds under sterile conditions, and grown within sterile potting mix in 50 × 50 × 125 mm plastic growth-tubes. Seedlings were of similar sizes prior to inoculation and introduction into the field, as measured by average (± 1 SD) vertical height of tallest growing leaf per plant: *L. longifolia*, 50.23 (± 8.84) cm; *P. labillardieri*, 44.83 (± 8.23) cm.

Prior to introduction to the field, seedlings were inoculated with a mix of soil extracted from remnant dunes in which residual vegetation had been present since European colonisation, following methods adapted from Utobo et al. (2011) and Johnson (1993). The three dunes from which soil was extracted were Puckey’s Estate (34°24’24”S, 150°54’5”E), Seven Mile Beach (34°50’7”S, 150°44’46”E) and
Bellambi Lagoon (34°22’31”S, 150°55’24”E). They were extracted from these sites on the 30th of April, 5th of May and 11th of May respectively. At each dune site, approximately 25 sub-samples of 250g of soil were extracted and bulked into one homogeneous soil sample of greater than 5 kg in weight. The 25 sub-samples were selected at random from along a 150 m transect running parallel to the shoreline. This sampling technique was chosen to take into account AMF propagule variability across each site, as studies have shown that mycorrhizae tend to be spatially aggregate at fine scales (Sylvia 1986; Friese and Koske 1991; Pringle & Bever, 2002).

To create the inoculate, 5 kg of the collected soil from each site was suspended in 25 l of tap water and mixed vigorously. This process frees the mycorrhizal spores from the soil particles and root fragments (Utobo et al., 2011). The mixture was then left for approximately 45 s so that the heavier particles in the suspension could settle out. After the mixture had settled, the supernatant was then decanted through a 1mm sieve. This sieve size let both mycorrhizal and bacterial spores through whilst removing particulate soil material (Gerdemann & Nicolson, 1963; Utobo et al., 2011). Prior to the application of the inoculate, ten seedlings of each species were harvested in order to determine a baseline rate of fungal root colonisation from seedlings obtained from the nursery. Due to time constraints, I was unable to analyse these, but was able to examine root samples obtained from the nursery one year prior. For *L. longifolia* 0.11% of plants were associated with mycorrhizae and of these the average colonisation rate was 34.75 ± 7.62% (B. Gooden, unpub. data). For *P. labillardieri* 0.06% of plants were associated with mycorrhizae and the average colonisation rate was 1.75 ± 1.25% (B. Gooden, unpub. data). From this we can infer that my plants would have been predominantly non-mycorrhizal before application of the mycorrhizal inoculate.
For each species the remaining 120 seedlings were then randomly allocated to either the inoculate treatment or left as un-inoculated controls. The inoculate was applied by slowly pouring 125 ml of the supernatant over each of the 60 seedlings per species until the soil was saturated. The control seedlings were given 125ml of water. The plants were inoculated three times over a 3-week period from 30\textsuperscript{th} of April to the 11\textsuperscript{th} of May.

Two plots of 20 m x 20 m were positioned at each of the 6 dune sites, with one plot used for the establishment of inoculated plants and the other for the non-inoculated control plants. These two plots were separated from one another by at least 40 m along the shoreline, in order to limit the potential connection of the inoculated and control plants via existing mycorrhizal networks (Sawyer \textit{et al.}, 2001; Simmard and Durall, 2004). Previous studies on the spatial distribution of mycorrhizal networks in coastal dunes have shown that fungi within the soil are highly spatially aggregated, with spores forming very small (<10 cm scale) and dense clusters within the soil, often not associated with existing plant roots (Sylvia 1986; Friese and Koske 1991). Given that mycorrhizal spores are not readily dispersed from their points of origin (Friese and Koske 1991) and the short period of this experiment (~4 months), it is unlikely that the fungi within the inoculated seedlings influenced the growth of the control seedlings that were planted over 40 m away within the same dune system.

Plants were introduced at the six dune sites from the 14\textsuperscript{th} of May to the 19\textsuperscript{th} of May. Ten plants of each species (i.e. 20 plants in total) were planted randomly within each 20 m x 20 m plot, equating to a total of 40 seedlings per dune site (Fig. 11). Within each plot the seedlings were planted greater than 1 m apart. After each plant had been planted they had their height measured, were watered and then tagged. On the 7th of October each of the sites were visited and plant survival and predation were
recorded for later analysis. Unfortunately, many of the plants were either dead or had
been severely attacked by rabbits and I was thus not able to harvest these plants and
gain meaningful information on their biomass. I decided to analyse whether
inoculation influences likelihood of plant survival on the dune, which is an important
and often costly component of a restoration programme. My future aspiration is to
monitor these plants and harvest them once they have grown to reproductive maturity.

2.3.3 Data analyses

General linear mixed models were used to examine the variation in survival
rates of the plants between treatment types (i.e. inoculated and uninoculated,
considered fixed effects) and species (i.e. L. longifolia and P. labillardieri, random
effects) using the statistical package JMP 11. Treatment type was considered a fixed
factor and species a random factor nested within treatment type. Sites where plants no
longer existed due to anthropogenic interference were discarded. Further, percentage
survival was calculated from those left at the sites, as I could not determine whether
the ones missing had died or had been removed by anthropogenic interference.
Figure 11 Sampling design used to examine the facilitative effects of mycorrhizal inoculation on dune restoration. The field experiment was conducted at six sites currently undergoing revegetation programs, the mesocosm experiment was carried out at the Nowra mesocosms. Experimental species were *Lomandra longifolia* (L) and *Poa labillardierei* (P). Plants were either treated with a fungal inoculate (YES) or treated with a control (NO).
An additional inoculation experiment was undertaken using dune mesocosms, located at the University of Wollongong’s Shoalhaven Campus (34°53’16”S, 150°34’4”E). The mesocosm facility consisted of 18 galvanised iron tanks (height: 120 cm; radius: 105 cm) filled with marine-derived sand, similar to that used for dune reconstruction. This experiment was done for three main reasons: (1) it permitted the inoculated and non-inoculated control seedlings to be grown separately, ensuring that seedling growth responses were truly independent of one another; (2) it enabled me to examine the sole effects of the addition of mycorrhizae on seedling establishment, growth and root colonisation, without the additional influences of attack by native and introduced herbivores; (3) it reduced the influence of variable soil and climatic conditions on seedling growth, so that plant responses to inoculation could be standardised across the seedlings.

On the 25th of May 108 *Lomandra longifolia* seedlings were planted at the Nowra dune mesocosms, 54 of which were inoculated and 54 of which were non-inoculated control plants (Fig. 11). The procedures for plant purchase, growth and inoculation were identical to those used in the field experiment. Within each mesocosm I planted six seedlings, with nine mesocosms containing inoculated plants and nine mesocosms containing non-inoculated control plants. Seedlings were harvested on 15th September, by carefully excavated around the roots. After excavation the roots and shoots were separately bagged. The roots were rinsed to remove excess soil and then a small handful were taken (<3mm in width) and placed in 70% ethanol for later AMF assessment. Roots and shoots were placed in the oven at 60°C for 4 and 3 days respectively. After drying root and shoot biomass were recorded for each plant.
2.3.5 *data analyses*

A general linear mixed model was used to examine the variation in root and shoot biomass between treatment types (inoculated and uninoculated) and tanks using the statistical package JMP 11. Treatment type was considered a fixed factor and tank a random factor nested within treatment type.
Chapter 3 – Results

3.1 Study 1 – Comparison of rates of fungal colonisation of native roots between restored and remnant dune sites

3.1.1 General description of rates of fungal colonisation

In total I quantified rates of colonisation of plant roots by fungi across 8,400 root intersections. Across species, sites and habitat types, the most commonly identified fungal structures were aseptate mycorrhizal hyphae (38.87 ± 2.4%), followed by septate hyphae of dark septate endophytes (23.82 ± 1.9%).

Relative rates of fungal colonization varied between C. glaucescens and L. longifolia (Fig 12). For C. glaucescens the most common fungal structures identified were aseptate mycorrhizal hyphae, which had an average root colonization value of 31.38 ± 3.8%. This was closely followed by the hyphae of the dark septate endophytes, which had an average root colonization value of 29.88 ± 2.8%.

Arbuscles were the least common fungal structure with only two being identified over the 4,200 intersections examined. For L. longifolia aseptate mycorrhizal hyphae were also the most common fungal structure at 46.36 ± 2.5%, followed by arbuscles with 22.83 ± 2.0%. Chytrid spores were the least common structure at 0.38 ± 0.2%.

There was no significant correlation between levels of mycorrhizal colonisation (vesicles, arbuscles, aseptate hyphae) and size of plant for L. longifolia (height: $F_{1,54} = 0.4531$, $p = 0.5037$; width: $F_{1,54} = 0.1297$, $p = 0.7202$) or C. glaucescens ($F_{1,54} = 1.6725$, $p = 0.2014$). Neither were there any correlation between levels of endophytic colonization (septate hyphae and chytrid spores) and size of plant, L. longifolia (height: $F_{1,54} = 0.0037$, $p = 0.9519$; width: $F_{1,54} = 0.0004$, $p = 0.9845$), C. glaucescens ($F_{1,54} = 0.9526$, $p = 0.3334$).
3.1.2 Comparison of fungal colonisation rates between remnant and reconstructed dunes

There were no significant differences in rates of colonisation of *C. glaucescens* roots by any fungal group between remnant and reconstructed habitats (Table 4, Fig. 13). There were, however, differences in rates of *C. glaucescens* root colonisation by mycorrhizal vesicles and aseptate hyphae amongst study sites (Table 4, Fig. 13). Vesicles were detected in *C. glaucescens* roots at only nine of 14 sites, and at sites where vesicles were detected the rates of root colonisation ranged from approximately 1 to 15%. Tukeys HSD test could not be used to determine which sites were significantly different, but we can assume the site with the highest rate of colonisation was significantly different from the site with the lowest. In contrast, aseptate hyphae were detected at all sites, but colonisation rates varied substantially, ranging from approximately 2 ± 1.6% at Perkins Beach (34°31’31”S, 150°52’37”E) to
70 ± 6.7% at Corrima Beach (34°22’43”S, 150°55’11”E). There was also a trend (i.e. $P = 0.0506$) towards significant variation in rates of chytrid spore colonisation across sites (Table 4, Fig. 13). Arbuscules were detected at only two of the 14 sites at extremely low abundances (i.e. < 1%), and thus were not included in analyses. Interestingly there was some correlation between fungal structures, with sites with high percent colonization of aseptate hyphae also having a high percent colonisation of septate hyphae ($F_{1,54} = 19.835$, $p = <.0001^*$) (Fig. 13).

Table 4 Results of general linear mixed models comparing the abundance of specific fungal structures for *Carpobrotus glaucescens* between habitat types (reconstructed and remnant) and sites. Bold values indicate significant effects

<table>
<thead>
<tr>
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<th>Predictor variable</th>
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<th>SS</th>
<th>F</th>
<th>p</th>
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For *L. longifolia*, there were also no significant difference in rates of fungal colonisation between remnant and reconstructed habitats, although there was a trend towards significance for septate hyphae ($p = 0.0556$) (Table 5, Fig. 14). There were, however, statistically significant differences in rates of *L. longifolia* root colonisation.
between study sites for septate hyphae (Table 5, Fig. 14). Septate hyphae were detected at all sites with average root colonization values ranging from 3% at Puckey’s Estate (34°24’24”S, 150°54’5”E) to 46% at Killalea Beach (34°20’35”S, 150°55’25”E). Chytrid spores were only detected at 3 of the 14 sites at abundances no higher than 3% and were thus not included in the analyses. There were again correlations between fungal structures, with sites with high percent colonization of arbuscles also having high percent colonization of aseptate hyphae (F$_{1,54}$ = 59.145, p = <.0001*) (Fig. 14).

Table 5 Results of general linear mixed models comparing the abundance of specific fungal structures for *Lomandra longifolia* between habitat types (reconstructed and remnant) and sites. Bold values indicate significant effects.

<table>
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Figure 13 Mean (±SE) abundance of fungal structures for *C. glaucescens* by sites (each bar, n=4). Sites are further broken down by habitat type (remnant and reconstructed). Letters denote significant differences in fungal structure abundance between sites, determined by a two-way ANOVA and Tukeys HSD tests. There was a significant difference of vesicle colonization rates between sites, but these differences were not picked up by the Tukeys HSD tests, at the very least the site with the highest colonization is different from the site with the lowest. Please note the difference in y-axis ranges between graphs.
Figure 14 Mean (±SE) abundance of fungal structures for *L. longifolia* by sites (each bar, n=4). Sites are further broken down by habitat type (remnant and reconstructed). Letters denote significant differences in fungal structure abundance between sites, determined by a two-way ANOVA and Tukeys HSD tests. Please note the difference in y-axis ranges between graphs.
3.1.3 Comparison of fungal communities between remnant and reconstructed dunes

The composition of fungal structures within *C. glaucescens* roots varied significantly amongst sites, based on the relative presence and abundance of each fungal structure (Table 6). However, fungal composition did not vary significantly between remnant and reconstructed dune habitats (Table 6). Compositional differences amongst sites, based on fungal abundance, are clearly visible in nMDS plots, with clustering of Bellambi Lagoon, Corrimal Beach and Killalea Beach being the most pronounced (Fig. 15). However, based on fungal presence/absence data, most sites overlap completely within the nMDS plot, indicating that most sites have exactly the same suite of fungal structures present with plant roots (Fig. 15). Presence/absence analysis revealed that sites varied by 21.76% based on what fungal structures were present, with abundance explaining a further 29.63% of the variation.

The composition of fungal structures within *L. longifolia* roots also varied significantly amongst sites, based on both abundance and presence of each fungal structure (Table 6). Composition did not however, differ significantly between remnant and reconstructed habitats (Table 6). Compositional differences in fungal structure abundance between sites are clearly visible in nMDS plots, with clustering of Bellambi Beach, Seven Mile Beach and Bellambi Lagoon being the most distinct (Fig. 16). As with *C. glaucescens*, nMDS plots based on fungal presence/absence data show a high level of overlapping, suggesting that most sites have the exact same suite of fungal structures present (Fig. 16). Presence/absence analysis determined that 10.18% of variation between sites was explained by which fungal structures were present, a further 24.74% by their abundance.
Table 6 Results of PERMANOVA models of the variation of fungal structure assemblages for *Carpobrotus glaucescens* and *Lomandra longifolia* versus habitat type: reconstructed and remnant, and site. Bold indicates significant effects. Parenthesis in response variable indicates data transformation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Response variable</th>
<th>df</th>
<th>SS</th>
<th>Pseudo-F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. glaucescens</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predictor variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal structures (Presence/absence)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>1</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Site(Habitat)</td>
<td>12</td>
<td>7273.7</td>
<td>1.8705</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td>13286</td>
<td></td>
<td></td>
<td></td>
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<td>Fungal Structures (abundance)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
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<td>1176.5</td>
<td>0.46438</td>
<td>0.747</td>
<td></td>
</tr>
<tr>
<td>Site(Habitat)</td>
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<td>30472</td>
<td>1.8936</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>41</td>
<td>54981</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. longifolia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal structures (Presence/absence)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>1</td>
<td>130.72</td>
<td>0.86016</td>
<td>0.468</td>
<td></td>
</tr>
<tr>
<td>Site(Habitat)</td>
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<td>1823.7</td>
<td>1.9696</td>
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<td></td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>3240.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal Structures (abundance)</td>
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<td></td>
<td></td>
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<tr>
<td>Habitat</td>
<td>1</td>
<td>1360</td>
<td>1.0807</td>
<td>0.392</td>
<td></td>
</tr>
<tr>
<td>Site(Habitat)</td>
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<td>15102</td>
<td>2.0516</td>
<td>0.006</td>
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</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>25763</td>
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</tr>
</tbody>
</table>
Figure 15 Non-metric multidimensional scaling ordination (nMDS) of a. presence/absence and b. abundance of fungal structure assemblages in *Carpobrotus glaucescens* by site (n=4). Each point signifies a plant. Points closer together indicate more similar fungal structures assemblages based on the Bray-Curtis indices of dissimilarity.
Figure 16 Non-metric multidimensional scaling ordination (nMDS) of a. presence/absence and b. abundance of fungal structure assemblages in *Lomandra longifolia* by site (n=4). Each point signifies a plant. Points closer together indicate more similar fungal structures assemblages based on the Bray-Curtis indices of dissimilarity.
3.2 Study 2 – Testing the facilitative effects of mycorrhizal inoculation on dune restoration, using field and mesocosm experiments

3.2.1 Field based experiment

The effect of inoculation on survival rate differed amongst species ($F_{1,12} = 6.52, p = 0.0253$) (Figure 17). Inoculation made no difference to L. longifolia as it had 100% survival in both treatments, but did improve survival in P. labillardieri. These results must be interpreted carefully as they are based off only a small number plants dying (7/75 P. labillardieri).

Figure 17 Comparison of survival rate ($\pm$SE) between species (Poa labillardieri and Lomandra longifolia) and treatment type (inoculated (Y), uninoculated (N)) in the field (For Poa n=75 (38=Y, 37=N); For Lomandra n = 77 (34=Y, 43=N). Darker shaded columns indicate mycorrhizal inoculation; lighter shaded columns are uninoculated controls. Letters denote significant differences between treatment types, determined by a two-way ANOVA and Tukeys HSD tests.
3.2.2 Mesocosm experiment

There were no significant differences detected between inoculated and uninoculated treatments for any of the biomass measures. There was however a trend towards significance for root/shoot ratio, with the root/shoot ratio of the uninoculated treatments being on average slightly higher (Table 7, Fig. 19). There were also no significant differences detected between tanks, although there was a trend towards significance for total biomass ($p = 0.0631$) and root biomass ($p = 0.0997$) (Table 7). Although not significant inoculated treatments average biomass measures were often greater than the uninoculated controls (Fig. 18).

Table 7 Results of general linear mixed models comparing root and shoot biomass for *Lomandra longifolia* between treatments (inoculated and control) and tanks. Bold values indicate significant effects.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Predictor variable</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>p</th>
<th>$r^2$</th>
<th>Figure reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Shoot biomass</strong></td>
<td>Model</td>
<td>17</td>
<td>363.69</td>
<td>1.6451</td>
<td>0.0691</td>
<td>0.146</td>
<td>Fig. 18a</td>
</tr>
<tr>
<td></td>
<td>Inoculate treatment</td>
<td>1</td>
<td>55.580</td>
<td>2.8852</td>
<td>0.1088</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tank(Inoculate treatment)</td>
<td>16</td>
<td>307.90</td>
<td>1.4798</td>
<td>0.1249</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>90</td>
<td>1396.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Root biomass</strong></td>
<td>Model</td>
<td>17</td>
<td>152.75</td>
<td>1.4846</td>
<td>0.1185</td>
<td>0.129</td>
<td>Fig. 18b</td>
</tr>
<tr>
<td></td>
<td>Inoculate treatment</td>
<td>1</td>
<td>2.6058</td>
<td>0.2753</td>
<td>0.6070</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tank(Inoculate treatment)</td>
<td>16</td>
<td>150.13</td>
<td>1.5504</td>
<td>0.0997</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>90</td>
<td>544.69</td>
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<tr>
<td><strong>Total biomass</strong></td>
<td>Model</td>
<td>17</td>
<td>891.82</td>
<td>1.7517</td>
<td><strong>0.0475</strong></td>
<td>0.170</td>
<td>Fig. 18c</td>
</tr>
<tr>
<td></td>
<td>Inoculate treatment</td>
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<td>82.255</td>
<td>1.6224</td>
<td>0.2209</td>
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</tr>
<tr>
<td></td>
<td>Tank(Inoculate treatment)</td>
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<td>1.6889</td>
<td>0.0631</td>
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<td></td>
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<td>2695.2</td>
<td></td>
<td></td>
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<tr>
<td><strong>Root/shoot ratio</strong></td>
<td>Model</td>
<td>17</td>
<td>0.8889</td>
<td>1.0507</td>
<td>0.4139</td>
<td>0.0006</td>
<td>Fig. 19</td>
</tr>
<tr>
<td></td>
<td>Inoculate treatment</td>
<td>1</td>
<td>0.1758</td>
<td>3.7578</td>
<td>0.0707</td>
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<tr>
<td></td>
<td>Tank(Inoculate treatment)</td>
<td>16</td>
<td>0.7220</td>
<td>0.9045</td>
<td>0.5664</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>90</td>
<td>4.4787</td>
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</tbody>
</table>
Figure 18 Comparison of mean (±SE) biomass for Lomandra longifolia between the two treatments: uninoculated and inoculated (n=54). Note different y-axis scale between biomass types.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot</th>
<th>Root</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td><img src="imagea.png" alt="Graph a. Shoot" /></td>
<td><img src="imageb.png" alt="Graph b. Root" /></td>
<td><img src="imagec.png" alt="Graph c. Total" /></td>
</tr>
<tr>
<td>Inoculated</td>
<td><img src="imagea.png" alt="Graph a. Shoot" /></td>
<td><img src="imageb.png" alt="Graph b. Root" /></td>
<td><img src="imagec.png" alt="Graph c. Total" /></td>
</tr>
</tbody>
</table>
Figure 19 Comparison of mean (±SE) root/shoot ratio’s for *Lomandra longifolia* between the two treatments: uninoculated and inoculated (n=54).
Chapter 4 – Discussion

4.1 Comparison of rates of fungal colonisation of native roots between restored and remnant dune sites

I found that there was no significant difference in either rates of fungal colonisation or composition of the suite of fungal structures within plant roots between remnant and reconstructed coastal dunes. There was considerable variation in fungal structures and colonisation rates of plant roots between adjacent beaches, but this variation did not depend on whether or not the beach contained remnant vegetation or had been reconstructed and revegetated. This result was contrary to what was hypothesized. I predicted that fungal colonisation, particularly of mycorrhizal fungi, would be higher within native plant roots from remnant coastal dune systems than those from native plant roots in reconstructed coastal dune systems. This prediction was made because reconstructed dunes are (1) fabricated with soil that has been modified during sand mining, with little to no residual roots of plants to disperse the mycorrhizal spores and hyphae, (2) revegetated with native seedlings that are propagated in inert growing conditions within a commercial nursery, using sterilised growing media devoid of fungal contaminants, and (3) were almost completely devoid of residual soil or remnant vegetation at the time of construction. Environmental disturbance involving the removal of soil and vegetation has been shown to have a negative effect on AMF communities within coastal sand dune systems (Gemma and Koske, 1992). In a study conducted on Fraser Island, Kurtböke et al. (2007) found that previously mined and rehabilitated dunes had significantly lower percentages of AMF colonisation (65-95%) than natural dune sites (90-95%).
My null findings do support those of other recent studies (Johnson & Wedin, 1997; Picone, 2000; Zhang et al., 2004; Sturmer & Siqueira, 2011; de Souza et al., 2013; da Silva et al., 2015). da Silva et al. (2015) found that revegetated coastal dunes within the Atlantic forest of north-eastern Brazil had higher AMF richness than their remnant counterparts, with twenty nine species registering within the revegetated areas and only seventeen in the remnant. de Souza et al. (2013) within the same study region identified twenty-eight species in revegetated areas and only 10 in their natural counterparts. In both these studies the revegetated dunes had been previously used for mineral extraction, which involves the removal of all vegetation and the majority of the dune material, which is very similar to my study system (de Souza et al., 2013; da Silva et al., 2015). Higher species richness could be explained by the heterogeneity of fungal propagule distribution within the soil as a result of disruptive mining practices along with the introduction of AMF species with the seedlings used for revegetation (de Souza et al., 2013; da Silva et al., 2015). It was also suggested that AMF fungal diversity was higher in these revegetated areas as they found themselves in an enrichment process of species; not only were species arriving with the seedlings used for revegetation but also via nearby native stands of vegetation (de Souza et al., 2013). This may in part explain why we found no variation in fungal colonisation between the habitat types. Although we can discount the arrival of species through revegetation practices due to soil sterilization, it is possible that my sites experienced immigration of fungal propagules from nearby stands of native vegetation over scales of 100s of metres to kilometres. It is well documented that some mycorrhizae have evolved the ability to disperse long distances by small mammals (Gerdemann & Trappe, 1974; Mangan & Adler, 2000; Mangan & Adler, 2002). For example Mangan and Adler (2002) found that the Central American spiny rat (Proechimys
*semispinosus* was a ready consumer and disperser of AMF spores, with spores isolated and cultured from faeces still being viable. It is not unfathomable that something similar may be happening within my study system, with the dispersers in this case being the common rabbit (*Oryctolagus cuniculus*), bush rat (*Rattus fuscipes*), southern brown bandicoot (*Isoodon obesulus*) and other native marsupials known to consume fungi.

Aside from this it has long been thought that dispersal of spores from the immediate soil around the infected root system is limited for the majority of mycorrhizal species (Friese & Koske, 1991; Bever *et al.*, 1996; Pringle & Bever, 2002). This is based off a number of studies finding that both common and rare AMF species spatially aggregate at a fine scale (Friese & Koske, 1991; Bever *et al.*, 1996; Pringle & Bever, 2002) and it is suggested to be, in part, a product of their limited movement, due to underground spore formation (Verbruggen *et al.*, 2013). Recently however it has been suggested that wind dispersal may be the mechanism by which many AMF pioneer species enter disturbed landscapes (Oehl *et al.*, 2011). In a study of the succession of AMF communities in the foreland of retreating glaciers, Oehl *et al.* (2011) found that pioneer AMF species were basically all within a specific size range (80-140µm) and globose in shape, and suggested that this strong selection criteria pointed to wind dispersal. As of yet no studies have been conducted using anemochorous traps to confirm whether AMF spore are indeed being carried on the air currents. In summary, although the reconstructed dunes and the native seedlings used to revegetate them may have initially been devoid of mycorrhizal fungi, the potentially high level of connectivity of fungal communities across the landscape via wind and animal dispersal may have resulted in no difference in fungal communities between remnant and reconstructed dunes.
The level of fungal abundance I found may not be just a question of habitat type but of length of time that the habitat in question had been vegetated and thus susceptible to fungal immigration. This leads into the second explanation as to why no difference in fungi was found between the two dune habitats: that is, the timeframe of reconstruction. It is highly likely that the dune systems that I used as my sites had been vegetated for long enough (>20 years) that the fungal community had had enough time to completely repopulate the area. This would concur with a study by Greipsson and El-Mayas (2000) on the occurrence of AMF at natural and reclaimed sand dune sites in Iceland. They found that there were no AMF spores in barren sands (vegetation free areas), low levels of spores in the 1-5 year old reclamation sites and then significantly higher levels of spore abundance and root colonization levels in the 10 year of reclamation site and natural old dune system (Greipsson & El-Mayas, 2000). AMF colonization and spore numbers did not in fact significantly differ between the 10 year old site and the natural dune system (Greipsson & El-Mayas, 2000). This suggests that after ten years immigration of AMF propagules into reclamation sites is high enough that the AMF communities become functionally similar to a natural reference dune that contains remnant vegetation. Likewise Jasper et al. (1987) found that the level of viable AMF propagules in mine-disturbed areas returned to that of the nearby native forest after only 4 years. Greipsson and El-Mayas (2000) suggested that such a quick AMF community recovery may be a result of wind dispersal, but otherwise the mechanisms behind this have not been explored. Based on previous research (Jasper et al., 1987; Greipsson & El-Mayas, 2000; de Souza et al., 2013) it is likely that reconstructed dunes are initially limited in the availability of mycorrhizal fungi, which may hamper the establishment and growth of native
seedlings at early stages of revegetation. However, given sufficient time, it is probable that the mycorrhizal network of plants and fungi becomes sufficiently reconnected.

### 4.2 The facilitative effects of mycorrhizal inoculation on dune restoration, using field and mesocosm experiments

Despite inoculation of nursery seedlings prior to establishment, there were no discernible differences in shoot and root biomass between inoculated and uninoculated *Lomandra longifolia* plants established at the mesocosms. Neither were there any differences in survival rate, with all plants from both treatments surviving up until harvest. Interestingly the application of a mycorrhizal inoculate to *P. labillardierei* prior to field transplantation, significantly but only very moderately improved their chance of survival. Of the 75 seedlings that were still present at the reconstructed dunes, seven had died, six of which were uninoculated. This result wasn’t common across species with both inoculated and uninoculated *L. longifolia* treatments recording 100% survival.

Aside from the moderate improvement in survival recorded for *P. labillardierei*, these results did not agree with my predictions nor were they consistent with the majority of the literature on this subject. I predicted that inoculated plants would have a higher survival rate and greater overall shoot and root biomass. It was assumed that this would occur as it is well established that AMF fungi can improve plant growth and survival by increasing the interface between them and the biological and physical environment (van der Heijden *et al.*, 1998; Harris, 2009). They enhance plant nutrient uptake allowing them to reallocate valuable resources to growth and reproduction rather than nutrient acquisition (van der Heijden *et al.*, 1998; Harris, 2009). Further to this, 82% of all the studies (n= 28; Table 1) that I found, that
investigated the use of AMF inoculation in landscape restoration found a significant improvement in plant growth and survival along with plant root colonization when plants were inoculated with AMF propagules. Inoculated plants had greater shoot and root biomass, nutrient uptake, basal diameter, inflorescence production, survival and tiller and panicle production (Richter & Stutz, 2002; Caravaca et al., 2003; Zhang et al., 2011). Only 18% of the papers I reviewed found that inoculation did not significantly improve plant growth and survival or root length colonization. Of these papers all gave reasons as to why inoculation showed no improvement. In their study on the restoration of a semi-arid degraded steppe, Maestre et al. (2002) suggested that the effect of inoculation on seedling survival in the field was most likely circumvented by drought summer conditions increasing fungal mortality rates. Other explanations given for such a result included that the potential benefit of nursery inoculation was masked by the natural colonization of seedlings by remnant AMF populations within the soil (Maestre et al., 2002; White et al., 2008; Cook et al., 2011), that the inoculum used was not adapted for the site-specific conditions, or that the inoculation procedure was not successful (Walker, 2003).

In my study, most of these reasons seem highly unlikely. The AMF inoculate used was taken from a number of natural reference sites that were as close to the experimental site as possible suggesting that the inoculum would have been adapted to the site-specific conditions. It is improbable that the inoculation procedure was unsuccessful as it was adapted from techniques successfully used for the collection of fungal spores (Gerdemann & Nicolson 1963; Utobo et al., 2011), further we found a moderate but significant effect of inoculation on plant survival within the field experiment for P. labillardierei. It is possible that resident AMF populations masked some of the benefits of inoculation within the field as I determined in study one that
fungal abundance did not significantly differ between remnant and reconstructed habitat types. This could also be the case at the mesocosms, with AMF being introduced to the tanks via past experiments and through wind borne dispersal of fungal propagules.

What is more likely is that the temporal limitations of the study did not allow enough time to see a benefit in terms of growth. Currently very little is known about how long it takes for plants to experience benefits after initial AMF colonisation. In terms of the rate of root length colonisation, we know that it increases exponentially with time before eventually plateauing (Buwalda et al., 1982; Stahl et al., 1988). In the two species that Buwalda et al. (1982) examined, spring wheat (*Triticum aestivum* cv. Highbury) and white clover (*Trifolium repens* cv. Huia), the plateau of root length colonisation was reached after approximately 50 days. In the literature I reviewed on AMF inoculation the earliest point at which a measure of plant fitness was taken for a greenhouse experiment and returned a significant result was 45 days (Stahl et al., 1988) and for a field experiment 84 days (Allen et al., 2005). In Stahl et al.’s (1988) study plant growth parameters were recorded earlier, but there analysis was not noted within the paper. In Allen et al.’s (2005) study no earlier measures were taken. As we do not have analysis of these growth parameters from initial plant inoculation we cannot determine at which point the treatment began to significantly improve plant performance. It might, in fact, be the case that plant performance does not improve measurably until after root length colonisation has plateaued. Another factor that might influence the point at which plant performance begins to measurably improve is the conditions under which inoculation occurred. Within the published literature plants were grown in the greenhouse after inoculation for 174 days (on average) before being transplanted into the field. The shortest timeframe over which plants
were grown after inoculation under nursery conditions was 30 days (Zhang et al., 2011). In this experiment plants were grown under nursery conditions for only 14 days after initial inoculation. As a result root length colonisation most likely had not plateaued before they were transplanted into their field sites. As the biophysical parameters were much harsher within the field, plant root length colonisation may have considerably slowed meaning that the benefits of AMF colonisations had a slower onset. This being the case an inoculation period of 126 days, of which this study had, may not have been long enough to see a significant difference in plant growth.

What is interesting though is that it seems to have been long enough for the inoculate to have a moderate but significant effect on the survival rate of *P. labillardierei*. That this effect was not the same for *L. longifolia* is not entirely surprising, as our species are likely to have different physiological tolerances. In Allen et al.’s (2005) study on the effect of inoculum type on the restoration of a seasonal tropical forest, there was also some species survival rates which were unaffected by inoculation. *Piscidia piscipula* for example retained its 100% survival rate for all treatment types for the three years in the field (Allen et al., 2005). Both *Piscidia piscipula* and *L. longifolia* are hardy, drought tolerant plants so it is unlikely that the stressors involved in transplantation to a disturbed landscape would be high enough for AMF inoculation to significantly affect survival. *P. labillardierei* on the other hand has been shown to be less tolerant to stressful conditions (M. Davies, unpub data). It is however, likely that the effects of inoculation would be more apparent for other growth parameters such as above and below ground biomass or plant height. For *Piscidia piscipula* in Allen et al.’s (2005) study this was indeed the case, with plant height being significantly greater in inoculated treatments after three
years in the field. Based on these results it appears as though pre-inoculation does not measurably improve plant performance in the field for the first four months after transplantation. However, it is possible that transplanting the seedlings so soon after inoculation may have slowed AMF colonisation and thus the benefits it confers.

4.4 Study constraints and future research

My first study looked at the level of AMF colonization by investigating the presence and abundance of a number of fungal structures within plant roots. While this may have been an adequate means by which to discern functional differences in plant-fungal interactions in the soil between recently disturbed and remnant coastal dune sites, I was not able to determine whether the identity or diversity of the fungal species differed across dune habitats. Differences in AMF identity and diversity between disturbed and undisturbed landscapes are well documented (Allen et al., 1998; Greipsson and El-Mayas, 2000; Allen et al., 2003). Allen et al. (1998) found that AMF communities shifted from diverse suites of fungi to ones dominated by fewer *Glomus* species with large-scale conversion of tropical forests to grassland. It has been suggested that disturbed environments have higher proportions of sporulating fungi, as soil disturbance selects for the more easily cultivatable species (Ohsowski et al., 2014). Indeed, in studies where no significant difference in AMF diversity was recorded between remnant and reconstructed habitats it was suggested that this may have been the result of the analyses relying heavily on sporulating mycorrhizae (Picone, 2000; Sturmer & Siqueira, 2011). Natural areas could potentially have higher levels of non-cultivatable mycorrhizae, but as these are difficult to isolate and identify these differences aren’t being documented (da Silva et al., 2015). Future research should examine whether remnant and reconstructed
habitats contain different assemblages of soil fungi, based on relative abundances of different species, rather than simply fungal structures.

The first study was also limited in that it only represented a point in time assessment of AMF colonization within coastal dunes, the scale at which AMF colonization increases from day 1 of dune restoration was not examined. A few studies examining AMF community succession have been conducted overseas (Greipsson and El-Mayas, 2000; Oehl et al., 2011), but as yet such research has not been conducted within Australia. A future avenue of research could thus be looking at AMF colonization rates over a chronosequence of coastal sand dunes. Such a study would involve analysis of AMF colonization and spore abundance at a number of dunes at day one of the reconstruction process (bare sand) and then comparing this to dunes across a range of reconstruction ages (1-20 years since revegetation). This could establish the rate at which AMF colonization occurs across the lifetime of a restoration project, along with the timeframe over which AMF diversity increases before reaching a threshold. This information would allow restoration practitioners to develop a framework that outlines at which point AMF inoculation would be most cost effective, but also best improve restoration potential.

The second study was limited substantially by the timeframe over which it could be completed. Plants were established in the field and mesocosms for just less than 4 months, and although we saw a significant effect of inoculation on survival in the field for *P. labillardierei*, no differences in growth parameters or survival were detected for *L. longifolia* in either the field or mesocosms. To detect these potential differences in growth parameters it would have been ideal to run the experiment for a much longer time period. My review of the literature on AMF inoculation in restoration found that on average inoculation experiments run for approximately two
years (White et al., 2008; Pagano et al., 2009; del Mar et al., 2011), so perhaps this should be used as a yardstick for future experiments. In not finding a result within the given timeframe my experiment highlighted the need for a better understanding of the timescale over which AMF benefits become measureable. Finding a result for survival but not any other growth parameters suggests that perhaps some benefits of mycorrhizal inoculation have an earlier onset than others. Thus it is suggested that future studies should focus on determining a timescale over which plant performance is improved by mycorrhizal inoculation both in the greenhouse and field. It is also suggested that these studies should compare the onset of each plant performance improvement (survival, height, biomass) over the experimental period.

As mycorrhizae are not limited in these reconstructed dunes, nor does mycorrhizal inoculation appear to improve growth and survivorship of seedlings, a focus of future research should be on determining the other limits to dune restoration and the best means by which to manage them. In these systems the other immediate threats to the restoration potential of dunes are chronic disturbance processes such as invasive weeds, introduced predators and human vandalism. For instance, rabbits have been identified by the Wollongong City Council as being highly damaging to both the revegetation and natural regeneration of native revegetation communities. A potential research avenue could be determining the relative costs and benefits of the complete eradication of rabbits from revegetation sites. Beltran et al. (2014) found that significant recovery of native vegetation communities could occur with very little restoration management after herbivore removal. In their study they found a significant transition in vegetation cover from ~74% bare ground/grass to ~77% woody plants, 28 years after herbivore eradication (Beltran et al., 2014). Exclusionary fences as a means of herbivore removal have demonstrated promising results.
(Opperman & Merenlender, 2000; Burns et al., 2012). Oppermann and Merenlender (2000) found that the mean density of saplings inside exclusionary fences was 0.49±0.15/m² in comparison to 0.05±0.02/m² for those outside. Research could compare the recovery of reconstructed dunes with and without herbivore exclusion over a period of time, doing a cost benefit analysis on its validity as a future management tool.

4.3 Conclusions and management implications

The aim of this study was to first determine whether rates of fungal colonization varied between reconstructed and remnant coastal dunes and then assess whether the application of a mycorrhizal inoculate to plants prior to revegetation facilitated their establishment. In conclusion, I found that there were no significant variation in fungal colonization between remnant and reconstructed coastal dunes. It is suggested that this may be a product of the age of the reconstructed dunes (>20 years old) as well as a result of only looking at presence and abundance of fungal structures and not fungal diversity. I also found that inoculation of plants prior to establishment in disturbed coastal landscapes had a variable effect on their growth and establishment. Inoculation seemed to influence the likelihood of survival of *P. labillardierei* in the field but had no effect on *L. longifolia*. Pre-inoculation of *L. longifolia* seedlings before establishment within mesocosms also did not influence their likelihood of survival or improve their growth. It was suggested that no variation in growth was detected between treatments as the length of the study was not adequate enough to see the mycorrhizal connections provide a measureable benefit to the plants growth.
One of the key limitations in the reestablishment of vegetation following disturbance is the health of the soil microbial community, in particular the mycorrhizae (Kardol & Wardle, 2010). Mycorrhizae are essential for the establishment of plant communities, and they also play an important role in management and maintenance of their diversity (van der Heijden et al., 1998; Harris, 2009). These mycorrhizae have been shown, in some cases, to be highly sensitive to disturbance, particularly the removal of vegetation (Allen et al., 1998; Greipsson and El-Mayas, 2000; Allen et al., 2003). Thus recent studies have suggested that their reintroduction should be a critical component of dune reconstruction programmes across disturbed landscapes. My results suggest that AMF inoculation may not be warranted under all circumstances. Similar levels in fungal colonization between reconstructed and remnant habitat types suggests that AMF may not be the limiting factor in the revegetation of these coastal sand dunes at this particular point in time. Thus it might not be cost effective or necessary to use AMF inoculation as a restoration tool in this situation. Instead, restoration should focus on reducing and eliminating the other disturbances that are known to cause declines of native seedlings, including attack from introduced herbivores and vandalism by humans. However, given the short-term nature of my study, it is possible that benefits of AMF inoculation may occur over a much longer time frame and thus should not be completely discounted as a management tool in coastal dune restoration.
References


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(*Chrysanthemoides monilifera* ssp. *rotundata*) in Australia. Department of Environment and Climate Change (NSW), Sydney.


