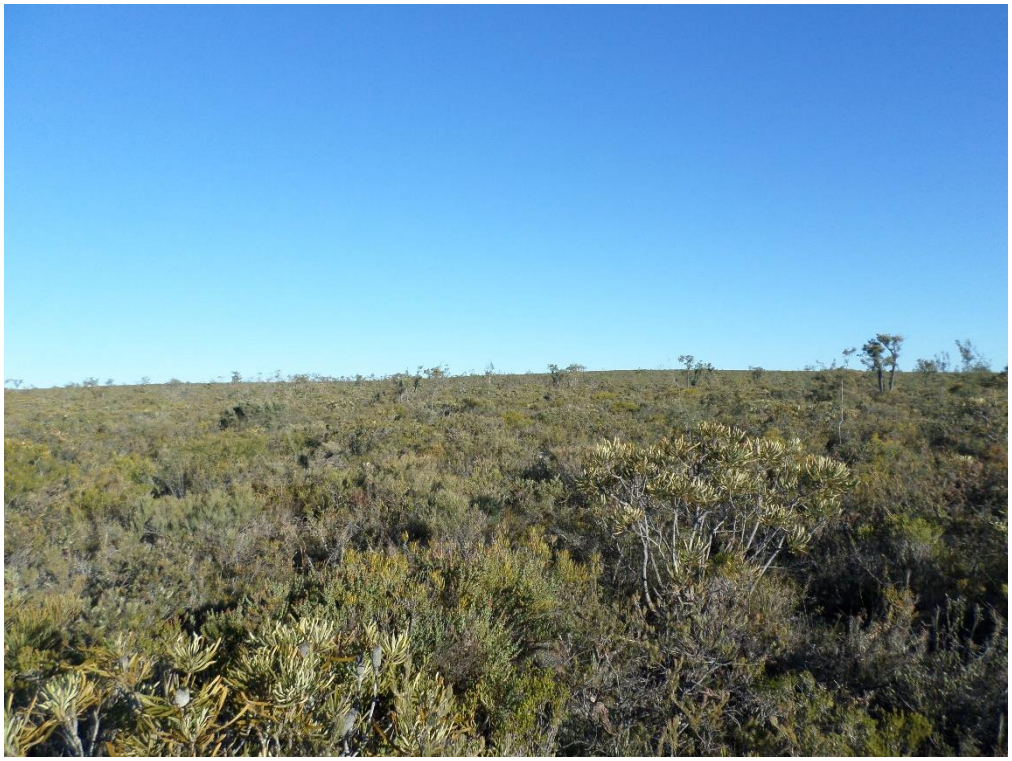
# Damping-off within natural and disturbed kwongan plant communities

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# Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution. To the best of my knowledge, all work performed by others, published or unpublished, has been acknowledged.

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# Abstract

Fungal and oomycete damping-off pathogens kill pre- and post-emergent seedlings and can regulate the abundance of plant species to help maintain diversity in natural ecosystems. However, damping-off pathogens may be detrimental to post-mining ecological restoration, a key hurdle in the process is the loss of pre- and post-emergent seedlings. Recently, several putatively native *Phytophthora* species, soil-borne oomycete plant pathogens, have been recovered from hyper-diverse kwongan vegetation in southwest Australia and may be damping-off pathogens. Damping-off pathogens may contribute to maintaining the diversity of natural kwongan plant communities and reduce seedling establishment within ecological restoration. Four experiments were designed to identify the distribution and role of *Phytophthora* and other potential damping-off oomycetes in natural and restored kwongan plant communities. Putatively native *Phytophthora arenaria*, introduced *P. cinnamomi* and *Pythium irregulare* were identified as damping-off pathogens with wide host ranges of native kwongan plant species through a glasshouse pathogenicity trial. Fungicide seed coat treatments improved seedling emergence for five of the 14 plant species studied in ecological restoration field trials, identifying low to moderate levels of pre-emergent damping-off caused by oomycetes and fungi. Natural kwongan soils collected from different plant species affected seedling emergence and survival in a glasshouse experiment. Damping-off caused conspecific negative plant-soil feedback for *Jacksonia floribunda* and *Xanthorrhoea* sp. Lesueur, a process that contributes to the maintenance of diversity in plant communities. Although, the presence and abundance of oomycetes detected using metabarcoding from the same natural kwongan soils were not associated with reduced seedling emergence or survival. In a regional metabarcoding survey of natural kwongan plant communities, the *Phytophthora* species richness and abundance were far lower than previously hypothesised due to the elimination of sources of sampling biases. Plant species and host age were strong drivers of the oomycete communities detected at a local scale using metabarcoding. These studies provided an insight into the distribution of important plant pathogens in a species rich Mediterranean shrubland and identified damping-off pathogens could be a mechanism contributing to maintaining the diversity of natural kwongan plant communities and low seedling establishment in ecological restoration.

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# List of publications

## Journal articles

Chapter 2 under review as: Shaw, C., Dunstan, W., Hardy, G.E.St.J., Burgess, T.I. 2019. Putative native *Phytophthora* species as damping-off pathogens and functional plant traits associated with susceptibility. Austral Ecology.

## Conference presentations

Shaw, C., Hardy, G.E.St.J., Burgess, T.I. Damping-off of native southwest Australian plant species by *Phytophthora* and *Pythium* species. 2017 8th Meeting of IUFRO Working Party 7.02.09, *Phytophthora* in Forests and Natural Ecosystems. 19th – 24th March 2017, Sapa, Vietnam. Oral presentation.

Shaw, C. Damping-off of native southwest Australian plant species by *Phytophthora* and *Pythium* species. 2017 South Coast *Phytophthora* Dieback Forum. 26 October 2017, Albany, Australia. Oral presentation.

Shaw, C., Dobrowolski, M., Hardy, G.E.St.J., Burgess, T.I. The role of damping-off on the survival of seedlings in conspecific and heterospecific soils. 2018. Conference for the Ecological Society of Australia (ESA). 26th – 30th November 2018, Brisbane, Australia. Oral presentation.

Shaw, C., Dobrowolski, M., Hardy, G.E.St.J., Burgess, T.I. *Phytophthora* and damping-off within natural and rehabilitated plant communities. 2019. 9th Meeting of IUFRO Working Party 7.02.09, *Phytophthora* in Forests and Natural Ecosystems. 19th – 26th March 2019, Sardinia, Italy. Oral presentation.

# Statement of contributors

This thesis was co-supervised by Professor Treena Burgess, Professor Giles Hardy, and Dr. Mark Dobrowolski. Treena, Giles and Mark contributed in the form of ideas, design and editorial assistance. Dr. William Dunstan provided co-supervision to data included in Chapter 2 and is a co-author on the manuscript submitted to Austral Ecology. Diane White completed molecular laboratory work in Chapter 3 and Chapter 5. Volunteers (listed by name in the acknowledgements) provided assistance in the field, glasshouse or laboratory. All chapters that have not been submitted to a journal for review will be published in collaboration with all my supervisors Treena Burgess, Giles Hardy and Mark Dobrowolski.

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All research was conducted with the appropriate approval and reported within the chapters.

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# Chapter 1: Introduction to regional and local drivers of diversity in southwest Australia and kwongan plant communities.

## Introduction

Introduced fungal and oomycete pathogens can have a severe impact on plant species that have not evolved a level of resistance. For example, *Phytophthora cinnamomi* and *P. ramorum* are introduced invasive oomycete plant pathogens and highly destructive to the natural vegetation of Australia and the USA, respectively (Shearer et al. 2007, Grünwald et al. 2012). Comparatively, the impact of native plant pathogens on the composition of natural plant communities may be more subtle but just as influential (Gilbert 2002, Bever et al. 2015)). Understanding the range and ecological role of native plant pathogens is important in a period of increased anthropogenic disturbance (Lewis and Maslin 2015). As plant communities and disturbances change over time understanding the historical vegetation and ecology informs management and helps predict the outcome of new interactions. Research into the native distribution of plant pathogens is crucial due to past and future introductions through international trade (Brasier 2008, Hulbert et al. 2017). The ecological role and distribution of native plant pathogens should be studied thoroughly in plant communities of southwest Australia, particularly on the Geraldton Sandplain, given the floristic diversity (Hopper and Gioia 2004) and the susceptibility of plant species to previous introduced pathogens (Shearer et al. 2004).

## Southwest Australia

The southwest of Australia (SWA) has high plant species richness and diversity. The reported regional species richness of SWA varies depending on the sources reviewed and the level of the taxonomic classification. Beard et al. (2000) estimated that there are 5710 plant species (to the species level and ignoring hybrids) within SWA, of which 3000 (52%) are endemic to the region. When subspecies are included, Hopper and Gioia (2004) estimated that there are 7380 vascular plant species, of which 49% are endemic to the region. Regardless of the taxonomic level assessed, these Mediterranean plant communities are incredibly rich in flora and have a high proportion of endemism. Many of the Mediterranean climate regions

are host to distinct species rich plant communities that rank amongst the highest in the world in terms of regional diversity (Cowling et al. 1996). Of the five Mediterranean climate regions, only the Southwestern Cape of South Africa has greater diversity than SWA (Cowling et al. 1996). Kwongan and other shrubland plant communities of SWA have greater species richness and diversity than woodlands and forests, and this is typical for the majority of the Mediterranean climate regions (Cowling et al. 1996, Hopper and Gioia 2004). For example, Banksia woodlands in SWA typically have 70–80% of the species richness of adjacent kwongan vegetation (George et al. 1979, Brown and Hopkins 1983).

The old, climatically buffered, infertile landscape (OCBIL) theory is a developing series of integrated hypotheses used to explain the evolution, ecology and conservation of landscapes (Hopper 2009). The southwest of Australia is one of 12 OCBIL terrestrial biodiversity hotspots and six of the seven integrated hypotheses can be used to explain the high species richness and diversity of the region (Hopper 2009, Hopper et al. 2016). The reduced dispersability, increased local endemism and common rarity of plant species contributes to the high richness and diversity (Hopper 2009). The seed of SWA plant species have fewer adaptations for long distance dispersal in comparison to young often disturbed, fertile landscapes (YODFL) (He et al. 2004, Hopper 2009). This reduced dispersibility should encourage local genetic divergence and allopatric speciation, resulting in a rapid increase of ancient populations with high levels of interpopulation genetic divergence (Hopper 2009).

Given that the limited dispersal hypothesis leads to increased local endemism, Hopper (2009) states OCBILs should have elevated persistent lineages (Gondwanan Heritage Hypothesis) and long lived individuals (Ultimate Self Hypothesis). The Ultimate Self Hypothesis describes some taxa have had the chance to produce a genotype that has evolved to overcome all environmental challenges and have little need to produce genetic variation to cope with and evolve through environmental change (James 2000, Hopper 2009). The most diverse Mediterranean OCBILs, the Cape Floristic Region and SWA, have had climatically stable histories due to fewer topographic upheavals leading to greater species accumulation and persistence (Cowling et al. 2015). The stability of the SWA climate has given several lineages the chance to persist to the present day (Coates 2000, Yates et al. 2007, Hopper 2009, Mucina and Wardell-Johnson 2011).

Adaptation to the unique environment of SWA has led to high speciation. The deeply weathered and infertile soils of SWA have had a pervasive outcome given the long period of time organisms have had to evolve (Hopper 2009, Hopper et al. 2016). Adaptations of underground structures are prominent and include cluster roots, dauciform roots, increased mutualism with ectomycorrhizal fungi, geophytism, extensive water foraging strategies (Hopper 2009, Hopper et al. 2016) and dual mycorrhizal mutualisms (Teste et al. 2020). There are a high proportion of carnivorous plant species in the most nutrient impoverished soils of SWA. A number of plant species have adapted to become tolerant of saline soils associated with palaeoriver systems of SWA (Hopper 2009, Hopper et al. 2016). Additionally, the Semiarid Cradle hypothesis results in speciation at the margins of SWA driven by the variable climate of semi-arid regions (Hopper et al. 2016).

Climatic and soil gradients partially explain the regional plant species turnover across SWA. Uniform predictors (wet quarter precipitation and radiation seasonality) were the most important climate factors explaining deviance in species turnover (Jones et al. 2016). In contrast, dry quarterly precipitation predicted abrupt transitions in species turnover and suggests tipping points exist where small variations in climate result in large floristic changes (Jones et al. 2016). The composition of the plant community was predicted best by changes in soil nutrients, in particular phosphorus availability (Jones et al. 2016). Rainfall and soil characteristics interact to produce an explanation for the transition of common families and ecosystems through space. The transition from Banksia woodland towards kwongan follows a declining rainfall gradient. Woodlands cannot be supported below an annual rainfall of 900mm on deep sands and 625mm on sands with ironstone gravel (Beard 1984, Pignatti and Pignatti 1997). The proportion of different life forms often changes in kwongan communities depending on rainfall and moisture availability (Brown 1989).

## Kwongan plant communities on the Geraldton Sandplain

Kwongan (or kwongkan) plant communities are primarily heath, shrubland and thicket vegetation. They are distributed across the SWA biodiversity hotspot (Hopper and Gioia 2004, Beard et al. 2013). These plant communities are located on the Geraldton Sandplain, Avon Wheatbelt, Coolgardie, Mallee and Esperance Plains IBRA regions in Western Australia

(Lamont et al. 1984, Beard et al. 2013) (Figure 1.1). On average, kwongan plant communities may receive between 350mm and 1000mm of rainfall annually, depending on their location (Pignatti and Pignatti 1997). Kwongan plant communities along the south coast of Western Australia receive the highest annual rainfall (Beard 1984). Kwongan plant communities are characteristically uniform in structure (Cowling et al. 1996), comprised primarily of shrubs below 1m (Hnatiuk and Hopkins 1981), with a sparse over storey (above 2m) and 65 to 95% projective foliage cover (Griffin et al. 1983, Beard 1984). The most common plant families, Proteaceae, Myrtaceae and Fabaceae contain a large proportion of the recorded plant species. Many of the plant families may only be represented by one or a small number of species (Hnatiuk and Hopkins 1981, Brown and Hopkins 1983, Griffin et al. 1983, Brown 1989). Kwongan plant communities are highly diverse and variable at individual community, site and regional levels (Lamont et al. 1984, Hopper and Gioia 2004, Zemunik et al. 2016). The floristic composition of kwongan plant communities' changes substantially between the regions.

Characteristically, few individual species dominate in kwongan plant communities on the Geraldton Sandplain. Kwongan vegetation in the region contains an estimated 2450 plant species (Lamont et al. 1984). Between 80 and 105 plant species have been recorded over 0.1 ha on lateritic soils (George et al. 1979, Hnatiuk and Hopkins 1981, Griffin et al. 1983, Griffin and Hopkins 1985). Within 60$m^2$ plots, the total number of plant species ranged between 48–176 from phosphorus rich to the most deficient soils (Zemunik et al. 2016). In vegetation surveys undertaken by Griffin et al. (1983) and Brown (1989), half of the sampling plots contained fewer than 10% of all recorded plant species and half of all plant species occurred once or very infrequently. Studies of homogeneity between closely located stands identified few species were shared despite the small distance and few alterations in physical factors (Hnatiuk and Hopkins 1981, Griffin et al. 1983, Lamont et al. 1984). Studies indicate all kwongan subregions with SWA are diverse and non-uniform plant communities (Brown 1989).

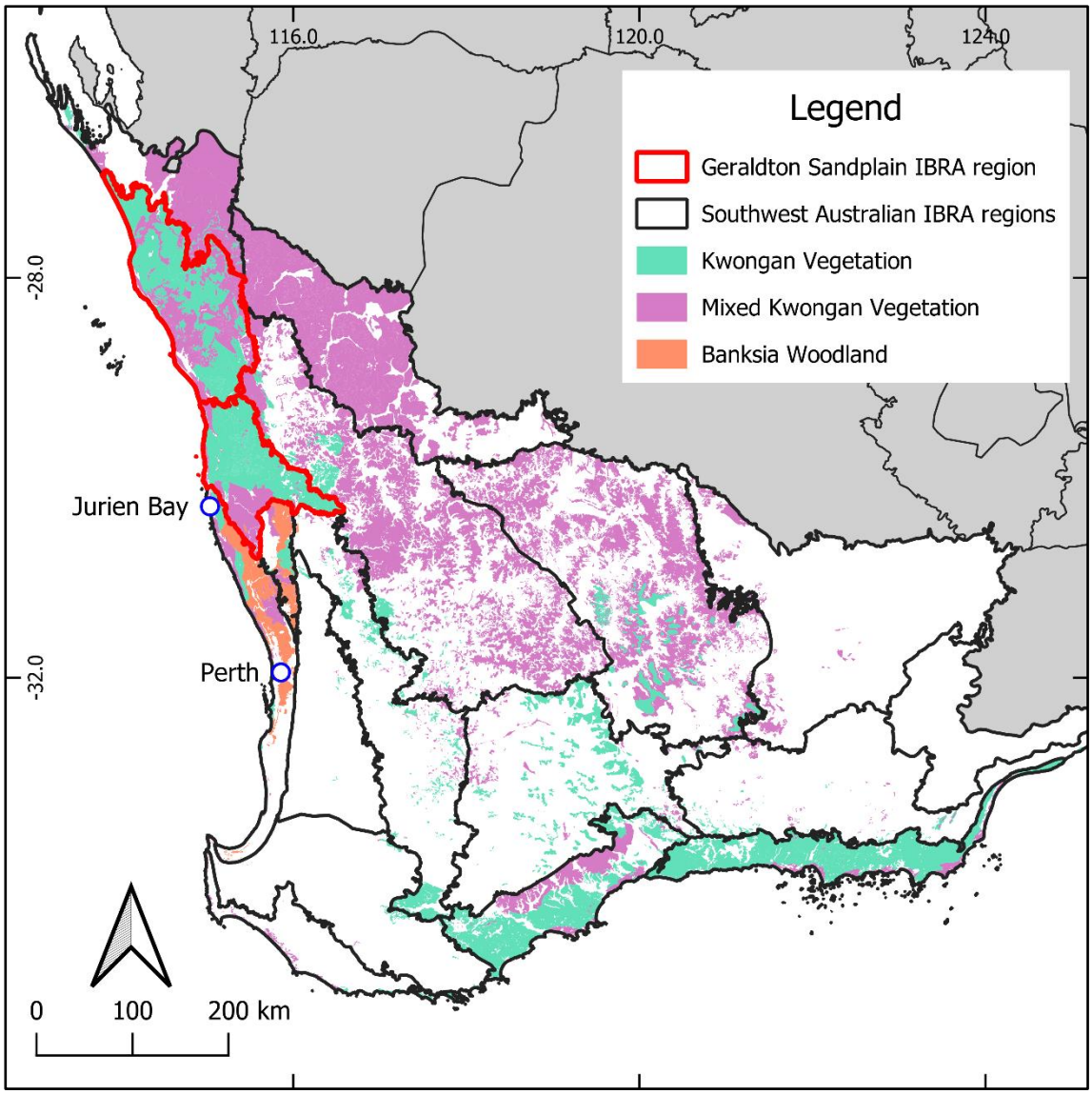


Figure 1.1: Southwest Australia and plant communities described as kwongan, mixed kwongan, and Banksia woodland by (Mucina et al. 2014). Spatial vegetation data was published by Beard et al. (2013).

## Abiotic influences on kwongan diversity

Fire has a temporal effect on the richness of kwongan plant communities on the Geraldton Sandplain. A fire will initiate a short window of increased availability of soil nutrients, moisture and light, creating gaps for recruitment (Keith et al. 2014). Plant species have adapted to persist after fire through soil and canopy stored seed banks, and through above and below ground resprouting (Miller and Dixon 2014). Two years post-fire, species richness may significantly increase by 4–29% in comparison to the pre-fire community (Herath et al.

2009b). The increase in species richness is likely attributable to the recruitment of annual plant species that ended their life cycle before the fire event and remained at the site in the soil seed bank (Herath et al. 2009b). Species persistence two years after fire is high (88–96%); however, fire-response influences species recovery as 67–85% of reseeders and 96–100% of resprouters remain (Herath et al. 2009b). Fire intervals are particularly important to maintain diversity in kwongan plant communities. Some plant species die or may suffer local extinction if fire intervals are changed (Keith et al. 2014). Short fire intervals prevent plants from reaching reproductive maturity, subsequently seed banks are not replaced and resprouting capacity is not developed (Miller and Dixon 2014). Long fire intervals can result in the decline of seed viability, reduced seed bank input and the increased mortality due to resprouting capacity senesces (Miller and Dixon 2014). Short and long fire intervals may cause populations to decline through preventing necessary development in juveniles and attrition of mature plants, respectively.

Moisture availability can influence the diversity, richness, composition and structure of kwongan plant communities. Plant communities on the lateritic hills of Mt Lesueur are distinctive in comparison to those on the same substrate elsewhere (Griffin et al. 1983). Slightly higher rainfall, and lower evaporation and temperatures likely result in higher moisture availability which is thought to be the factor responsible for the composition of the plant community (Griffin et al. 1983). Wetland depressions and wet heath plant communities differ in structure, diversity and richness from dry kwongan vegetation types (Hnatiuk and Hopkins 1981, Elkington 1988). They typically have lower species richness, more sparse vegetation and larger areas of bare ground. Wetter sites have their own particular suite of plant species, there are fewer woody shrubs and in mid-September they are occupied by various annuals (Elkington 1988).

Subtle changes in the local topography can lead to complex interactions between plant species. Dune height influences the genetic diversity of *Banksia attenuata,* and the diversity of larger shrubs and small trees (He et al. 2008). Tall dunes have deeper sands and store a greater volume of ground water. This promotes the survival of all dune restricted species during hot and dry summers. Local extinction rates are lower on dune crests and more individuals of *B. attenuata* are supported, leading to increased reproduction and seed sets, therefore promoting a greater range of genotypes (He et al. 2008). The abundances of

*B. attenuata* and *B. hookeriana* are positively correlated as they often share and compete for the same niche space due to their similar environmental tolerances (He and Lamont 2014). Conversely, population allelic richness of the two species is negatively correlated as inferior genotypes are likely excluded due to competition at the genotypic level. Plant species sharing similar environmental tolerances can co-exist as only inferior genotypes are excluded instead of the plant species (He and Lamont 2014). Water availability and niche spaces have an important influence on genetic and species diversity.

Drought may have a similar effect on kwongan plant communities as fire (Hnatiuk and Hopkins 1980). Aseasonal drought stress has been reported in a large number of plant species after consecutive years of below average rainfall (Hnatiuk and Hopkins 1980). Seedling regeneration and resprouting was used by some plant families to overcome the conditions. Drought can affect vegetation over large areas. Hnatiuk and Hopkins (1980) found sites with greater access to ground water were least affected by drought and the sensitivity of plant families varied substantially. Similar to fire, drought contributes to a complex patchwork of plant communities across the landscape (Hnatiuk and Hopkins 1980).

Soil nutrients have a large effect on the diversity and richness of kwongan plant communities regionally. The greatest species richness and diversity is found on soils with the lowest levels of phosphorus (P) (Cowling et al. 1994, Lambers et al. 2010, Zemunik et al. 2016). This is common for many kwongan plant communities across SWA. Laliberté et al. (2014) reported nutrient and physical properties of the soil explain the difference in the diversity and richness between kwongan plant communities along the Jurien Bay chronosequence. Soil age, plant species richness and diversity increase with distance from the coast (Zemunik et al. 2016). Nitrogen (N) availability increases, total P declines continuously to extremely low levels, and soil pH declines with soil age (Turner and Laliberté 2015). Environmental filtering from the regional species pool was driven by soil acidification, which was strongly correlated with declining total P and soil age (Laliberté et al. 2014). Many plant species have a poor capacity to acquire P from young alkaline soils as they have evolved on old strongly weathered acidic soil (Laliberté et al. 2014). Environmental filtering best explained the variation in diversity of plant communities along the strong resource gradient compared to local resource heterogeneity, resource partitioning, nutrient

stoichiometry or soil fertility ecological theories (Laliberté et al. 2014). Variation in soil nutrients are highly influential in shaping kwongan plant communities.

Historically, kwongan plant communities have been difficult to group or assign subclassifications at fine scales (Brown 1989). Recent studies of kwongan shrublands around Eneabba in the north and Cooljarloo in the south of the Geraldton Sandplain IBRA region have placed vegetation into four and 17 community subclassifications, respectively (Tsakalos et al. 2018, Tsakalos et al. 2019). All environmental variables collected from a subset of relevés in the study areas explained 18% and 29% of compositional variance at Cooljarloo and Eneabba, respectively (Tsakalos et al. 2018, Tsakalos et al. 2019). Soil physical and chemical properties non-exclusively explained the greatest amount compositional variation in both studies.

### Biotic influences on kwongan diversity

The functional diversity of below ground nutrient acquisition strategies increases with soil age along the Jurien Bay chronosequence (Zemunik et al. 2015). Plant species with non-mycorrhizal cluster roots (NMCR) are thought to be the most effective at acquiring P the main limiting nutrient in the plant communities found on the oldest soils (Lambers et al. 2015). All nutrient acquisition strategies can be found in the most P deprived soils and the most common traits are equally abundant (Zemunik et al. 2015). Each nutrient acquisition strategy has trade-offs (Laliberté et al. 2015, Lambers et al. 2018) and suggest that there is no single superior strategy for acquiring P. Biological interactions may reduce the competitive ability of NMCR plant species and promote coexistence (Laliberté et al. 2015, Lambers et al. 2018).

Nutrient exchange and facilitation between plant species with different acquisition strategies may be a mechanism contributing to the coexistence of kwongan plant species (Lambers et al. 2018). Nitrogen transfer occurred between plant species with different nutrient acquisition strategies after foliar feeding a donor plant (Teste et al. 2015). Plant species with mycorrhizal and NMCR could not fix nitrogen, and the exchange of nutrients occurred regardless of root intermingling and the nutrients available in the soil (Teste et al. 2015). Plant growth may also increase when the nearest neighbours have different nutrient acquisition strategies (Teste et al. 2014). Nutrient availability can be facilitated by NMCR plant species. The magnesium uptake, height and weight of the arbuscular mycorrhizal plant

species, *Scholtzia involucrata* (Myrtaceae) increased in the presence of *Banksia attenuata* (Muler et al. 2014). Nutrient transfer and facilitation between plant species with contrasting nutrient acquisition strategies may be a mechanism that promotes co-existence and the maintenance of diversity in kwongan plant communities (Lambers et al. 2018).

Soil-borne plant pathogens may equalise the competitive ability of plant species with NMCR and prevent their dominance in nutrient deficient soils (Laliberté et al. 2015). Plant species with NMCR have ephemeral roots without a complete suberised exodermis that are particularly susceptible to soil-borne plant pathogens (Lambers et al. 2015, Lambers et al. 2018). Conversely, other plant species may be protected by their association with mycorrhizal fungi (Laliberté et al. 2015, Lambers et al. 2018). Ectomycorrhizal (Myrtaceae) and NMCR (Proteaceae) plant species were grown together with and without a mixture of putatively native soil-borne plant pathogens (Albornoz et al. 2016). The competitive ability of the NMCR plant species was reduced in the presence of plant pathogens, while Myrtaceae were not negatively affected by pathogens and ectomycorrhizal colonisation increased (Albornoz et al. 2016). The survival and growth of NMCR (and nitrogen-fixing) plant species were poorer when grown in natural soils collected from the same plant species or nutrient acquisition strategy (Teste et al. 2017). The trade-off between nutrient acquisition strategy and susceptibility to soil-borne plant pathogens likely plays an important role in promoting co-existence between plant species and the diversity of kwongan plant communities.

### Native plant pathogens in kwongan plant communities

Soil-borne plant pathogens have been detected in kwongan plant communities for several decades. Western Australian vegetation has extensively been surveyed for *Phytophthora cinnamomi* after it was first identified by Podger et al. (1965), and the introduced invasive plant pathogen was found widely distributed across SWA (Shearer et al. 2007). The diversity and structure of kwongan vegetation and similar plant communities are severely affected by *P. cinnamomi* (Shearer and Dillon 1996, Bishop et al. 2010, Shearer et al. 2012) and 40% of all plant species in SWA are susceptible to the pathogen (Shearer et al. 2004). Plant communities on the coast are the least affected by *P. cinnamomi* and disease expression is hypothesised to be suppressed by alkaline calcareous soils (Shearer and Crane 2014). Since 2000, many new *Phytophthora* species have been identified using molecular

detection tools and through surveys of natural vegetation (Hansen et al. 2012, Scott et al. 2013). In SWA, *Phytophthora* species were identified from natural plant communities by re-evaluating isolates found during surveys for *P. cinnamomi* (Burgess et al. 2009). Several *Phytophthora* species are hypothesised to be native to kwongan vegetation on the Geraldton Sandplain and may be influencing the diversity of these plant communities.

For example, *P. arenaria* belongs within clade 4 and has been isolated in kwongan plant communities since 1986 (Rea et al. 2011). Additionally, clade 6a *Phytophthora*, such as *P. cooljarloo, P. kwongonina*, *P. pseudorosacearum*, and *P. rosacearum* have been detected primarily in kwongan plant communities on the Geraldton Sandplain (Burgess et al. 2018c). A large metabarcoding survey indicated *P. arenaria* was distributed throughout Australia, and *P. rosacearum* was present in several states (Burgess et al. 2017b, Burgess et al. 2018b). *Phytophthora arenaria* has been detected from a number of *Banksia* species in dry vegetation (Rea et al. 2011), and clade 6a have been detected in dry and wet kwongan vegetation types (Burgess et al. 2018c). *Phytophthora arenaria* does not cause dieback fronts typical of introduced *Phytophthora* species (Rea et al. 2011). The maximum growth temperatures of *P. arenaria* and clade 6a species are 32.5–37.5$^{o}$C (Rea et al. 2011, Burgess et al. 2018c). These *Phytophthora* species are all homothallic, they can self-fertilise to produce oospores for long-term survival. Homothallism is a beneficial adaptation in stressful environments as damaged DNA may be repaired (Bernstein and Bernstein 2010) and allow these species to produce survival structures without enduring the cost of locating a second mating type (Billiard et al. 2012). The biological characteristics and distribution of *Phytophthora arenaria* and clade 6a suggest these species have adapted to the difficult environmental conditions of SWA, and are native to kwongan plant communities on the Geraldton Sandplains (Rea et al. 2011, Burgess et al. 2018c).

Putatively native *Phytophthora* species may be damping-off pathogens in natural plant communities. Damping-off pathogens are responsible for the death of seedlings pre- and post-emergence (Agrios 2005). The most common damping-off pathogens are from the oomycete genera *Pythium* and *Phytophthora*, and the fungal genera *Fusarium* and *Rhizoctonia* (Tainter and Baker 1996). Simamora et al. (2017) identified *P. arenaria* and closely related *P. boodjera* as damping-off pathogens of mallee *Eucalyptus* species. *Phytophthora* and *Pythium* species are widely recognised damping-off pathogens with a

large host range. These oomycete genera are responsible for damping-off in agricultural (Hendrix and Campbell 1973, Tainter and Baker 1996, Savita and Nagpal 2012, Matny 2013), silvicultural (Heather et al. 1977) and greenhouse industries worldwide (Schroeder et al. 2013). Damping-off has been reported in the SWA, *Pythium* species have reduced yields of agricultural crops (Li et al. 2014) and *Phytophthora* species may be responsible for poor germination and survival of seed used in mine site restoration (Woodman 1993). Additionally, native damping-off plant pathogens are important within natural plant communities as they contribute to the maintenance of diversity by promoting co-existence between plant species (Bever et al. 2015, Teste et al. 2017).

## Mechanisms for pathogen driven diversity

The interaction between soil microorganisms and plants form the basis of a biotic feedback model that was first adapted by Bever (1994). This model offers a general explanation for the outcomes and direction of plant-soil interactions driven by abiotic and biotic mechanisms (Ehrenfeld et al. 2005). Natural ecosystems experience fluctuations in plant-soil interactions, and subtle variations in plant and microbial composition are continuous (Bonanomi et al. 2005). Variation in the abundance of a plant species may subsequently result in changes to the health, survival and abundance of the plant population mediated by the response of the microbial community (Bever 1994). The soil microbial community may respond to the increased abundance of a plant species in two distinct ways, either through negative or positive feedback (Bever 1994, Bever et al. 1997, Bever 2003). Negative feedback restricts the abundance of a plant species by reducing growth and survival within the population if numbers increase (Kulmatiski et al. 2008). Whilst, positive feedback mechanisms provide support that increases the abundance and health of a plant species. Negative feedback is viewed as a mechanism for promoting diversity as it prevents the dominance of an individual species and out-competing rarer species (Bever 1994, Bonanomi et al. 2005, Mangan et al. 2010, Reinhart 2012). Mature and damping-off plant pathogens are drivers of negative plant soil feedback as they respond to changes in abundance and reduce the survival and health of plant populations (Gilbert 2002). Relationships between plant and soil community may either be detrimental or nourish plant diversity within an

ecosystem. Plant-soil feedback categorizes the underlying mechanism determining the outcome of the interaction.

The Janzen-Connell (J-C) hypothesis (Janzen 1970, Connell 1971) accounts for the regular spacing of tree species and contributes to the maintenance of diversity for some plant communities. The J-C hypothesis describes a high density of specialist natural enemies, such as soil-borne plant pathogens beneath a parent due to a reservoir of host tissue supplied by seed input or the adult plant that leads to large scale seedling mortality (Gilbert 2002). Both seed and pathogen density decreases with distance from the parent, seeds dispersed further away from the parent are more likely to establish due to lower pathogen densities (Augspurger 1983, Augspurger and Kelly 1984). The J-C effect represents a negative plant-soil feedback, driven by a negative density or distance dependent relationship (Bever et al. 2012). If seedling mortality increases disproportionately in response to higher seedling density (overcompensating density-dependence), this force will exclude conspecific seedlings in close proximity to the parent plant (Freckleton and Lewis 2006). Overcompensating density-dependent relationships help maintain the species richness and diversity of plant communities by creating openings for uncommon plant species alongside heterospecific species, promoting coexistence (Freckleton and Lewis 2006).

Specific natural enemies are believed to best promote coexistence between plant species through negative plant-soil feedback or the J-C effect (Gilbert 2002); although, the interaction between a natural virulent plant pathogen and a single host is uncommon (Barrett et al. 2009). Generalists or multi-host pathogens may remove other host species regardless of their abundance (Bever et al. 2015). However, natural generalist plant pathogens may still contribute to coexistence if they affect plant species differently, i.e. the fittest hosts experience the largest negative plant-soil feedback (Gilbert 2002, Augspurger and Wilkinson 2007). The susceptibility of a heterospecific plant species to the pathogen community of another host tends to decrease when phylogenetic distance or functional trait dissimilarity increase (Liu et al. 2012a, Schweizer et al. 2013, Liu et al. 2015). Local environmental conditions, such as moisture and temperature, will lead to further differences in the virulence of a plant pathogen or the host-pathogen interaction (Barrett et al. 2009). Hersh et al. (2012) suggest host-specific pathogens may be uncommon and unique

fungal compositions or differential effects of generalists, influenced by the plant species and environmental conditions, may frequently drive negative plant-soil feedback in seedlings.

There is substantial evidence to support the J-C hypothesis and negative plant-soil feedback in natural plant communities driven by plant pathogens and promoting co-existence (Comita et al. 2014, Crawford et al. 2019). Density and distance-dependent mortality has been identified in the literature within temperate and tropical plant communities (Comita et al. 2014). Negative plant-soil feedback has been studied primarily in grassland plant communities and appears to be an important force promoting coexistence (Kulmatiski et al. 2008, Crawford et al. 2019). Plant pathogens are responsible for driving the J-C effect and negative plant-soil feedback in natural plant communities (Augspurger 1983, Augspurger and Kelly 1984, Mills and Bever 1998, Packer and Clay 2000, 2003, Hood et al. 2004, Bell et al. 2006, Bagchi et al. 2010, Mangan et al. 2010, Martin and Canham 2010, Liu et al. 2012a, Liu et al. 2012b, Terborgh 2012, Miller et al. 2019). Bagchi et al. (2014) provided direct evidence of the J-C hypothesis promoting co-existence, as damping-off pathogens increased the diversity of seedling assemblages. However, many studies do not identify the specific member of soil microbial community responsible for driving negative feedback and it is assumed plant pathogens are responsible (Mordecai 2011, Hodge and Fitter 2013).

## Seedling establishment in restored kwongan plant communities

Post-mining ecological restoration is common on the Geraldton Sandplains and may be affected by damping-off. A large proportion of the topsoil stored and broadcast seed is lost during the rehabilitation process of kwongan and Banksia woodland plant communities. Before mining commences topsoil is stripped and stockpiled, but the viability of the topsoil seedbank declines over time (Rokich et al. 2000) and abiotic conditions, such as high soil moisture increase seed decay (Pakeman et al. 2012, Golos and Dixon 2014). Topsoil and broadcast seed contribute 3% and 1% of total germinable seed of perennial plant species in kwongan restoration, respectively (Bellairs and Bell 1993). Although broadcast seed increases the species richness of kwongan restoration substantially, mulch harvested from the canopy of kwongan vegetation can contribute 96% of germinable seed of perennial plant species (Bellairs and Bell 1993). Additionally, Rokich et al. (2002) found broadcast seed

had an efficiency of 7% in the restoration of a similar SWA plant community, Banksia woodland. Hallett et al. (2014) identified < 1–20% of seedlings emerged across 15 Banksia woodland genera, and of the total seed input, < 1–7% of seedlings remained after summer. In other biomes, the greatest losses occur between the germination of seed and emergence of seedlings (James et al. 2011). The microbial community is responsible for reducing seed viability and seedling deaths in natural topsoil (Wagner and Mitschunas 2008, Bever et al. 2015). Low seedling emergence and survival in kwongan ecological restoration may be caused by damping-off pathogens, and seedling establishment is a major hurdle in returning plant communities that reflect reference or historic vegetation.

## Thesis aims and outline

The southwest of Australia and kwongan plant communities have high floristic diversity. Soil-borne plant and damping-off pathogens can contribute to the maintenance of diversity in forest and grassland plant communities through negative feedback mechanisms. Several putatively native damping-off pathogens have been detected within kwongan vegetation. Previous studies have indicated native plant pathogens may play a role in promoting co-existence between plant species in kwongan plant communities. Given the ability of some *Phytophthora* and other oomycetes to cause pre- and post-emergent damping-off, these pathogens may have a large influence on natural and restored kwongan plant communities. Four experiments were designed to further explore the role of damping-off and *Phytophthora* in kwongan plant communities.

Chapter 2 tested if putatively native and introduced *Phytophthora* found in kwongan plant communities are damping-off pathogens and their native host ranges.

Chapter 3 examined the distribution, richness and abundance of *Phytophthora* in natural and disturbed kwongan plant communities on the Geraldton Sandplain using metabarcoding.

Chapter 4 investigated the effect of fungicide seed coats on the seedling emergence and survival of kwongan plant species in post-mining ecological restoration.

Chapter 5 examined if plant-soil feedback is driven by damping-off for five kwongan plant species in a glasshouse experiment. Additionally, the oomycete communities associated with roots of mature plants in the field and seedlings harvested from conspecific soils were studied.

Chapter 6 summarised the major findings of the experimental chapters and discusses the outcomes related to damping-off disease and plant pathogens in kwongan plant communities. Derived from the

Chapter 6 summarised the major findings of the experimental chapters and incorporated results from the current and previous studies to discuss damping-off disease and plant pathogens in kwongan plant communities.

# Chapter 2: Putative native *Phytophthora* species as damping-off pathogens and functional plant traits linked to susceptibility


## Abstract

Damping-off oomycete and fungal plant pathogens reduce the germination, emergence, and survival of seedlings. In agriculture this reduces productivity and is considered a major problem; however, in natural ecosystems the impact is more subtle and may be important for maintaining the structure and diversity of natural plant communities. The oomycete genus *Phytophthora* is frequently detected in natural plant communities causing disease in mature plants, but it is rarely assessed as a damping-off pathogen. Pathogenicity experiments were established with eight oomycete treatments, six putatively native *Phytophthora*, the invasive *P. cinnamomi*, and *Pythium irregulare*, to determine the damping-off host range in selected plant species from a hyper-diverse Mediterranean type ecosystem. Additionally, plant and seed traits were analysed to determine if they were correlated with susceptibility to pre-emergent damping-off. *Phytophthora arenaria* was the only generalist putatively native damping-off pathogen, causing 13 of 19 plant species to experience significantly reduced seedling emergence or survival by 36–98% in its presence. *Pythium irregulare* and the introduced *P. cinnamomi* caused significant damping-off in 10 and 11 plant species by 50–90% and 42–88%, respectively. Plant species that were slowest to germinate and emerge were the most susceptible to pre-emergence damping-off caused by *P. arenaria*. These results suggest native and invasive *Phytophthora* may substantially influence the structure and diversity of natural plant communities through damping-off. While mature plant species are the most common focus in *Phytophthora* research, our study indicates damping-off should be considered to determine the complete impact of these soil-borne plant pathogens.


## Introduction

*Phytophthora* and *Pythium* are closely related genera of oomycetes and many species are plant pathogens with global distributions (Thines and Kamoun 2010). In managed ecosystems, soil-borne *Pythium* spp. are regarded primarily as damping-off pathogens

(Hendrix and Campbell 1973, Lamichhane et al. 2017). Plant species in agricultural, forestry and natural ecosystems can experience damping-off from soil-borne *Phytophthora* spp. (Heather et al. 1977, Camilo-Alves et al. 2013, Matny 2013, Domínguez-Begines et al. 2017). Soil-borne damping-off pathogens can kill seedlings pre- and post-emergence, infecting the seed, hypocotyl, roots, and stems (Tainter and Baker 1996). For example, *Phytophthora boodjera* reduced seedling emergence of several mallee *Eucalyptus* species by 100% (Simamora et al. 2017). Despite the negative impacts of damping-off pathogens in managed systems, they are an integral component of the microbial community in natural plant communities (Ehrenfeld et al. 2005).

Native plant pathogens, including *Phytophthora* and *Pythium* species, have increasingly been studied in relation to a variety of related mechanisms that maintain biodiversity in natural plant communities (Gilbert 2002, Bever et al. 2012). Plant-soil feedback (PSF) hypotheses state that pathogens maintain plant community diversity by responding to changes in the abundance of different plant species (Bever 2003). There are different variations of PSF mechanisms which are governed by the number, relationship and specificity of soil microbes (Bever et al. 2012, Bever et al. 2015). Damping-off by *Pythium* spp., for example, has been attributed to negative-feedback that maintains diversity in grassland plant communities (Bever 1994, Bever et al. 1997, Mills and Bever 1998). The Janzen-Connell hypothesis (JCH) suggests that plant community diversity may be maintained by seedling predators, such as pathogens, reducing seedling recruitment in close proximity to mature conspecific individuals (Janzen 1970, Connell 1971). The JCH represents a negative PSF mechanism where an abundance of host tissue supports seedling predation by herbivores and virulent pathogen populations (Comita et al. 2014). For example, native *Pythium* spp. and unknown oomycetes have been identified as the drivers of seedling recruitment distributions predicted by the JCH through damping-off in temperate and neotropical tree species, respectively (Packer and Clay 2000, 2003, Bell et al. 2006). Few *Phytophthora* spp. have directly been identified as drivers of negative density dependent plant-soil feedback, despite evidence for diverse *Phytophthora* communities in natural systems (Hansen et al. 2012).

Plant functional traits have been used in ecology to identify similar responses across plant species to abiotic and biotic factors and the subsequent effect on the ecosystem

(Cornelissen et al. 2003). Soft traits, those that are easily quantifiable and relate indirectly to specific functional mechanisms, are often used when studying a large number of species or sites (Hodgson et al. 1999). The susceptibility of plant species to pathogens and the impact on the diversity of plant communities has been studied in relation to the phylogenetic distance between plants (Liu et al. 2012a, Parker et al. 2015, Zhu et al. 2015). Often, closely related plant species are more likely to be susceptible to the same pathogen (Gilbert and Webb 2007). However, functional traits and their relationship to plant defence and the influence on PSF has received less attention (Kardol et al. 2013). Trade-offs between competitive ability and plant defence can promote coexistence (Laliberté et al. 2015, Lambers et al. 2018). Plant species with superior nutrient acquisition strategies can be more susceptible to pathogens that equalise this competitive advantage (Albornoz et al. 2016), and experience negative feedback mechanisms which promotes coexistence and maintains diversity (Teste et al. 2017). Plant functional traits may be important for understanding the role of pathogens in diverse natural plant communities.

*Phytophthora* spp. have commonly been detected in kwongan (or kwongkan) plant communities on the Geraldton Sandplain, and Swan Coastal Plain biogeographic regions within southwest Australia (Burgess et al. 2009, Burgess et al. 2017b, Burgess et al. 2018c). The impact of the invasive *P. cinnamomi* on kwongan and similar Banksia woodland plant communities is severe (Shearer et al. 2004, Shearer et al. 2007). Several of the *Phytophthora* spp. found in kwongan plant communities are hypothesised to be native to either the Geraldton Sandplains bioregion or more broadly Australia (Rea et al. 2011). Putatively native *Phytophthora* spp. that are detected frequently in natural kwongan vegetation, do not produce dieback fronts associated with the invasive *P. cinnamomi*, and their phenotypic traits are well suited to harsh environmental conditions (Rea et al. 2011, Burgess et al. 2018c). Additionally, putatively native *Phytophthora* were found to reduce growth, root health of native hosts, and *Eucalyptus* seedling emergence and survival (Simamora et al. 2017, Belhaj et al. 2018). Given the unique plant community in which these *Phytophthora* species are detected and their ability to cause disease in a number of hosts, their impact on native kwongan plant species should be further studied.

Kwongan plant communities are variable at individual, site and regional levels and have high species diversity, endemism and turnover (Hnatiuk and Hopkins 1981, Lamont et al. 1984,

Beard et al. 2013, Mucina et al. 2014). Several studies have recently been devoted to exploring and explaining the diversity across and within kwongan plant communities at different spatial scales (Laliberté et al. 2014, Zemunik et al. 2015, 2016, Tsakalos et al. 2018). Plant-soil feedback mechanisms have been shown to broadly contribute to maintaining diversity between plant species with different functional traits (Teste et al. 2017, Png et al. 2019). It is possible that native oomycete pathogens contribute to the maintenance of kwongan diversity (Laliberté et al. 2015, Teste et al. 2017). There are several examples of oomycete pathogens driving the Janzen-Connell hypothesis, and density-dependent seedling mortality through damping-off in other vegetation communities (Packer and Clay 2003, Bell et al. 2006). The potential impacts of these pathogens on seedling emergence and survival may be important for the success of post-mining rehabilitation and horticultural industries. Oomycete plant pathogens are frequently found across the southwest of Western Australia (WA) and are managed by mining and horticultural industries (Davison et al. 2006, Colquhoun and Kerp 2007). The pathogenicity of some of the *Phytophthora* species have been examined on older seedlings and saplings (Simamora et al. 2017, Belhaj et al. 2018); however, no studies have determined their pathogenicity as pre- and post-emergent damping-off pathogens on a wide range of kwongan hosts. Studying the effect of *Phytophthora* and *Pythium* spp. on seedlings of hosts native to southwest Australia will identify the potential impact the pathogens have on the natural plant communities and influence management guidelines in industry.

This chapter investigates the effect of several putatively native oomycetes on the emergence and survival of seedlings from a range of native kwongan hosts. Host specificity and susceptibility are important assumptions of the various ecological theories that suggest that plant community diversity can be driven by pathogens (Gilbert 2002, Bever et al. 2012). This knowledge may be important in future research into the role of these pathogens in natural plant communities. Additionally, a range of hosts allows for the study of functional traits that may be associated with susceptibility to damping-off. Plant traits allow analysis of various properties regardless of species identity which increase the generality of the results. These correlations between damping-off susceptibility and plant traits may be a first step in providing insights into evolutionary trade-offs, success of regenerative traits in the presence

of pathogens and how these may structure plant communities. Our study aimed to answer the following questions:

1) Are putatively native *Phytophthora* and *Pythium* species damping-off pathogens of native kwongan plant species?
2) Are there seed or plant traits that indicate susceptibility to damping-off?
3) How does the host range differ between the *Phytophthora* and *Pythium* species tested in the experiment?

## Methods

Two experiments were conducted using plant species native to kwongan plant communities in southwest Australia. Experiment 1 was a preliminary study and used a variety of *Phytophthora* species and isolates (Table 1) to test the susceptibility of plant species to damping-off. Experiment 2 aimed to confirm the results of Experiment 1, test an additional oomycete (*Pythium irregulare*) (Table 1), and perform a statistical analysis to identify traits correlated to damping-off susceptibility.

### Oomycetes used and Inoculum preparation

*Phytophthora* species selected were isolated communities through soil bating in kwongan plant which are native to southwest Australia (Table 2.1). *Phytophthora cinnamomi* was included in the experiment as its invasive and due to its wide distribution across southwest Australia. *Pythium irregulare* has often been isolated from kwongan communities in our own preliminary studies, and by Laliberté et al. (2015), and from agricultural systems in WA (Li et al. 2014). *Phytophthora* and *Pythium* species inocula were grown in a vermiculite substrate (1 L vermiculite, 10 g millet seed, 600 mL V8 broth) for five weeks at 20°C in the dark (Simamora et al. 2017). Additional sterile vermiculite substrate was prepared in the same way for the inoculation of negative control treatments. Trays and punnets used in Experiments 1 and 2 were inoculated with the oomycete or negative control treatments at 1% of the dry sand weight.

**Table 2.1:** The clade, date of isolation, location and the status of Western Australian isolates of *Phytophthora* and *Pythium* tested in the experiments.

| Isolate[1] | Identity | Clade | Date | Location | Status[2] | Experiment |
|---|---|---|---|---|---|---|
| CBS 127950 | *P. arenaria* | 4 | 2009 | Eneabba, W.A. | Native | 1 and 2 |
| VHS 26806; CBS 138637 | *P. boodjera* | 4 | 2012 | Tincurrin, W.A. | Native | 2 |
| MP 94-48 | *P. cinnamomi* | 7 | 1994 | Willowdale, W.A. | Invasive | 1 and 2 |
| HSA 2313 | *P. cooljarloo* | 6a | 1996 | Cooljarloo, W.A. | Native | 1 |
| CBS 143062 | *P. cooljarloo* | 6a | 2008 | Cooljarloo, W.A. | Native | 1 |
| IMI 329669 | *P. kwonganina* | 6a | 1986 | Cervantes, W.A. | Native | 1 |
| HSA 2530 | *P. pseudorosacearum* | 6a | 1998 | Cooljarloo, W.A. | Native | 1 |
| VHS 24266 | *P. pseudorosacearum* | 6a | 2010 | Cooljarloo, W.A. | Native | 1 and 2 |
| HSA 1658 | *P. rosacearum* | 6a | 1993 | Albany, W.A. | Native | 1 |
| HSA 2529 | *P. rosacearum* | 6a | 1998 | Cooljarloo, W.A. | Native | 1 |
| MUCC 829 | *Pythium irregulare* | n/a | 2015 | Perth, W.A. | Unknown | 2 |

[1] Culture collections were abbreviated: CBS = Centraalbureau voor Schimmelcultures, the Netherlands; HAS = Hart Simpson and Associates, Perth, Australia; IMI = CABI Bioscience (Imperial Mycological Institute), UK; MUCC = Murdoch University Culture Collection, Perth, Australia; VHS = Vegetation Health Service Collection, Department of Parks and Wildlife, Perth, Australia.
[2] The current status of *Phytophthora* species in Australia designated by Burgess et al. (2017b) or described in Burgess et al. (2018c).

### Experiment 1

A range of plant species native to kwongan plant communities were selected for the preliminary experiment (Table 2.2). Twenty-one plant species belonging to nine families and 18 genera were chosen. The plant species were selected for the trial if they met the following criteria: availability of seed, poor rehabilitation results based on data provided by mineral sands mining companies, high germination rates and are common in the kwongan vegetation communities present around Cooljarloo, WA. Several plant species were included as they fit most of the selection criteria. A balance between plant species known to be susceptible and resistant to *P. cinnamomi* were selected to test if susceptibility to the pathogen was different at early life stages.

Seedling emergence and survival of 21 plant species were tested with nine isolates of *Phytophthora* and a negative control (Table 2.1). River sand was steam sterilised twice at 98°C in hessian bags for two hours on consecutive days. The river sand was given 24 hours to cool before being mixed with inoculum and placed in free draining seedling trays (350 × 295 × 50 mm, 5,162 ml) produced by Garden City Plastics (https://www.gardencityplastics.com). The plant species were sown into four replicates of

each *Phytophthora* isolate. Each plant species was then sown in rows containing 30 seeds. Rows were spaced at 25 mm intervals and the order of the plant species within the replicate was randomly allocated. The experiment was run between the 10th of April and the 12th of August 2014. The mean minimum and maximum glasshouse temperatures ranged from 12°C and 25°C, respectively. The trays were hand watered with de-ionised water every other day to run-off. The emergence and death of seedlings were monitored daily for 38 days and then weekly until the conclusion of the experiment. Seedlings were recorded as emergent when both cotyledons were visible above the soil surface and then marked with a wooden toothpick to indicate when a death had occurred for a specific seedling.

**Table 2.2:** Southwest Australian kwongan plant species selected for the damping-off susceptibility preliminary trial, Experiment 1. The selection criteria and plant species trait information is displayed.

| Family | Species | Common species | Treatment[1] | *P. cinnamomi* Susceptibility[2] | Rehabilitation germination | Individual seed (mg)[3] |
|---|---|---|---|---|---|---|
| Casuarinaceae | *Allocasuarina humilis* | Yes | None | Conflicting | Poor | 2.4 |
| Dasypogonaceae | *Dasypogon obliquifolius* | Yes | None | Unknown | Poor | 7.7 |
| Ericaceae | *Astroloma xerophyllum* | Yes | Sm | Susceptible | Good | 26.3 |
| Fabaceae | *Gompholobium tomentosum* | Yes | HW, S | Resistant | Unknown | 1.7 |
| Iridaceae | *Patersonia occidentalis* | Yes | Sm | Conflicting | Poor | 3.3 |
| Loranthaceae | *Nuytsia floribunda* | Yes | None | Resistant | Poor | 27.0 |
| Myrtaceae | *Calothamnus quadrifidus* | No | None | Resistant | Good | 0.1 |
| Myrtaceae | *Calytrix flavescens* | Yes | Sm | Resistant | Poor | 2.0 |
| Myrtaceae | *Eucalyptus todtiana* | Yes | None | Susceptible | Poor | 10.0 |
| Myrtaceae | *Eremaea pauciflora* | Yes | Sm | Unknown | Good | 1.1 |
| Myrtaceae | *Hypocalymma angustifolium* | No | Sm | Resistant | Good | 1.0 |
| Myrtaceae | *Leptospermum erubescens* | No | Sm | Resistant | Average | 0.4 |
| Myrtaceae | *Melaleuca brevifolia* | Yes | None | Unknown | Unknown | 0.4 |
| Myrtaceae | *Melaleuca seriata* | Yes | None | Resistant | Good | 0.4 |
| Proteaceae | *Banksia attenuata* | Yes | None | Susceptible | Poor | 90.9 |
| Proteaceae | *Banksia telmatiaea* | Yes | None | Susceptible | Poor | 16.7 |
| Proteaceae | *Hakea trifurcata* | Yes | None | Susceptible | Poor | 12.7 |
| Proteaceae | *Petrophile brevifolia* | Yes | Sm | Unknown | Poor | 3.7 |
| Proteaceae | *Peterophile linearis* | Yes | Sm | Susceptible | Bad | 4.5 |
| Proteaceae | *Stirlingia latifolia* | Yes | None | Susceptible | Poor | 11.8 |
| Asphodelaceae | *Xanthorrhoea preissii* | Yes | Sm | Susceptible | Poor | 18.2 |

[1] Pre-germination treatments as outlined in Sweedman and Merritt (2006); HW, Hot Water; S, Scarify; and Sm, Smoke Water.
[2] *P. cinnamomi* susceptibility was determined using O'Gara et al. (2005b).
[3] Individual seed mass

## Experiment 2

The plant species selected are native to the southwest of Australia and are found in kwongan plant communities. The selection criteria were altered to broaden the scope of the study to identify if functional traits reflected susceptibility and 30 plant species were selected to provide sufficient data. The 30 species of plants (Table 2.3) were selected from

three dominant plant families, Fabaceae, Myrtaceae and Proteaceae (Zemunik et al. 2016). Species were selected based on seed availability, previous viability tests, importance in rehabilitation in the region, abundant in natural communities, and seed and plant traits. Seed mass, seed storage mechanism, nutrient acquisition strategy, symbiotic association with nitrogen fixing bacteria, response to fire and mature plant size were considered and balanced as evenly as possible when selecting plant species for the experiment (Table 2.3; Table S2.1). Traits selected for the analysis were related to recruitment or hypothesised to affect mature plant species susceptibility to soil-borne plant pathogens (Lambers et al. 2018). Germination treatments were undertaken as outlined in Sweedman and Merritt (2006).

The emergence and survival of 30 plant species was monitored in five oomycete treatments and a negative control. Given that *Phytophthora kwonganina*, *P. cooljarloo*, and *P. rosacearum* (HSA 2529) did not significantly reduce the emergence and survival of any plant species in Experiment 1 (Table 2.4), they were replaced with *Phytophthora boodjera* and *Pythium irregulare* in Experiment 2 (Table 2.1). Seedling emergence and deaths were recorded to determine host susceptibility to the oomycete treatments compared to the negative control (Table 2.1). Seedlings were recorded as emergent when both cotyledons were visible above the soil surface and then marked with a wooden toothpick to indicate when a death had occurred for a specific seedling. The experiment ran between the 23$^{rd}$ of October 2016 and the 21$^{st}$ of March 2017. Experiment 2 was conducted in an evaporatively cooled glasshouse, temperatures ranged from 17–30°C. Each treatment (6) and plant species (30) combination were replicated 10 times (1800 punnet cells). Each replicate consisted of three free draining 10 cell punnets (345 × 144 × 80 mm) produced by Garden City Plastics (https://www.gardencityplastics.com). Punnets with individual cells replaced seedling trays used in Experiment 1 to avoid potential interactions between different plant species and their effects on inoculum levels. River sand was steam sterilized twice in the punnets at 98°C for two hours on consecutive days and allowed to cool before inoculation. Vermiculite inoculum was mixed into the sterile river sand before sowing. Then ten seed of each plant species were sown into a single punnet cell (70 × 70 × 80 mm, 250 ml). Seeds were sown using a tool to produce a consistent pattern and hole depth. The position of the plant species within the replicates were randomly chosen. Replicates were arranged in a

randomised block design in the glasshouse and moved every 10 days. The punnets were hand watered with de-ionised water every other day to run-off. The presence of the *Phytophthora* treatments was confirmed by removing dead seedlings from the cell and plating the roots on amended NARH agar (Simamora et al. 2018). *Pythium irregulare* was isolated using NARH agar with the hymexazol removed as it suppresses *Pythium* spp. (Tsao 1983, Kato et al. 1990). At the end of the experiment all punnets were baited, as described by O'Brien et al. (2009), to confirm the presence and survival of the *Phytophthora* and *Pythium* species.

**Table 2.3:** Southwest Australian kwongan plant species selected to test their susceptibility to damping-off by soil-borne oomycetes in Experiment 2. Columns contain information about the different plant and seed traits selected to be analysed in a pre-emergent susceptibility model.

| Family | Species | Treatment[1] | Primary NS[2] | Secondary NS | N-fixing | Seed Storage | Fire Response | Individual seed (mg)[3] |
|---|---|---|---|---|---|---|---|---|
| Fabaceae | *Acacia pulchella* | HW, S | AM | None | Yes | Soil | Death | 4.3 |
| Fabaceae | *Bossiaea eriocarpa* | HW, S | AM | Cluster | Yes | Soil | Death | 2.1 |
| Fabaceae | *Daviesia nudiflora* | HW, S | Cluster | AM | Yes | Soil | Resprouter | 23.9 |
| Fabaceae | *Gastrolobium spinosum* | HW | ECM | AM | Yes | Soil | Death | 8 |
| Fabaceae | *Gompholobium knightianum* | HW, S | AM | ECM | Yes | Soil | Death | 2.2 |
| Fabaceae | *Gompholobium tomentosum* | HW, S | AM | ECM | Yes | Soil | Death | 1.3 |
| Fabaceae | *Jacksonia floribunda* | HW, S | AM | ECM | Yes | Soil | Resprouter | 7.6 |
| Fabaceae | *Jacksonia sternbergiana* | HW, S | AM | Cluster | Yes | Soil | Death | 9.8 |
| Fabaceae | *Kennedia prostrata* | HW, S | AM | Cluster | Yes | Soil | Death | 36.3 |
| Fabaceae | *Viminaria juncea* | None | Cluster | AM | Yes | Soil | Death | 6 |
| Myrtaceae | *Beaufortia elegans* | None | ECM | AM | No | Canopy | Death | 0.5 |
| Myrtaceae | *Calothamnus hirsutus* | None | ECM | None | No | Canopy | Resprouter | 0.04 |
| Myrtaceae | *Calytrix flavescens* | Sm | AM | None | No | Soil | Death | 1.7 |
| Myrtaceae | *Eremaea asterocarpa* | None | ECM | AM | No | Canopy | Resprouter | 1.3 |
| Myrtaceae | *Eucalyptus todtiana* | None | ECM | AM | No | Canopy | Resprouter | 8.2 |
| Myrtaceae | *Hypocalymma angustifolium* | Sm | AM | ECM | No | Soil | Resprouter | 0.32 |
| Myrtaceae | *Leptospermum erubescens* | None | ECM | None | No | Canopy | Resprouter | 0.04 |
| Myrtaceae | *Melaleuca seriata* | None | ECM | AM | No | Canopy | Resprouter | 0.04 |
| Myrtaceae | *Scholtzia laxiflora* | None | AM | None | No | Soil | Resprouter | 1.1 |
| Myrtaceae | *Verticordia densiflora* | Sm | AM | None | No | Soil | Resprouter | 2.5 |
| Proteaceae | *Banksia attenuata* | None | Cluster | None | No | Canopy | Resprouter | 103.7 |
| Proteaceae | *Banksia telmatiaea* | None | Cluster | None | No | Canopy | Death | 18.5 |
| Proteaceae | *Conospermum stoechadis* | Sm | Cluster | None | No | Soil | Resprouter | 5.1 |
| Proteaceae | *Grevillea eriostachya* | Sm | Cluster | None | No | Soil | Resprouter | 23.2 |
| Proteaceae | *Grevillea shuttleworthiana* | Sm | Cluster | None | No | Soil | Resprouter | 3.1 |
| Proteaceae | *Hakea costata* | None | Cluster | None | No | Canopy | Death | 8.9 |
| Proteaceae | *Hakea trifurcata* | None | Cluster | None | No | Canopy | Death | 16.3 |
| Proteaceae | *Lambertia multiflora* | None | Cluster | None | No | Soil | Resprouter | 26.3 |
| Proteaceae | *Petrophile drummondii* | Sm | Cluster | None | No | Canopy | Death | 11.7 |
| Proteaceae | *Stirlingia latifolia* | None | Cluster | None | No | Soil | Resprouter | 14.1 |

[1] Pre-germination treatments as outlined in Sweedman and Merritt (2006); HW, Hot Water; S, Scarify; and Sm, Smoke Water.
[2] NS, Nutrient acquisition strategy. AM, arbuscular mycorrhizal fungi; Cluster, non-mycorrhizal cluster roots; and ECM, ectomycorrhizal fungi.
[3] Individual seed mass

## Statistical Analysis

A One-Way Analysis of Variance (ANOVA), and Welch ANOVA were used to determine if there was a significant difference in the seedling emergence and survival between the oomycete treatments and the negative control for each plant species in Experiments 1 and 2. A Welch ANOVA was performed when assumptions of homogeneity of variance were violated (Welch 1951). The number of emergent and alive seedlings at the end of the experiments were used as the response variables. Seedling survival, or the number of alive seedlings at the end of both experiments indicated the overall impact of pre- and post-emergent damping-off. The effect of pre- and post-emergent damping off could not properly be separated, seedling deaths of the total emergent seedlings strongly violated statistical assumptions, likely due to the variable seedling emergence between treatments. However, post-emergent damping-off effects were indicated when the impact of the oomycete treatments either became significant or remained significant with a substantial drop in seedling survival. Additionally, an analysis was conducted determine the consistency of results for plant species and treatments used in Experiment 1 and Experiment 2. Each plant species and measurement (emergence and survival) were analysed individually. The seedling emergence and survival relative effect sizes (oomycete treatment / negative control) were calculated and used as response variables in two-way ANOVAs with experiment and oomycete treatment as predictors. A Levene's test, Bartlett's test, and assessment of fitted values vs residuals were used to evaluate homoscedasticity, and to determine normality a Shapiro-Wilk test, observed frequency histograms, and Q-Q plots were performed. Leniency was given when assessing normality as the treatments were not always normally distributed for each plant species, especially in Experiment 1 due to low replication. Although, it was assumed ANOVA tests are robust enough to handle moderate normality violations (Schminder et al. 2010, Blanca et al. 2017). Tukey and Games-Howell post-hoc analyses with *P* adjusted values were performed on parametric and non-parametric ANOVA tests, respectively. The analysis was carried out with R (R Core Team 2018), using the "car" (Fox and Weisberg 2019), "graphics", "emmeans" (Lenth 2018), "stats", and "userfriendlyscience" (Peters 2018) packages.

To determine if there were any plant or seed traits that were more likely to indicate susceptibility to pre-emergent damping-off pathogens, a Binomial Generalized Linear Mixed

Model (GLMM) was performed. Individual models were constructed for the *P. arenaria* and *Py. irregulare* treatments as they were virulent damping-off pathogens. The number of emergent seedlings and non-emergent seedlings were used as the binomial response variable and 11 soft (i.e. easily quantifiable) functional traits were identified as predictors *a priori*. Fixed covariates consisted of primary nutrient acquisition strategy (categorical with three levels), secondary nutrient acquisition strategy (categorical with four levels), plant family (categorical with three levels), seed mass (continuous), symbiotic relationship with nitrogen fixing bacteria (categorical with two levels), the fire response of the plant (categorical with two levels), seed storage mechanism (categorical with two levels), mean control germination time (continuous), germination treatment (categorical with three levels), mature plant minimum height (continuous) and mature plant maximum height (continuous). The negative control treatment was additionally added to the model as an interaction term to indicate if there were any significant changes caused by the oomycete treatment. Plant species was used as a random intercept within the model to account for the inherent differences in base line seed viability. In addition, group and replicate were included separately to account for the dependency structure in the randomised block design. All continuous covariates were standardised before fitting the model. The data-exploration protocol followed was described by Zuur et al. (2010). Collinearity was determined by assessing $GVIF^{1(2/df)}$ values, graphical representation of the data structure and by calculating appropriate correlation coefficients. Fixed factors were removed sequentially if $GVIF^{1(2/df)}$ and/or correlation coefficients values were greater than two and 0.6, respectively (Dormann et al. 2013). The models were validated by calculating a dispersion statistic, plotting the residuals versus fitted values, and residuals versus each covariate included and removed from the model (Zuur and Ieno 2016). A Tukey p-adjusted *post-hoc* analysis averaged across the other fixed factors was performed on the categorical factors in the model. Model selection was carried out by following the protocol outlined in Zuur et al. (2009). The standardised estimates and their standard errors, Z and *P* values were reported. Graphical representations of the models were constructed using the fitted mean and 95% confidence intervals averaged across other covariates within the optimal model. All statistical analysis were performed in R (R Core Team 2018), using the dplyr (Wickham et al. 2018), effects (Fox 2003), emmeans (Lenth 2018), ggplot2 (Wickham 2016), ICC (Wolak et

al. 2012), lme4 (Bates et al. 2014), MuMIn (Barton 2018), psych (Revelle 2018), and questionr (Barnier et al. 2018) packages.

## Results

### Experimental comparison

The results of Experiments 1 and 2 were consistent despite small differences in the effect size of several isolates on seedling emergence and survival (Figure 2.1). Although six plant species were included in both experiments, two species (*Eucalyptus todtiana* and *Melaleuca seriata*) had substantially different seed viability and the data were discarded when assessing the consistency of the results between the experiments. Isolates from three *Phytophthora* species used in both experiments were considered for the comparison. The relative effect size of the *P. arenaria* and *P. pseudorosacearum* treatments on the emergence and survival of seedlings was consistent in both experiments ($P > 0.05$, Figure 2.1). A significant ($P \leq 0.05$) difference between the results of the experiments was produced by *P. cinnamomi*. The seedling emergence effect size of *Banksia attenuata* and *Banksia telmatiaea* in the *P. cinnamomi* treatment was significantly ($P \leq 0.05$) different between experiments. However, the seedling survival relative effect size was not significant ($P > 0.05$) different between experiments for all four of the species tested in the *P. cinnamomi* treatment.

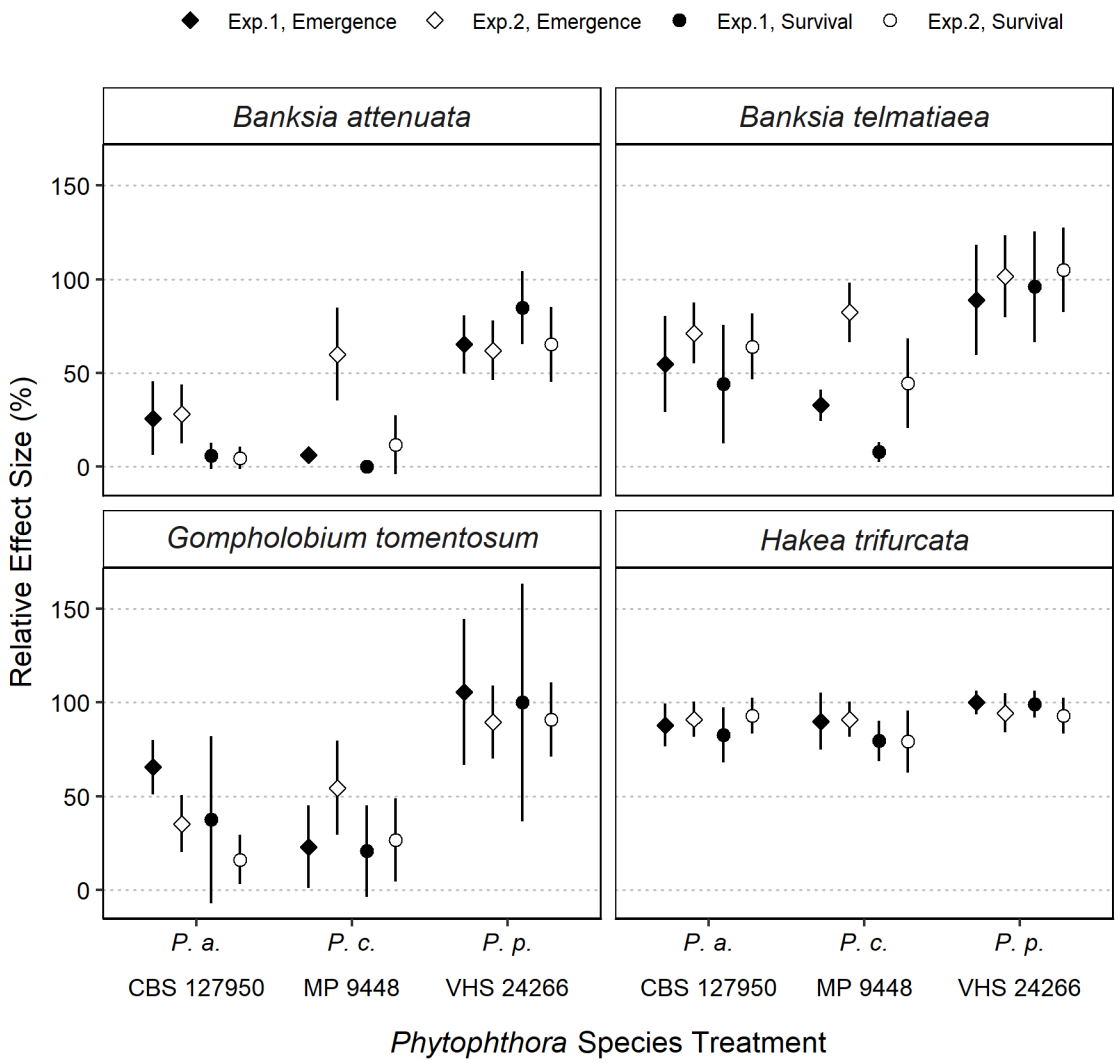


**Figure 2.1:** The mean effect size relative to the control treatments (*Phytophthora* treatment/control treatment × 100) and the 95% confidence interval of seedling emergence and survival. Four plant species and three *Phytophthora* species, *P. arenaria* (*P. a.*), *P. cinnamomi* (*P. c.*) and *P. pseudorosacearum* (*P. p.*) were included in Experiments 1 and 2, and used to compare the results between experiments. Experiments and measurements are differentiated by shape and colour; Experiment 1 (black), Experiment 2 (white), seedling emergence (diamond) and seedling survival (circle).

### Experiment 1: Pre- and post-emergent damping-off

Seed viability varied and 14 of the 21 plant species selected germinated adequately to analyse the data (Table 2.4). This preliminary experiment indicated that *Phytophthora* spp. significantly reduced the emergence and survival of seed and seedlings. *Phytophthora cinnamomi* had the largest host range of the *Phytophthora* spp. selected, significantly effecting either the germination and/or survival of seven plant species ($P \leq 0.05$) (Table 2.4). *Phytophthora arenaria* significantly affected five plant species ($P \leq 0.05$), all of which were

similarly susceptible to *P. cinnamomi* (Table 2.4). *Phytophthora rosacearum* I (HAS 1658) and *P. pseudorosacearum* (VHS 24266) were the only other *Phytophthora* spp. to significantly affect the emergence and survival of seedlings ($P \leq 0.05$), reducing the germination and survival of *Banksia attenuata* and *Melaleuca seriata*, respectively. The two isolates of *P. pseudorosacearum* produced similar results except for the emergence and survival of *Melaleuca seriata* as HSA 2530 had no significant effect (Table S2.2). The isolates of *P. cooljarloo*, *P. kwonganina* and *P. rosacearum* (HSA2529) did not significantly reduce the seedling emergence or survival of any plant species (Table S2.2).

**Table 2.4:** The mean percentage of emergent and surviving seedlings sown into soils inoculated with *Phytophthora* species relative to the negative control treatment. Only plant species with adequate seedling emergence for analysis in Experiment 1 are displayed. Significant ($P < 0.05$) post-hoc adjusted *P*-values between the treatment and the negative control are displayed in bold and grey cells. Data for several isolates of *Phytophthora* species (*P. cooljarloo*, *P. kwonganina*, *P. rosacearum* and *P. pseudorosacearum*) are not presented as they had no significant impact on seedling emergence or survival.

| | *Phytophthora* Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *P. arenaria* CBS127950 | | *P. cinnamomi* MP 94-48 | | *P. rosacearum* HSA1658 | | *P. pseudorosacearum* VHS24266 | |
| Plant species | Emergence | Survival | Emergence | Survival | Emergence | Survival | Emergence | Survival |
| *Allocasuarina humilis* | **14** | **2** | **9** | **5** | 67 | 62 | 93 | 83 |
| *Banksia attenuata* | **26** | **6** | **6** | **0** | **64** | **57** | 65 | 85 |
| *Banksia telmatiaea* | 55 | **44** | **33** | **8** | 83 | 81 | 89 | 96 |
| *Calothamnus quadrifidus* | 56 | 18 | 19 | 9 | 115 | 118 | 70 | 45 |
| *Eremaea pauciflora* | 58 | 5 | 30 | 16 | 153 | 113 | 142 | 150 |
| *Eucalyptus todtiana* | 25 | 10 | 108 | 0 | 100 | 120 | 117 | 100 |
| *Gompholobium tomentosum* | 65 | 38 | **23** | 21 | 67 | 73 | 106 | 100 |
| *Hypocalymma angustifolium* | 83 | 0 | 33 | 0 | 283 | 700 | 17 | 50 |
| *Hakea trifurcata* | 88 | 83 | 90 | 80 | 82 | 78 | 100 | 99 |
| *Leptospermum erubescens* | 35 | 20 | 26 | 27 | 178 | 233 | 35 | 27 |
| *Melaleuca brevifolia* | 21 | **18** | **17** | **0** | 104 | 129 | 29 | 29 |
| *Melaleuca seriata* | **52** | **15** | **27** | **1** | 100 | 74 | **36** | **35** |
| *Patersonia occidentalis* | 84 | 81 | 56 | 56 | 81 | 77 | 96 | 91 |
| *Xanthorrhoea preissii* | 129 | 121 | **40** | **8** | 78 | 75 | 125 | 142 |

## Experiment 2: Pre- and post-emergent damping-off

Seed viability varied, 19 of the 30 plant species germinated adequately to analyse their data (Table 2.5). The oomycete treatments had a varied effect on seedling emergence of the species tested (plant species × oomycete treatment interaction, $F_{72, 807} = 2.265$, $P \leq 0.001$; Table 2.5). *Phytophthora arenaria* (CBS 127950), *P. cinnamomi* (MP 94-48), and *Py. irregulare* (IK 1) had a significant ($P \leq 0.05$) negative effect on the seedling emergence on 11, 6, and 10 plant species, respectively (Table 2.5). However*, P. boodjera* (VHS 26806) and

*P. pseudorosacearum* (VHS 24266) treatments only negatively affected the seedling emergence of two and one plant species, respectively.

The *P. cinnamomi* treatment significantly ($P \leq 0.05$) reduced seedling emergence and survival of six and 11 plant species relative to the negative control, respectively. This indicated along with differences in effect size that *P. cinnamomi* may be a more virulent post-emergent than pre-emergent damping-off pathogen. In contrast, across the plant species seedling survival was only marginally lower than seedling emergence in the *Py. irregulare* treatment (Table 2.5). *Phytophthora arenaria* treatments substantially reduced the number of seedlings for most of the plant species that experienced pre-emergent damping-off (Table 2.5). The seedling survival of plant species affected by *P. arenaria*, *P. cinnamomi* and *Py. irregulare* were reduced by 36–98%, 42–88% and 50–90%, respectively. Additionally, *Banksia telmatiaea* and *Jacksonia sternbergiana* in several oomycete treatments had significantly ($P \leq 0.05$) lower numbers of surviving seedlings compared to controls, whilst neither had significantly lower seedling emergence.

*Banksia attenuata* seedling emergence was significantly ($P \leq 0.05$) reduced by all the oomycete treatments (Table 2.5). The survival of *Banksia attenuata* seedlings was not affected by *P. pseudorosacearum* and *Py. irregulare*, and a small number of deaths in the negative control treatments increased the *P*-values above the significance threshold. The seedling emergence of *Banksia telmatiaea*, *Grevillea eriostachya*, *Hakea costata* and *Hakea trifurcata* were not significantly ($P \geq 0.05$) impacted by any of the oomycete treatments. *Phytophthora arenaria, P. cinnamomi* and *Py. irregulare* reduced the survival of 7–8 of the 10 Fabaceae plant species. Myrtaceae plant species were all negatively affected by the oomycete treatments. The seedling emergence and survival of *Eucalyptus todtiana* and *Calothamnus hirsutus* were each significantly reduced by three treatments.

**Table 2.5:** The mean percentage of emergent and surviving seedlings sown into soils inoculated with oomycete treatments relative to the negative control. Only plant species with adequate seedling emergence for analysis in Experiment 1 are displayed. Significant ($P < 0.05$) post-hoc adjusted *P*-values between the treatment and the negative control are displayed in bold and grey cells.

| | Oomycete Treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *P. arenaria* CBS127950 | | *P. boodjera* VHS26806 | | *P. cinnamomi* MP 94-48 | | *P. pseudorosacearum* VHS24266 | | *Py. irregulare* MUCC 829 | |
| Plant Species | Emergence | Survival | Emergence | Survival | Emergence | Survival | Emergence | Survival | Emergence | Survival |
| *Acacia pulchella* | **42** | **26** | 94 | 92 | **59** | **53** | 89 | 87 | 85 | 84 |
| *Bossiaea eriocarpa* | **33** | **33** | 63 | 58 | 65 | 54 | 83 | 83 | **44** | **42** |
| *Daviesia nudiflora* | **44** | **29** | 76 | 74 | 79 | **58** | 74 | 72 | **11** | **10** |
| *Gastrolobium spinosum* | **49** | **17** | 84 | 84 | **58** | **33** | 87 | 86 | **51** | **50** |
| *Gompholobium knightianum* | **24** | **10** | 70 | 66 | 80 | 64 | 82 | 82 | 72 | 66 |
| *Gompholobium tomentosum* | **35** | **16** | 93 | 91 | **54** | **25** | 89 | 91 | **26** | **18** |
| *Jacksonia floribunda* | **66** | **29** | 86 | 83 | **44** | **14** | 96 | 96 | **42** | **38** |
| *Jacksonia sternbergiana* | 66 | **22** | 74 | 67 | 70 | **33** | 62 | 62 | **43** | **38** |
| *Kennedia prostrata* | 82 | 82 | 90 | 92 | 95 | 78 | 85 | 87 | **36** | **20** |
| *Viminaria juncea* | 59 | 52 | 66 | 66 | 28 | **17** | 79 | 79 | 24 | 24 |
| *Calothamnus hirsutus* | **32** | **21** | 91 | 88 | **62** | **44** | 86 | 86 | **32** | **17** |
| *Eucalyptus todtiana* | **50** | **21** | **23** | **2** | 64 | 29 | 96 | 94 | **30** | **27** |
| *Melaleuca seriata* | 48 | 45 | 97 | 94 | 26 | 23 | 94 | 90 | 26 | 19 |
| *Banksia attenuata* | **28** | **2** | **40** | **16** | **60** | **12** | **62** | 65 | **46** | 42 |
| *Banksia telmatiaea* | 71 | **64** | 65 | **62** | 83 | **44** | 102 | 105 | 87 | 62 |
| *Grevillea eriostachya* | 91 | 91 | 126 | 122 | 78 | 13 | 117 | 117 | 87 | 65 |
| *Hakea costata* | 97 | 97 | 99 | 99 | 88 | 84 | 97 | 97 | 108 | 107 |
| *Hakea trifurcata* | 91 | 93 | 95 | 97 | 91 | 79 | 94 | 93 | 74 | 74 |
| *Lambertia multiflora* | **60** | **38** | 90 | 90 | 61 | **13** | 99 | 87 | 93 | 89 |

## Re-isolation of oomycete treatments

There were no *Phytophthora* or *Pythium* spp. isolated from the roots of dead seedlings in the negative control treatments across Experiments 1 and 2. Individual *Phytophthora* and *Pythium* spp. were routinely isolated from their respective treatments. Additionally, baiting soils from the punnets indicated there was no cross contamination. The dead root and stem tissue of 20 plant species were harvested at intervals throughout Experiment 2. The corresponding oomycete treatment was isolated from 60.5% of the 208 total dead seedling samples collected. Often an isolate could not be recovered directly due to the small amount of suitable tissue harvested after extensive pathogen damage. In contrast, all of the *Phytophthora* and *Pythium* spp. were recovered from the corresponding treatment through baiting at the end of the experiments.

## Seed and plant trait models

Data exploration revealed no outliers. Several categorical covariates were unbalanced and this data in conjunction with collinearity testing justified their removal from the model. Model selection and validation tests additionally confirmed family, germination treatment and minimum mature plant species height were the best covariates to remove. Both models

fitted values and residuals were graphically checked and indicated they were valid. The *P. arenaria* and *Py. irregulare* models were under-dispersed, with dispersal statistics of 0.63 and 0.52, respectively. Due to the under-dispersion, *P*-values are likely to be slightly conservative (higher).

The optimal model indicated mean germination time was a significant ($P = 0.03$) indicator of seedling emergence success when soils were inoculated with *P. arenaria* (Table 2.6; Figure 2.2A). Plant species that took longer to germinate experienced higher levels of damping-off. Germination time was not collinear with any of the other fixed factors. Seedling emergence in response to germination time, nutrient acquisition strategy, and fire response changed significantly ($P \leq 0.001$) between the negative control and *P. arenaria* treatments (Table 2.6; Figure 2.2A–C). Significant interaction terms indicated the relationship between levels varied between the negative control and *P. arenaria* treatments, not that levels significantly varied within an oomycete treatment. The differences between the categories of primary nutrient acquisition strategy were largely driven by the decreased susceptibility of cluster root plant species to damping-off caused by *P. arenaria*, relative to arbuscular mycorrhizal and ectomycorrhizal plant species. The differences between fire response were increased by the susceptibility of resprouting plant species to *P. arenaria* damping-off.

Primary nutrient acquisition strategy was collinear with family (correlation coefficient $V = 0.79$, $P < 0.0001$), members of the Fabaceae family were the only arbuscular mycorrhizal plant species, and Proteaceae plant species have cluster roots (Table 2.3). Fire response was not collinear with any of the covariates. The collinear fixed factors were removed from the model. Therefore, the result indicating primary nutrient strategy changed significantly between negative control and *P. arenaria* treatments reflects that family would predict the same response in the model. The *P. arenaria* model marginal $R^2$ and conditional $R^2$ were 0.13 and 0.35, respectively.

The optimal *Py. irregulare* model indicated that none of the single terms (functional traits) were significant (Table 2.6). Tukey *post-hoc* p-adjusted values averaged across the other terms in the model specified there was only a significant ($P < 0.0001$) difference between the negative control and *Py. irregulare* treatment. No other fixed factor was a significant indicator of seedling emergence. The interaction term suggests that the relationship between levels within plant species that form symbiotic relationships with N-fixing bacteria,

primary nutrient acquisition strategy and fire response were significantly different between the negative control and the *Py. irregulare* treatments (Table 2.6; Figure 2.3A–C). Significant interaction terms indicated the relationship between levels varied between the negative control and *Py. Irregulare* treatments, not that levels significantly varied within an oomycete treatment. The increased difference between plant species with and without N-fixing bacteria associations in the *Py. irregulare* treatment was driven by the lower seedling emergence of N-fixing plant species (Figure 2.3A). N-fixing plant species were collinear with family (correlation coefficient V = 0.89, $P < 0.0001$), germination treatment (correlation coefficient V = 0.79, $P < 0.0001$) and seed storage strategy (correlation coefficient V = 0.72, $P < 0.0001$). N-fixing plant species were members of Fabaceae, required a hot water germination treatment and store seed in the soil (Table 2.3). The variation in relationship between levels of primary nutrient acquisition strategy was driven by the lower seedling emergence of ECM plant species in the *Py. irregulare* treatment. Primary nutrient acquisition strategy was collinear with family. Similar to the *P. arenaria* model, resprouting plant species were more susceptible to damping-off by *Py. irregulare* and increased the difference between treatments. The *Py. irregulare* model marginal $R^2$ and conditional $R^2$ were 0.14 and 0.41, respectively.

**Table 2.6:** Estimated regression parameters, standard error, Z values and *P* values for the optimal *P. arenaria* and *Py. irregulare* Binomial GLMMs. The seedling emergence of 19 plant species within oomycete treatments was modelled against seed and plant traits and the negative control treatment.

| Model | Fixed Effects | Estimate | Std. Error | Z Value | P Value | Sig[1] |
|---|---|---|---|---|---|---|
| *Phytophthora arenaria* | Intercept | 0.050 | 0.425 | 0.118 | 0.906 | |
| | Treatment: Control | 0.495 | 0.195 | 2.539 | 0.011 | * |
| | Primary Nutrient: AM | -0.580 | 0.506 | -1.144 | 0.252 | |
| | Primary Nutrient: ECM | -0.828 | 0.590 | -1.402 | 0.161 | |
| | Fire Response: Resprouter | -0.965 | 0.524 | -1.842 | 0.065 | |
| | Mean Germination Time | -0.530 | 0.245 | -2.166 | 0.030 | * |
| | Treatment × Primary Nutrient: AM | 0.916 | 0.236 | 3.882 | < 0.001 | *** |
| | Treatment × Primary Nutrient: ECM | 0.993 | 0.271 | 3.672 | < 0.001 | *** |
| | Treatment × Fire Response | 0.799 | 0.241 | 3.312 | 0.001 | *** |
| | Treatment × Germination Time | 0.426 | 0.113 | 3.769 | < 0.001 | *** |
| *Pythium irregulare* | Intercept | 0.130 | 0.500 | 0.261 | 0.794 | |
| | Treatment: Control | 0.253 | 0.234 | 1.084 | 0.278 | |
| | Primary Nutrient: AM | -0.063 | 0.663 | -0.096 | 0.924 | |
| | Primary Nutrient: ECM | -0.670 | 0.655 | -1.023 | 0.306 | |
| | N-Fix: 1 | -0.866 | 0.603 | -1.436 | 0.151 | |
| | Fire Response: Resprouter | -0.989 | 0.556 | -1.778 | 0.075 | |

| Model | Fixed Effects | Estimate | Std. Error | Z Value | P Value Sig[1] |
|---|---|---|---|---|---|
| | Treatment × Primary Nutrient: AM | 0.258 | 0.310 | 0.832 | 0.406 |
| | Treatment × Primary Nutrient: ECM | 0.947 | 0.307 | 3.082 | 0.002 ** |
| | Treatment × N-Fix | 1.243 | 0.282 | 4.411 | < 0.001 *** |
| | Treatment × Fire Response | 0.947 | 0.263 | 3.603 | < 0.001 *** |

[1] Asterisks indicate the different levels of significance, * P ≤ 0.05, ** P ≤ 0.01, and *** P ≤ 0.001.

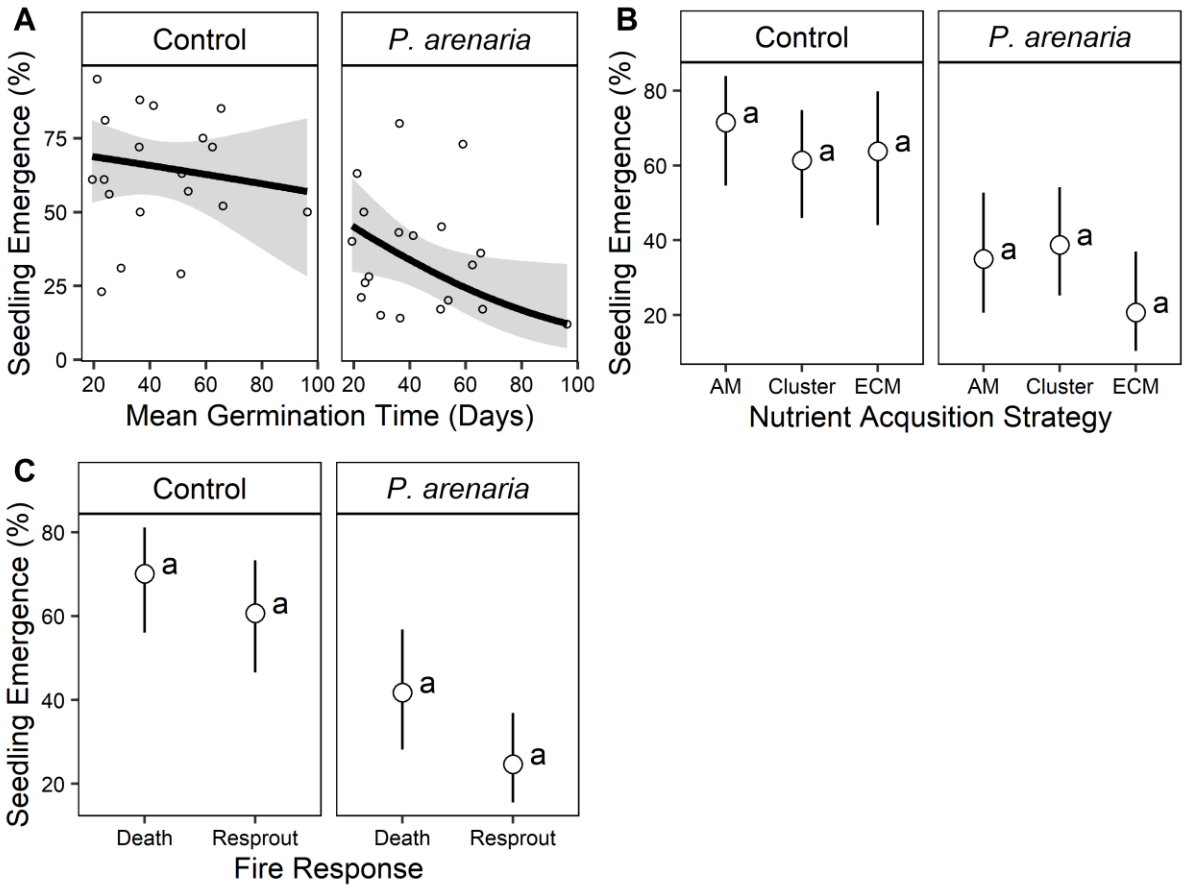


**Figure 2.2:** The mean seedling emergence percentage with 95% confidence intervals for the fixed factors extracted from the *P. arenaria* treatment model. The seedling emergence of functional traits are displayed in response to the control and *P. arenaria* treatments. **A.** Mean germination time (days) with raw data points, **B.** primary nutrient acqusition strategy, and **C.** fire response. Fitted values for each of the fixed factors are avaeraged across other covariates in the model. Homogenous subsets (P > 0.05) are represented by letters.

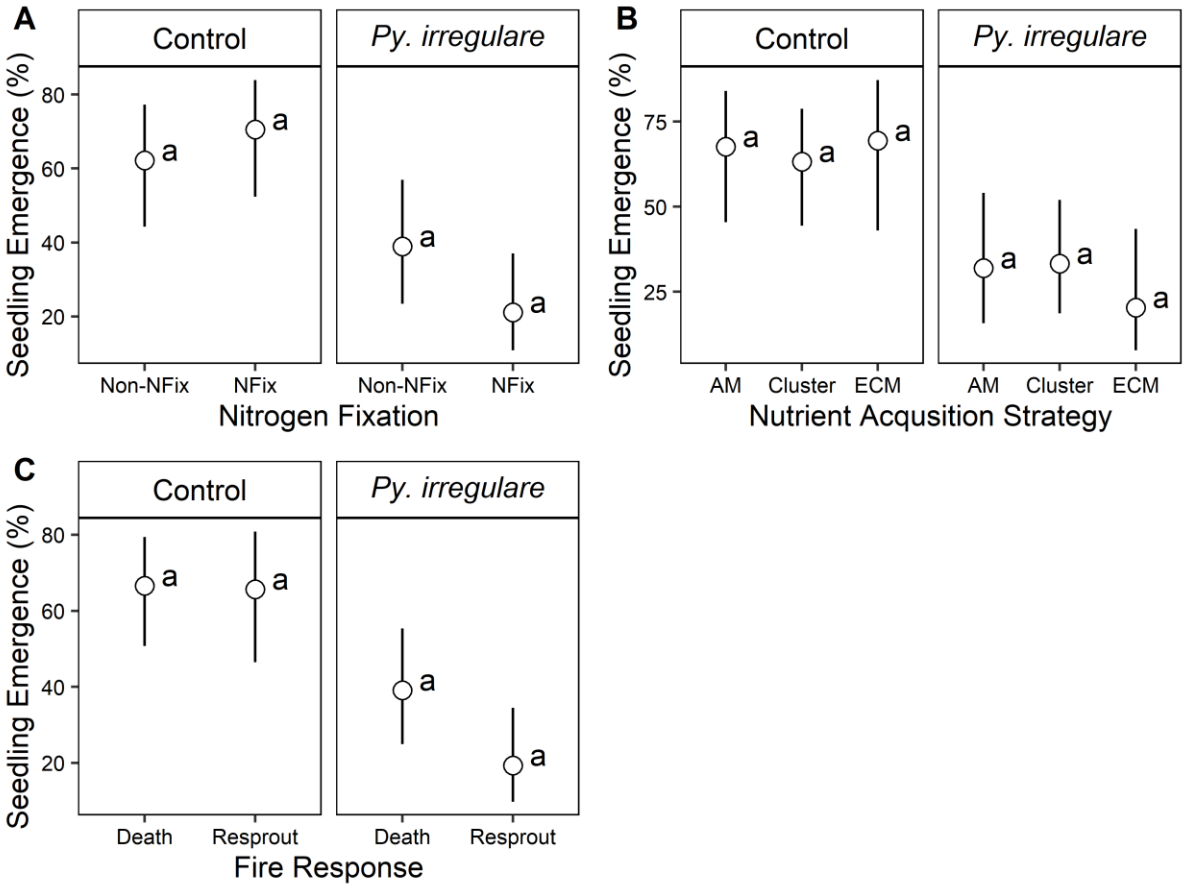


**Figure 2.3:** The mean seedling emergence percentage with 95% confidence intervals for the fixed factors extracted from the *Py. irregulare* treatment model. The seedling emergence of functional traits are displayed in response to the control and *Py. irregulare* treatments. **A.** Plant species with N-fixing bacteria association, **B.** primary nutrient aqusition strategy, and **C.** fire response. Fitted values for each of the fixed factors are avaraged across other covariates in the model. Homogenous subsets (P > 0.05) are represented by letters.

## Discussion

For the first time putatively native *Phytophthora* were shown to be damping-off pathogens of plant species found within a diverse Mediterranean plant community. Additionally, and not unexpectedly, the well-known invasive pathogen *P. cinnamomi* and the common *Pythium irregulare* were damping off pathogens. Very few of the plant and seed functional traits correlated with increased susceptibility to damping-off. Slower germination time was the only functional trait directly correlated with susceptibility to *P. arenaria* pre-emergent damping-off, and traits did not predict susceptibility to *Py. irregulare*. The host range of the *Phytophthora* species studied differed substantially. *Phytophthora arenaria*, *P. cinnamomi* and *Py. irregulare* were virulent and generalist damping-off pathogens, and the remaining

*Phytophthora* species negatively impacted two or fewer plant species. These *Phytophthora* species may play a substantial role in shaping the diversity of natural plant communities.

Not all putatively native *Phytophthora* species were damping-off pathogens and host range varied substantially. The findings were consistent with studies of *P. arenaria* and *P. boodjera*. Simamora et al. (2017) previously found *P. arenaria* and *P. boodjera* as damping-off pathogens of *Eucalyptus* species, and they also reduced the growth and health of native hosts (Belhaj et al. 2018). In another study, a mix of a number of putatively native *Phytophthora* species reduced the competitive ability of Proteacea plant species (Albornoz et al. 2016). The least pathogenic *Phytophthora* species, *P. cooljarloo*, *P. kwongonina*, *P. pseudorosacearum*, and *P. rosacearum* are separated by a small phylogenetic distance and represent a cluster within *Phytophthora* phylogenetic clade 6 (Burgess et al. 2018c). The small phylogenetic distance between the clade 6 *Phytophthora* species may reflect their similar pathogenicity observed in the present study. These clade 6 *Phytophthora* species appear to have a greater impact on root health of young seedlings compared to seedling emergence and survival (Belhaj et al. 2018). The impact of *Py. irregulare* on native kwongan hosts was consistent with studies from managed systems, where the pathogen caused damping-off disease in a large number of plant species (Bahramisharif et al. 2013, Weiland et al. 2013, Li et al. 2016, Infante et al. 2018).

Only one of the plant functional traits examined was associated with susceptibility to pre-emergent damping-off pathogens. Plant species which took longer to germinate were more susceptible to *P. arenaria* as a pre-emergent damping-off; however, no traits were related to *Py. irregulare* susceptibility. This may indicate that if *P. arenaria* is native to the plant community, it could have evolved to target specific plant species that remain dormant for longer or are slower to emerge. Rapid germination is a defence strategy employed to avoid pathogens as increased exposure leads to greater disease symptoms (Dalling et al. 2011). The interaction term between the oomycete treatment and several functional traits was significant, such as Fabaceae, resprouters or ECM plant species, and suggested that susceptibility may vary between the levels of these functional trait groups. The functional trait models only analysed seedling pre-emergence because it is likely that mechanisms for the post-emergent infection of seedling tissues can be different to that of pre-emergent seed (Hering et al. 1987). For example, several plant species used in Experiment 1 and 2

experienced reduced seedling emergence; however, successfully emergent seedlings were not affected by post-emergent damping-off, and vice-versa. The models identified functional traits that may indicate susceptibility to pre-emergent damping-off pathogens and may potentially influence the structure of plant communities in which the pathogens are found.

The oomycete species tested in this study appear to be a mix of potentially host specific and generalist plant pathogens. Plant pathogen host specificity plays an important role in plant-soil feedback and maintaining the diversity of plant communities (Mordecai 2011, Bever et al. 2015). It is difficult to determine the exact host specificity of *P. pseudorosacearum* and *P. boodjera* due to the limited number of plant species tested compared to the 2450 estimated to be present in the kwongan plant community (Lamont et al. 1984). Host specific pathogens maintain diversity by regulating the abundance of single plant species, alternatively, cross species infection or spill over by a multi-host pathogen can exclude a less competitive susceptible plant species (Gilbert 2002). *Phytophthora arenaria* and *Py. irregulare* appear to show a broad host range indicating they are generalist pathogens. Generalist plant pathogens must impact hosts differentially, competitive plant species should be more affected in order to contribute to coexistence between hosts (Bever et al. 2015). Most plant species affected by *P. arenaria* experienced similar losses of seedlings, while *Py. irregulare* showed varied impacts. Generalist pathogens can still lead to plant species coexistence if they have density-dependent distributions, mature species association and/or vector specific transmissions (Bever et al. 2015). Abiotic and biotic conditions can change the susceptibility of seedlings by increasing their health and decreasing the virulence of pathogens (Bell et al. 1995, Garbeva et al. 2004, Dalling et al. 2011, Scarlett et al. 2013, Liang et al. 2015). Therefore, the putatively native generalist plant pathogens need to be examined in a wider variety of environmental conditions to further define their impacts on the diversity of natural plant communities.

Invasive plant pathogens can reduce the diversity of natural plant communities through damping-off. This study confirmed *P. cinnamomi* reduces the number of seedlings for several native species. *P. cinnamomi* has previously been noted as an invasive damping-off pathogen in managed and unmanaged plant communities (Heather et al. 1977, Domínguez-Begines et al. 2017). Seedling susceptibility to *P. cinnamomi* was not consistent with the

response of mature plant species. For example, *Acacia pulchella* and *Gompholobium tomentosum* are regarded as resistant mature plant species, but were susceptible to damping-off. Invasive plant pathogens may remove resistant mature plant species over longer periods of time through damping-off. In contrast there were plant species, such as *Hakea costata* and *Hakea trifurcata*, known to be susceptible at mature life stages that were resistant to damping-off (O'Gara et al. 2005b). This is consistent with previous research in plant communities where the recruitment of susceptible mature plant species would still occur after the infestation of a site (Shearer and Dillon 1996, McDougall et al. 2002, Weste 2003). Susceptible plant species may still occur on *P. cinnamomi* infested sites if they mature quickly and produce large quantities of seed prior to death, allowing some individuals survive until maturity to reproduce (Rockel et al. 1982). Seedling recruitment occurs after fires in Australian plant communities (Gill 1981), and *P. cinnamomi* appears to be active in the post fire environment (Moore et al. 2015). Invasive damping-off pathogens may remove seed and seedlings from the post fire environment; however, no studies to date have compared seedling regeneration in burnt and unburnt *P. cinnamomi* infested sites. The available plant species lists and estimates of community susceptibility, such as Shearer et al. (2004) and O'Gara et al. (2005b), may need to be further developed to include species susceptible to *P. cinnamomi* damping-off. Understanding the impact invasive pathogens have on seedling establishment in natural ecosystems may provide insight into the long-term effects on plant communities and novel management strategies.

The experiments confidently identified the pathogenicity of the oomycete treatments in glasshouse conditions. The results for plant and *Phytophthora* species compared between experiments were consistent, and the relative effect sizes of the few treatments that differed, only varied slightly. The results of plant species and oomycete treatments used only in Experiment 2, are reliable due to the consistency between experiments and the large number of replications. However, a single isolate of each species was included to ensure as many plant species in the trial as possible. Isolates of the same *Phytophthora* spp. can show varying levels of pathogenicity (Linde et al. 1999). Results from Experiment 1 showed that isolates of *P. cooljarloo* and *P. pseudorosacearum* showed very similar pathogenicity. Experiments that have previously included multiple isolates of *P. arenaria* showed very few differences in damping-off pathogenicity (Simamora et al. 2017); however, small to

moderate differences between isolates have been reported in saplings (Rea et al. 2011, Belhaj et al. 2018). Examining the virulence of these pathogens in natural field conditions will likely be more informative with regards to the role they play in these plant communities.

The *Phytophthora* and *Pythium* damping-off pathogens identified can have a negative impact on industries that rely on the recruitment of native seedlings. Given the wide spread distributions of *P. arenaria*, *P. boodjera, P. cinnamomi* and *Py. irregulare* in Australia (Burgess et al. 2017a, Burgess et al. 2017b), these pathogens may pose a serious risk. Species of *Phytophthora* and *Pythium* can regularly be isolated from nursery plants (Hardy and Sivasithamparam 1988, Davison et al. 2006, Bienapfl and Balci 2014) and have caused damping-off disease (Simamora et al. 2017). Additionally, post-mining restoration could be impacted by these damping-off pathogens. Return of plant species in post-mining restoration from topsoil stored and broadcast seed may be low (Bellairs and Bell 1993, Hallett et al. 2014). These *Phytophthora* and *Pythium* species are distributed in kwongan plant communities (Rea et al. 2011, Laliberté et al. 2015, Burgess et al. 2017b). However, few studies have identified pathogens or quantified their impact in ecological restoration. Given the potential impact of these pathogens on the recruitment of native plant species there should be further investigation into their natural range and strict hygiene should implemented when dealing with the spread of these pathogens.

# Chapter 3: Terrestrial dispersal pathways and environmental predictors of the distribution of *Phytophthora* in kwongan plant communities, a diverse Mediterranean shrubland


## Abstract

Anthropogenic activities and the environment strongly influence the distribution of plant pathogens in natural plant communities. Native and invasive soil-borne plant pathogens can have a large impact on the composition of plant communities. Several recently described *Phytophthora* species are hypothesised to be native to diverse Mediterranean shrubland of southwest Australia. Although, little is known about the abundance, richness, and factors influencing the distribution of *Phytophthora* in these plant communities. A metabarcoding survey of plant roots collected from disturbed roadsides and natural kwongan vegetation was undertaken to determine the influence of pathogen dispersal pathways and environmental factors on the presence of *Phytophthora* species. Sources of common sampling biases and potential cross contamination were removed from the collection strategy and laboratory processing procedure to help set an accurate baseline of *Phytophthora* found in natural kwongan vegetation. Seven *Phytophthora* species were detected from 21.5% of the sample points located within the survey region. There was little difference in the composition of the *Phytophthora* community between disturbed roadsides and natural vegetation. Mean summer rainfall was the only significant predictor of *Phytophthora* presence; however, there was minimal variation in this variable across the survey region and it was strongly collinear with longitude or the distance from the coast. The low abundance and richness of *Phytophthora* spp. compared to previous studies was likely the result of removing sources of sampling bias and careful sample processing procedures. The establishment of soil-borne pathogens in roadside vegetation may be a key factor limiting the transmission of *Phytophthora* into natural plant communities. These results have ramifications for the monitoring of plant diseases and understanding the movement and presence of *Phytophthora*.

## Introduction

*Phytophthora* species are primarily soil-borne plant pathogens and these oomycetes are frequently detected within natural plant communities. Until recently, little was known about the occurrence and diversity of *Phytophthora* species within natural ecosystems; however, several plant disease epidemics caused by these pathogens triggered the sampling of natural plant communities (Hansen et al. 2012). Metabarcoding surveys of natural plant communities reveal high species richness and diversity (Burgess et al. 2017b, Redondo et al. 2017, Bose et al. 2018). A lack of accurate surveys before widespread anthropogenic disturbance makes it difficult to distinguish between native and introduced *Phytophthora* species in natural plant communities. The introduced or native status of many *Phytophthora* species can be approximated based on indirect indicators. For example, invasive pathogens generally lack genetic variability due to few introduction or establishment events. However, many *Phytophthora* species reproduce asexually and subsequently have less genetic variability (Hansen et al. 2012). The physical characteristics of native *Phytophthora* species should match the environmental conditions (Rea et al. 2011), and multiple putative native species detected from the same plant community may be closely phylogenetically related (Burgess et al. 2018c). *Phytophthora* native to a region tend not to be commonly detected from natural vegetation on other continents (Jung et al. 2016) nor have many associations with agricultural or nursery industries (Burgess et al. 2017b, Redondo et al. 2017). Surveys of natural plant communities worldwide are critical to determine the native range *Phytophthora* species due to their global distributions.

Anthropogenic pathways disperse *Phytophthora* species within landscapes and to new regions. The movement of soil and plant stock during exploration, colonisation in the early 20$^{th}$ century potentially introduced many *Phytophthora* species to new regions of the world (Brasier 2008, Scott et al. 2013). Despite modern biosecurity, nursery trade of ornamental plants is still a common source for the introduction of plant pathogens into urban environments (Hulbert et al. 2017). Infected plant stock may introduce pathogens into agricultural and forestry land uses, or identify native *Phytophthora* species through the movement and monitoring of exotic host plant species (Wingfield et al. 2015, Hulbert et al. 2017). *Phytophthora* may become established within disturbed vegetation, such as urban nature reserves, ecological restoration and amenity spaces through the out-planting of

infested nursery stock (Barber et al. 2013, Jung et al. 2016, Simamora et al. 2018). Urban and agricultural environments become a dispersal source for *Phytophthora* species if the pathogens become established amongst susceptible hosts (Lombaert et al. 2010). Introduced plant pathogens are spread into and within natural environments through the accidental movement of infested soil by earth moving machinery (Podger 1972, Colquhoun and Hardy 2000), vehicles (Jules et al. 2002) and shoes and equipment along recreational trails (Jules et al. 2002, Davidson et al. 2005, Cushman and Meentemeyer 2008). Once established on the edge or inside protected areas, *Phytophthora* species may move into plant communities via hyphal growth within the roots of susceptible hosts, surface and subsurface water flows, erosion and animal vectors (Ristaino and Gumpertz 2000). Anthropogenic dispersal pathways are the primary dispersal mechanism for many *Phytophthora* species into natural plant communities.

Climate and edaphic conditions play a key role in the establishment of *Phytophthora* species throughout the landscape. Temperature and moisture influence the reproduction, growth and survival of *Phytophthora* species (Erwin and Ribeiro 1996, Hardham and Blackman 2018). For example, the distribution of the destructive and invasive *P. cinnamomi* in Australia is primarily constrained by soil moisture driven by precipitation (Burgess et al. 2017a). Edaphic factors (e.g. pH) may limit disease expression and the establishment of *Phytophthora* (Alabouvette et al. 1996, Shearer and Crane 2014, Burgess et al. 2017a). Environmental filtering can determine if *Phytophthora* species survive along climate gradients in the landscape (Redondo et al. 2018); however, dispersal limitation and competitive exclusion also influence their establishment (Kraft et al. 2015, Cadotte and Tucker 2017). Some *Phytophthora* functional traits have been correlated with the establishment of invasive species and the presence of species in harsh cold and dry environments (Redondo et al. 2017, 2018). Within Australian natural plant communities, mean precipitation of the warmest quarter and mean temperature of the wettest quarter were the strongest climate predictors of *Phytophthora* community structure (Burgess et al. 2018b).

*Phytophthora* species are frequently detected in kwongan plant communities on the Geraldton Sandplain Biogeographic region in the southwest of Australia. In a recent metabarcoding survey, a total of 27 *Phytophthora* phylotypes were detected from a small

number of niche water gaining kwongan sites (Burgess et al. 2017b, Table S3.4). *Phytophthora arenaria*, *P. cooljarloo*, *P. kwongonina* and *P. pseudorosacearum* were first detected through baiting methods from kwongan vegetation and are hypothesised to be native to this plant community (Rea et al. 2011, Burgess et al. 2017b, Burgess et al. 2018c). The phenotypic traits, such as thick-walled oospores, homothallism, and tolerance to high temperatures of these *Phytophthora* species found in kwongan plant communities may be adaptations to the hot and dry Mediterranean summers of this region (Rea et al. 2011, Burgess et al. 2018c). Sample collection within the kwongan plant communities has favoured symptomatic vegetation located within disturbed roadside plant communities (Burgess et al. 2017a, Figure S3.1) and this bias has been identified in past surveys of *P. cinnamomi* and may skew the frequency of detection (Podger et al. 1990). *Phytophthora cinnamomi* has been the most commonly detected species (Burgess et al. 2017a) and substantially reduces the species richness, diversity and structure of kwongan plant communities (Wills 1993, Shearer et al. 2007, Barrett and Rathbone 2018). However, native oomycetes and *Phytophthora* are now hypothesised to drive plant-soil feedback mechanisms and contribute to the maintenance of the diversity of kwongan plant communities (Albornoz et al. 2016, Teste et al. 2017, Lambers et al. 2018). The distribution and abundance of several *Phytophthora* species that may shape the structure and diversity of kwongan plant communities are not fully quantified.

Protected areas of kwongan plant communities offer an opportunity to compare the *Phytophthora* species associated with dispersal pathways and natural vegetation in a Mediterranean climate. The closest city, Perth Western Australia (WA), has high *Phytophthora* species richness within urban nature reserves and amenity spaces (Barber et al. 2013, Khdiar 2018), and may act as a source for new infestations at protected areas in the region. Climate and edaphic factors appear to play a role in the structure of *Phytophthora* communities and the distribution of species in southwest Australia. Biases in the sampling of *Phytophthora* (Table S3.4), limitations of baiting detection methodologies (Davison and Tay 2005, O'Brien et al. 2009), and a narrow range of previous metabarcoding surveys have made it difficult to accurately determine the abundance, richness and status of *Phytophthora* species. Comparing the *Phytophthora* communities associated with dispersal pathways and natural vegetation may identify recent introductions and clarify the status of

putatively native species found within the region. This will benefit land managers as they can implement management and hygiene procedures to stop the further spread of potentially invasive *Phytophthora* species through the landscape. Furthermore, determining the abundance of native *Phytophthora* as opposed to just their presence in an area, may more accurately reflect their potential influence on the diversity of natural plant communities.

Using eDNA and metabarcoding to detect *Phytophthora* species, this study aimed to answer the following questions:

1. What is the species richness and abundance of *Phytophthora* in kwongan plant communities?
2. How does the *Phytophthora* richness, abundance and community structure change between natural plant communities and disturbed vegetation alongside dispersal pathways?
3. What influence do environmental factors have on the presence of *Phytophthora* species within the study region?

## Methods

### Sample collection and preparation

All of the sites (*n* = 20) were located in kwongan and Banksia woodland plant communities north of Perth, WA (Figure 3.1, Table S3.1). The region has a Mediterranean climate, nutrient deficient soils, and high floristic diversity and endemism (Hopper and Gioia 2004, Zemunik et al. 2016). The Department of Conservation, Biodiversity and Attractions (DCBA) approved a permit for the collection of root samples from protected areas under their management (CE005666). Main Roads Western Australia (MRWA) and local shires permitted sampling within roadside reserves. Sites and subsites were selected using aerial images prior to field visits to avoid sampling biases. The Dieback Information Delivery and Management System (DIDMS), an online *Phytophthora* sampling location database, was used to select areas previously not sampled for *Phytophthora* species (https://didms.gaiaresources.com.au).

At each site, root samples were collected from plants at disturbed and natural vegetation subsites. Disturbed subsites were located within 15 m of road edges where anthropogenic actives may have distributed *Phytophthora* species, such as unsealed and sealed roads, vehicle tracks, fire breaks, and walking trails. Whilst natural vegetation sites were more than 150m from roads and tracks, and displayed no signs of invasive *Phytophthora* dieback disease fronts. Aerial images and historic tracks recorded by Crook et al. (1982) were examined for disturbance in natural vegetation to reduce the likelihood of a miss-classified natural vegetation subsite.

The sites were sampled in July 2018. Root samples (*n* = 200) were collected from 20 sites, of the 10 samples collected at each site, five were taken at each of the disturbed and natural subsites. It was important to not bulk root samples, as the abundance of *Phytophthora* species at each site was important information with respect to understanding their potential role in the ecosystem. At individual sites, the disturbed and natural subsites shared similar plant communities. The plant communities could vary substantially between sites because of the distances travelled. Plant species were selected from a mix of families and life-forms (tree, shrub, grasses, and herbs) but were all common around the sampling point and within the community. Individual plants sampled were healthy and unhealthy. Sample points within a subsite were separated by 50 m, and approximately 20 g of fine roots (diameter < 5 mm) were collected from the rhizosphere of five plant species within a 5 m radius of a sample point and combined (100 g fresh weight). While all effort was made to collect from an individual, due to the density of roots within the rhizosphere, the collection of root material from other plant species likely occurred. Additionally, the variation inherent in the plant community and distance between points made it difficult to constantly sample the same five plant species at each sampling point.

Prior to processing and after collection, root samples were stored in paper bags for a maximum for ten days at room temperature (17°C ± 2). Root samples were air dried in a 35°C temperature-controlled room for an hour, shaken within the bag, and then washed to remove the remaining soil. Roots were cleaned with water in disposable containers and processed in a laboratory that had never contained *Phytophthora* or soil samples. Cleaned roots were mixed, a sub-sample of 30 g was separated and then stored frozen at -20°C. Each of the frozen root sub-samples were then cut into a homogenous mixture using a disposable

razor blade within a plastic Petri dish, 50 mg of root material was transferred to a bead tube for grinding and then stored at -20°C.

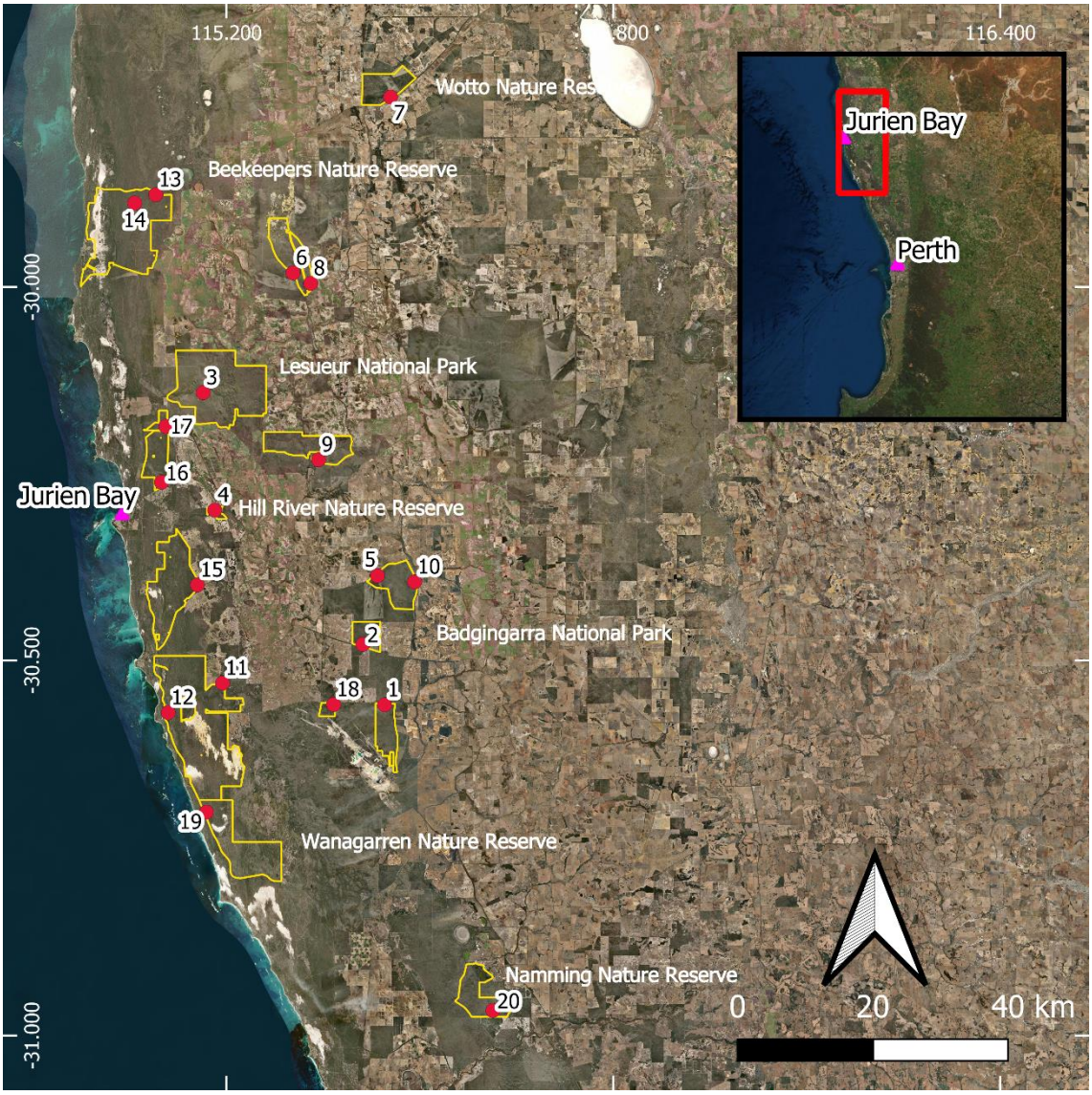


**Figure 3.1:** The location and distribution of sampling sites in Western Australia.The sites are located within the region highlighted in red on the inset map of Western Australia.

## eDNA extraction and HTS-sequencing

DNA was extracted using the DNeasy® PowerPlant® Pro Kit by Qiagen according to the manufacturer's protocol. Final elutions used 60 µL of TE buffer. Each batch of extractions contained a negative control. A single batch of *Phytophthora* eDNA extractions were previously performed in the laboratory, reducing the risk of cross contamination compared to high traffic laboratories. Disposable utensils or equipment that had never previously been

used for *Phytophthora* eDNA extraction were used. All DNA was stored at -20°C. All actions were carried out as described in the Illumina protocol (Illumina Demonstrated Protocols: 16S Metagenomic Sequencing Library Preparation). ITS 18s rRNA gene sequences (~250 bp) were amplified by a nested PCR. The first PCR used *Phytophthora* specific primers 18h2f and 5.8RBis. The first round PCR tubes contained 12.5 µl of PCR buffer KAPA HiFi HotStart ReadyMix (KAPA Biosystems), 8 µl of PCR grade water, 1 µM of each primer and 2.5 µl of genomic DNA. A second nested PCR used ITS6 and 5.8S-IR primers (Català et al. 2015) with Illumina MiSeq adapter sequences attached to the 5' end. The second round of PCR tubes contained 1 µl of PCR product. PCR cycling conditions were 94°C for 2 min, 30 cycles of 95°C for 20 s, 60°C for 25 s and 72°C for 1 min before a final 72°C for 7 min and holding at 4°C. First round PCR was conducted in duplicate and second round RCR products were combined based on intensity of bands on 2% agarose gels. Extraction negatives were run first to test for contamination. Negative PCR controls were checked and discarded if bands were visualised.

Amplicon library preparation was performed according to recommended protocols (Illumina Demonstrated Protocol: 16S Metagenomic Sequencing Library Preparation). Uniquely indexed libraries were pooled for the sequencing run, which was performed on an Illumina MiSeq using 500-cycle V2 chemistry (250 bp paired-end reads) following the manufacturer's recommendations.

### Bioinformatics analysis

Paired-end reads were imported and relabelled in Geneious Prime 2019.0.4 (https://www.geneious.com). Forward and reverse reads were merged using USEARCH v11.0.667 (Edgar 2010). Merged pairs < 175 bp, > 500 bp, > 10 miss matches, > 1% expected errors were discarded in the merging or quality filtering process. To prevent mismatches, the forward and reverse ends of the sequences were trimmed by 53 and 23 bp, respectively. Chimeras were discarded from the dataset. The reads were clustered with the UNOISE algorithm into zero-radius operational taxonomic units (ZOTUs) (Edgar and Flyvbjerg 2015). Individual sequences were assigned a ZOTU, and a ZOTU table was created with USEARCH v11.0.667.

To assign species names, ZOTUs were first imported into Geneious Prime and consensus sequences were aligned. Phylotypes were first matched to a species through an internal

blast search of a local reference database containing described, designated but undescribed taxa, and phylotypes previously detected only through metabarcoding surveys. Additionally, a GenBank database search was conducted for phylotypes that were not identified through the first curated search. Phylogenetic analyses within clades were performed using confirmed sequences of *Phytophthora* species with Geneious tree builder. Any phylotype that did not correspond with a known species was designated as a putative new species. Phylotypes corresponding to new or undescribed species were labelled as *Phytophthora* sp. nov followed by the clade number. The identified *Phytophthora* species are considered phylotypes due to their detection through sequencing compared to the collection of living isolates.

### Site variables and predictors of *Phytophthora* species

Soil and climate variables and site characteristics were collected for a statistical analysis to determine if these covariates predicted *Phytophthora* presence (Table 3.1). Soil properties at a depth of 5–15cm and 15–30cm were extracted from the Soil and Landscape Grid of Australia (SLGA) with a 90 $m^2$ resolution using the slga R package (Grundy et al. 2015, O'Brien 2019). Simulated mean monthly soil moisture and temperature between 1960 and 2014 at a resolution of 5 $km^2$ was collected from the Australian Water Availability Project (AWAP) (Jones et al. 2009). The mean summer (December to February), winter (June to August) and annual soil moisture and temperature was calculated from the AWAP dataset. Observational climate variables were extracted from a dataset of monthly gridded maximum and minimum air temperatures and precipitation produced by the Australian Bureau of Meteorology (BOM) for the AWAP (Raupach et al. 2009). The BOM variables were gridded at 5 $km^2$ and interpolated from a network of weather stations. The BOM monthly observations between 1970 and 2016 were used to calculate mean summer, winter and annual maximum and minimum temperature and precipitation covariates (Table 3.1). Site characteristics such as subsite (disturbed or natural), distance from disturbance, latitude, longitude, elevation above mean sea level and vegetation type were included in the analysis (Table 3.1).

**Table 3.1:** The soil, climate and site characteristic variables collected and extracted from databases and used for modelling the presence of *Phytophthora* species. The statistical analysis contained the variables in bold after testing for collinearity.

| Variable type | Predictor Variable | Measurement | Source[1] | Resolution |
|---|---|---|---|---|
| Continuous | Soil pH (5 - 15cm; 15 - 30cm) | | SLGA | 90m$^2$ |
| **Continuous** | **Soil available water capacity (5 - 15cm; 15 - 30cm)** | **%** | **SLGA** | **90m$^2$** |
| **Continuous** | **Soil electrical conductivity (5 - 15cm; 15 - 30cm)** | **dS/m** | **SLGA** | **90m$^2$** |
| **Continuous** | **Soil sand (5 - 15cm; 15 - 30cm)** | **%** | **SLGA** | **90m$^2$** |
| Continuous | Soil silt (5 - 15cm; 15 - 30cm) | % | SLGA | 90m$^2$ |
| Continuous | Soil clay (5 - 15cm; 15 - 30cm) | % | SLGA | 90m$^2$ |
| **Continuous** | **Mean summer precipitation** | **mm** | **BOM** | **5km$^2$** |
| **Continuous** | **Mean winter precipitation** | **mm** | **BOM** | **5km$^2$** |
| Continuous | Mean annual precipitation | mm | BOM | 5km$^2$ |
| Continuous | Mean summer temperature maximum | $^o$C | BOM | 5km$^2$ |
| Continuous | Mean winter temperature maximum | $^o$C | BOM | 5km$^2$ |
| Continuous | Mean annual temperature maximum | $^o$C | BOM | 5km$^2$ |
| Continuous | Mean summer temperature minimum | $^o$C | BOM | 5km$^2$ |
| Continuous | Mean winter temperature minimum | $^o$C | BOM | 5km$^2$ |
| Continuous | Mean annual temperature minimum | $^o$C | BOM | 5km$^2$ |
| **Continuous** | **Mean summer soil temperature (grass root density 0 - 0.5m)** | **$^o$C** | **AWAP** | **5km$^2$** |
| Continuous | Mean winter soil temperature (grass root density 0 - 0.5m) | $^o$C | AWAP | 5km$^2$ |
| Continuous | Mean annual soil temperature (grass root density 0 - 0.5m) | $^o$C | AWAP | 5km$^2$ |
| **Continuous** | **Mean summer soil moisture relative to saturation (grass root density 0 - 0.5m)** | **Proportion (0 - 1)** | **AWAP** | **5km$^2$** |
| Continuous | Mean winter soil moisture relative to saturation (grass root density 0 - 0.5m) | Proportion (0 - 1) | AWAP | 5km$^2$ |
| Continuous | Mean annual soil moisture relative to saturation (grass root density 0 - 0.5m) | Proportion (0 - 1) | AWAP | 5km$^2$ |
| Continuous | Latitude (S30.97036 to S29.74415) | | | |
| Continuous | Longitude (E115.05681 to E115.61680) | | | |
| Continuous | Elevation | m.a.m.s.l[2] | | |
| **Continuous** | **Distance from disturbance** | **m** | | |
| **Categorical** | **Subsite (disturbed/natural)** | | | |
| **Categorical** | **Plant community (Banksia woodland/kwongan)** | | | |

[1] Sources: Soil and Land Grid of Australia (SLGA), Bureau of Meteorology (BOM) and Australian Water Availability Project (AWAP)
[2] Metres above mean sea level.

## Statistical Analysis

Statistical analyses were used to determine if subsite (disturbed or natural) influenced the community of *Phytophthora* species. Samples with at least one detection were included in the analyses. Unconstrained ordination using non-metric multidimensional scaling (NMDS) was performed with the metaMDS function in the vegan R package (Oksanen et al. 2018). The NMDS coordinates for each sample were generated using both Jaccard (presence) and Bray-Curtis (abundance) indices in two dimensions. All NMDS coordinates generated using the Jaccard index were randomly jittered by 0.05 along the x and y axes to display the abundance of points plotted at the same location representing identical communities. A dummy species was added to each sample point or site due to denuded assemblages (Clarke et al. 2006). The NMDS coordinates were extracted and plotted using the GGplot2 R package (Wickham 2016). Ellipses were drawn into plots to display the extent of the *Phytophthora* communities with 95% confidence intervals for the site type using the

'stat_ellipse' function. A permutational multivariate analysis of variance (permanova) was performed to determine if subsite significantly influenced the composition of *Phytophthora* communities using the adonis function in the vegan R package. The dissimilarity matrices were produced with the Jaccard and Bray-Curtis method using the vegdist function. The permanova assumption of homogeneity of multivariate dispersions between factor levels was assessed using the betadisper and permutest functions in the vegan R package.

A binomial generalised linear mixed effect model (GLMM) was performed to determine if the presence of *Phytophthora* was correlated with climate, soil and site characteristics. A binary analysis was opted for over a Poisson response variable (*Phytophthora* species richness) due to the rarity of multiple detections from a single sample point. Two binomial GLMM analyses were performed on the presence of all *Phytophthora* species and the *P. versiformis* complex at each sample point. In both analyses, subsite was used as a random intercept to account for the dependency structure associated with observations from a similar location. The model included predictor variables soil, climate and site characteristics as fixed effects terms (Table 3.1). The data exploration and model validation protocols were conducted as outlined by Zuur et al. (2010) and Zuur and Ieno (2016), respectively. Correlation coefficients appropriate for the variable types, graphical representation, and variance inflation factor (VIF) values were used to assess the collinearity of covariates in order to prevent conservative *P*-values (Zuur et al. 2010). The model did not contain covariates if correlation coefficients were > 0.6 or < -0.6 (Dormann et al. 2013), or VIF values were above 3 (O'Brien 2007). The optimal model was selected by ranking the combinations of fixed effects in the model (Zuur et al. 2009), using AICc values with the dredge function in the MuMIn R package (Barton 2019). All statistical analyses were conducted in R (R Core Team 2018), using the dplyr (Wickham et al. 2018), effects (Fox 2003), emmeans (Lenth 2018), ggplot2 (Wickham 2016), and lme4 (Bates et al. 2014) packages.

## Results

### Sequencing throughput and quality control

A total of 233,172 reads were obtained from the run and 35.7% of wells produced good quality reads. From the 84.8% of read pairs that were merged successfully, the average

merged read length was 292 bp. The genus *Phytophthora* corresponded to 39.2% of reads. On average, there were 457 ± 123 *Phytophthora* reads per sample. The remaining reads were attributed to other oomycetes, *Peronospora* (6.5%) and *Pythium* (21.3%) phylotypes were identified (Table S3.4) but were not included in the statistical analysis. Three groups of phylotypes could not be separated on the basis of the ITS1 phylogeny, (1) *P. citricola* and *P. pachypleura*; (2) *P. versiformis*, *P. quercina* and *P. '*aff. ohioensis'; and (3) *P. inundata*, *P. condilina*, and *P. humicola*. The word 'complex' has been placed behind the first *Phytophthora* species listed when referencing these groups that could not be separated on the basis of the ITS1 phylogeny.

### *Phytophthora* species detected from root eDNA

A total of seven distinct phylotypes were detected that primarily matched a species or a complex (Table 3.2, Table S3.2). The phylotypes corresponding to *P. arenaria, P. cinnamomi*, *P. citricola* complex, *P. elongata*, *P. inundata* complex, and *P. versiformis* complex have previously been recorded in WA. This was the first study to record *P.* sp. nov 10 in Western Australia, it had previously only been detected in Queensland.

*Phytophthora* was detected at 18 (90%) sites and 43 (21.5%) sample points (Table 3.2). On average, 1.45 different phylotypes were detected at each site. The mean alpha diversity for disturbed and natural sites was 1.6 and 1.0, respectively. The detection of multiple phylotypes from a single sample point was uncommon, and seven (3.5%) of sample points contained either two or three phylotypes. *Phytophthora* was detected more frequently at disturbed than natural subsites (Table 3.2). *Phytophthora* phylotypes were detected from 24% of disturbed and 13% of natural subsites. Introduced *Phytophthora* phylotypes were detected at 3% and 4% of disturbed and natural subsites, respectively.

Several clades of *Phytophthora* were not represented in the survey. There were no detections of phylotypes belonging to *Phytophthora* Clades 1, 5, 8 and 9 (Table 3.2), and multiple phylotypes were only identified from Clade 2. The majority of the phylotypes were rare, five were detected at three or fewer sites (Table 3.2). The most common phylotype corresponded to the *P. versiformis* complex and was detected at 65% of sites and from 14.5% of sample points, followed by *P. elongata* which was found at 25% of sites and from 5% of sample points (Table 3.2). The phylotype corresponding to the invasive *P. cinnamomi* was detected in five samples, three of which were from the same site (Table 3.2).

The proportion of each *Phytophthora* species at disturbed and natural sites was similar. The phylotype corresponding to the *P. versiformis* complex comprised 53% and 60% of the total detections in disturbed and natural plant communities, respectively (Figure 3.2). Similarly, *P. elongata* did not differ substantially between the site types. Both *P. arenaria* and *P.* sp. nov 10 were not detected at natural sites (Figure 3.2; Table 3.2), and all species, apart from *P. elongata*, increased their representation as a proportion of total species in the natural site communities (Figure 3.2B).

**Table 3.2:** The phylotypes and corresponding *Phytophthora* species detected in kwongan and Banksia woodland plant communities in southwest Australia. The clade, first record in Australia, status, count and percentage of detections at sites and sample points for individual *Phytophthora* species are displayed. The number of detections at disturbed and natural subsite sample points is included. The total number of *Phytophthora* detected at sites and sample points is summarised below.

| | | | | Sites | | Sample points | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Phytophthora* species | Clade | First record | Status[1] | # | % | # | % | Disturbed | Natural |
| *P. citricola* complex | 2 | 1971 | Invasive | 2 | 10 | 2 | 1 | 1 | 1 |
| *P. elongata* | 2 | 1989 | Native? | 5 | 25 | 10 | 5 | 7 | 3 |
| *P. versiformis* complex | 3 | 2014 | Native | 14 | 65 | 29 | 14.5 | 17 | 12 |
| *P. arenaria* | 4 | 1986 | Native | 1 | 5 | 2 | 1 | 2 | 0 |
| *P. inundata* complex | 6 | 1984 | | 2 | 10 | 2 | 1 | 1 | 1 |
| *P. cinnamomi* | 7 | 1947 | Invasive | 3 | 15 | 5 | 2.5 | 2 | 3 |
| *P.* sp. nov 10 | 10 | 2017 | Native? | 2 | 10 | 2 | 1 | 2 | 0 |
| Total | | | | 18 | 90 | 43 | 21.5 | 24 | 17 |
| *n* samples | | | | 20 | | 200 | | 100 | 100 |

[1] The current status of *Phytophthora* species in Australia designated by Burgess et al. (2017b), a phylotype complex corresponding to a mix of putative native and invasive *Phytophthora* was not categorised.

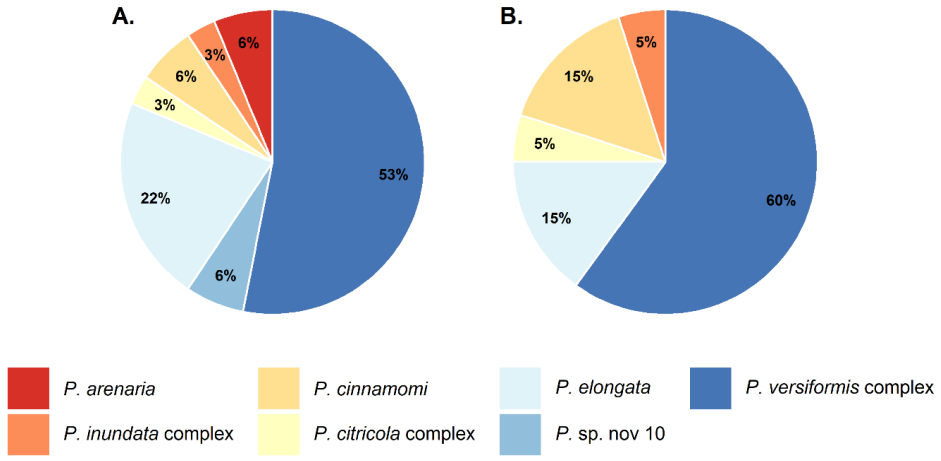


**Figure 3.2:** The proportion of *Phytophthora* species detected at **A.** disturbed and **B.** natural subsites.

*Phytophthora* community analysis

The presence permanova determined there was no significant (F = 0.5384, $R^2$ = 0.0129, *P* = 0.7065) difference between *Phytophthora* communities associated with natural and disturbed subsites (Figure 3.3A). Additionally, the permanova run using the abundance of *Phytophthora* reads produced similar results, and subsite was not significant (F = 1.1585, $R^2$ = 0.0275, *P* = 0.2777) (Figure 3.3B).

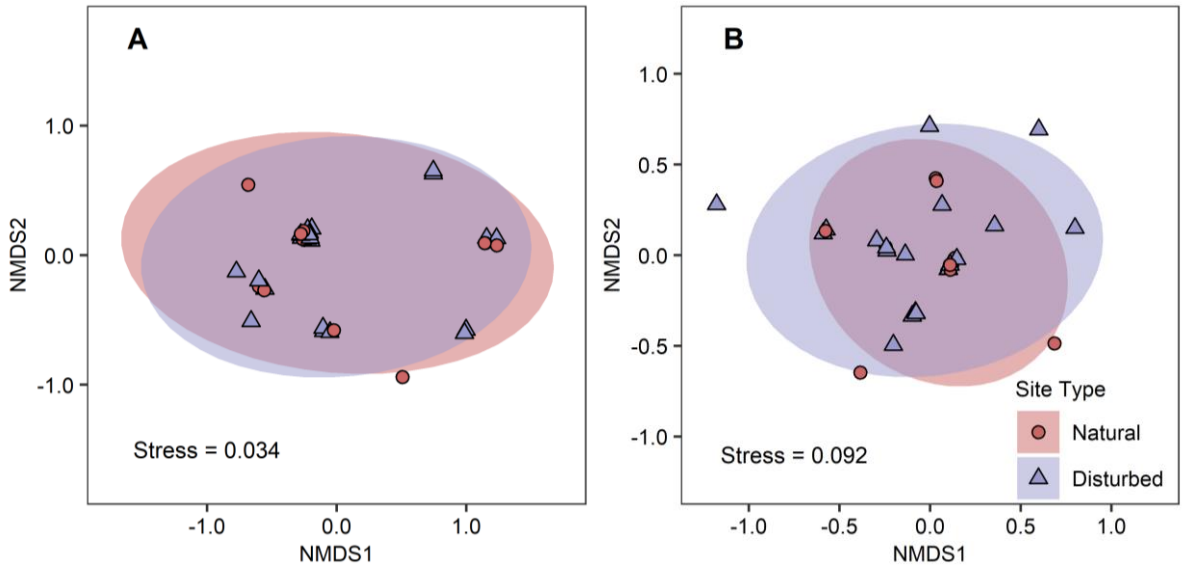


**Figure 3.3:** The non-metric multidimensional scaling ordination plots displaying the *Phytophthora* communities associated with natural and disturbed site types using **A.** Jaccard (presence); and **B.** Bray-Curtis (abundance) dissimilarity indices. Small clusters of points in figure **A.** represent the same community of *Phytophthora* spp. as positions were randomly jittered.

Predictors of *Phytophthora* presence

Binomial GLMMs were used to determine if soil, climate or site characteristics predicted the presence of any *Phytophthora* species and *P. versiformis* complex, the most commonly detected species. Data exploration revealed there were no outliers, non-linear relationships, or unbalanced covariates. Covariates were removed during model selection if they did not contribute to the explanation of the response variable. The selection of the optimal *P. versiformis* complex model indicated that none of the fixed effects helped explain the variation in the response variable or were significant. The optimal *Phytophthora* model contained four covariates after the selection process. The dispersion statistic was 0.98 and indicated that the optimal *Phytophthora* model had unbiased estimated parameters and

accurate standard errors. Patterns were not detected in the graphical representation of the Pearson residuals versus the fitted values and the included and excluded covariates.

The optimal binomial GLMM indicated that mean summer precipitation was a significant ($P$ = 0.036) predictor of *Phytophthora* presence and absence (Table 3.3). The probability of detecting a *Phytophthora* species was 21.2% higher at sample points with the greatest mean summer precipitation (35 mm) compared to the lowest (27 mm) (Figure 3.4A–B). Mean summer precipitation was highly collinear with longitude (correlation coefficient r = 0.92, $P$ < 0.001; Figure 3.4A, C). Additionally, mean summer precipitation was moderately collinear with elevation (correlation coefficient r = 0.59, $P$ < 0.001) and negatively correlated with summer, winter and annual minimum temperatures and maximum temperatures during winter. The correlation between these covariates indicates summer precipitation and *Phytophthora* presence increased with distance from the coast. The site type (disturbed or natural) had no significant ($P$ = 0.17) effect on the presence of *Phytophthora*. No other covariates were significant in the optimal model (Table 3.3). The *Phytophthora* presence model marginal and conditional $R^2$ values were 0.09 and 0.12, respectively.

**Table 3.3:** The presence and absence of all *Phytophthora* species detected in the survey region modelled against soil, climate and site characteristic variables. The covariates, estimated regression parameters, standard error, Z values and P values of the optimal Binomial GLMM are displayed.

| Fixed Effects | Estimate | Std. error | Z value | *P*-value | Sig |
|---|---|---|---|---|---|
| Intercept | -1.1555 | 0.2684 | -4.304 | 1.68e-05 | *** |
| Electrical conductivity | 0.3107 | 0.1811 | 1.716 | 0.0861 | |
| Subsite: Natural | -0.5182 | 0.3779 | -1.371 | 0.1703 | |
| Mean summer precipitation | 0.4099 | 0.1959 | 2.092 | 0.0364 | * |
| Mean summer soil moisture | 0.2794 | 0.1634 | 1.709 | 0.0874 | |

Asterisks indicate the different levels of significance, * P ≤ 0.05, ** P ≤ 0.01, and *** P ≤ 0.001.

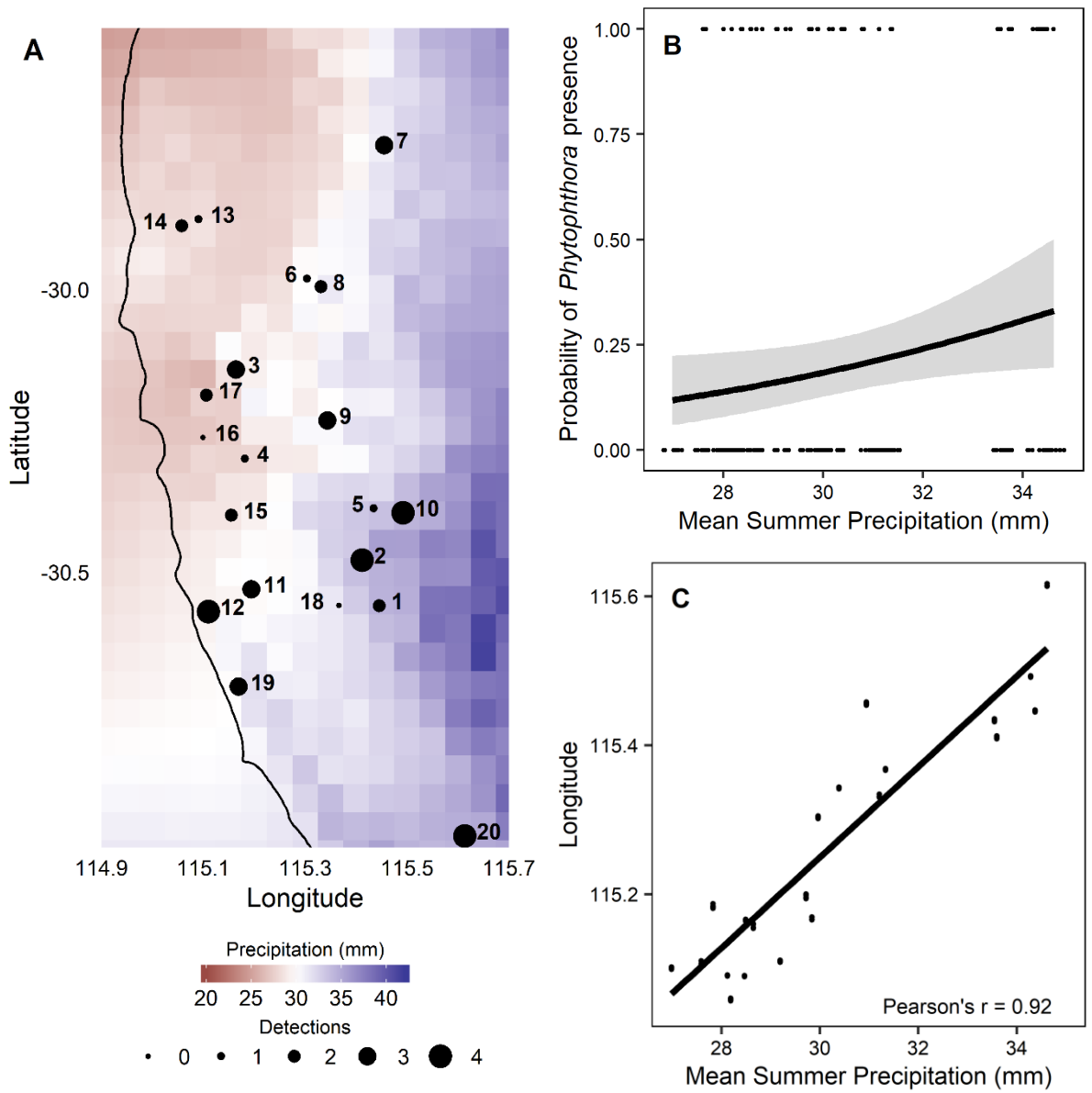


**Figure 3.4: A.** Mean summer precipitation and the number of *Phytophthora* detections made at each site. **B.** The predicted probability of *Phytophthora* presence with 95% confidence intervals in relation to mean summer precipitation. **C.** The relationship between the longitude and the mean summer precipitation at sample points.

## Discussion

Metabarcoding of eDNA extracted from the roots of plant species within a hyper-diverse Mediterranean type ecosystem revealed low *Phytophthora* richness and abundance. The survey detected seven distinct *Phytophthora* phylotypes corresponding to a species, of which *P. versiformis* complex was the only commonly detected species. The composition of *Phytophthora* communities at disturbed and natural subsites did not differ significantly; however, detections and species abundance were higher at disturbed sites. Mean summer

precipitation, highly correlated with longitude, was the only significant indicator of the presence of *Phytophthora* within the survey region. The *Phytophthora* species richness and abundance were a strong departure from the findings of previous studies and the hypothesised distribution of *Phytophthora* species in the region.

The results of metabarcoding *Phytophthora* surveys conducted within natural vegetation and the same region have produced conflicting results. Burgess et al. (2017b) collected bulked soil samples from 23 sites within the region and detected 27 *Phytophthora* phylotypes with an estimated Jackknife species richness of 31.78 (Table S3.4). Seven *Phytophthora* phylotypes were detected in the current survey with an identical Jackknife estimation of species richness (Table S3.4). The stark difference between the current and previous metabarcoding survey indicate roots may be a biological filter for many species, or variation in the location of sites, sample material and processing procedure produce substantially different results. *Phytophthora* species can be poor saprophytes (McCarren 2006), infect the roots of asymptomatic plant species (Crone et al. 2013, Belhaj et al. 2018), and spread through dry natural environments with permeable soils by root to root contact (Hill et al. 1994). Therefore, it is unlikely that the large number of *Phytophthora* species detected by Burgess et al. (2017b) are surviving exclusively in remote natural soils without the infection of roots. In the current study, sample sites were primarily located in dry kwongan and Banksia woodland plant communities; in contrast, Burgess et al. (2017b) mainly processed samples from water gaining sites which are not representative of the plant community as a whole (Tsakalos et al. 2018), and more likely to contain *Phytophthora* due to favourable environmental conditions and the transportation of spores via water flows. Sieving or grinding soil samples may have caused cross-contamination during the processing procedure employed by Burgess et al. (2017b). Whilst in the current study, the collection of root material avoided sieving and grinding activities, and utilised disposable equipment to considerably reduce the risk of cross-contamination. Sampling biases and processing procedures may substantially influence the outcome of metabarcoding HTS surveys, and further testing is required to quantify their impact on reported *Phytophthora* communities.

*Phytophthora* community structure and the number of introduced phylotype detections were similar between natural and disturbed roadside vegetation. The structure of the *Phytophthora* community did not change significantly between the natural and disturbed

roadside plant communities. Similar *Phytophthora* communities were detected when comparing natural and roadside vegetation in an Australian alpine environment (Khaliq 2019). The consistent structure between natural and disturbed subsites indicates that once introduced *Phytophthora* species establish within roadside plant communities they easily disperse into natural vegetation. Putatively native *Phytophthora* phylotypes were more abundant in disturbed vegetation compared to natural plant communities; however, subsite was not a significant predictor of *Phytophthora* presence. The transmission of both native and introduced *Phytophthora* species likely provides more opportunities to infect plant hosts adjacent to dispersal pathways. Anthropogenic dispersal pathways, such as roads alter the soil chemical properties and composition of the below-ground microbial community (Trombulak and Frissell 2000, Neher et al. 2013, Neher et al. 2017). Studies of roadside conditions in Western Australia suggests higher concentrations of mineral nutrients and soil moisture for longer periods may increase the abundance and health of *Banksia* species (Lamont et al. 1994a, Lamont et al. 1994b). However, changes to abiotic and biotic environmental conditions by disturbance may increase plant stress for some species and subsequently, susceptibility to pathogen infection (Cobb and Metz 2017). For example, disturbed roadsides are associated with increased canker disease incidence in marri (*Corymbia calophylla)* by the native pathogen *Quambalaria coyrecup* in the south-west of WA (Paap et al. 2017a, Paap et al. 2018). Sampling the roots of asymptomatic roadside vegetation in a dry Mediterranean plant community does not affect the observed *Phytophthora* community structure, but these environments may increase the reported species abundance due to plant stress and higher soil moisture.

The period of summer drought appears to be a limiting factor in the distribution of *Phytophthora* species in a dry Mediterranean climate. Mean summer precipitation was the only significant predictor of the presence of *Phytophthora* at a site. Sites with the highest mean summer precipitation had a higher probability of *Phytophthora* detection. However, mean summer precipitation did not vary substantially across the survey region, and the location and quantity of summer rainfall are stochastic between years. The term was highly collinear with other covariates, such as longitude which may reflect changes in the vegetation community (Laliberté et al. 2014, Zemunik et al. 2016) or land use and traffic. Therefore, it is difficult to confidently hypothesise summer precipitation is a key driver of

the presence of *Phytophthora* species. In other studies, precipitation is the primary factor shaping the distribution and community composition of *Phytophthora* species. *Phytophthora cinnamomi* is constrained by precipitation in the southwest of Australia (Burgess et al. 2017a), and the mean precipitation of the warmest quarter was the strongest climate predictor of *Phytophthora* community in Australia (Burgess et al. 2018b). Increased aridity is linked to the recovery of *P. ramorum* from hosts (Lione et al. 2017). Precipitation appears to be a stronger driver than temperature in shaping terrestrial *Phytophthora* communities in colder climates (Redondo et al. 2018). The low abundance and richness of *Phytophthora* species within the survey region is likely a result of the low annual and summer precipitation compared to other regions of southwest of Australia and the world. An extensive survey or a review of *Phytophthora* species detected in southwest of Australia is required to accurately determine the influence of a Mediterranean climate on the functional diversity and distribution of *Phytophthora*.

The status of *Phytophthora* species found in the survey region can be more accurately estimated based on the frequency and location of detections. The *P. versiformis* complex was the only abundant native phylotype and was first found to be distributed across the southwest of Australia in natural and disturbed vegetation (Paap et al. 2017c, Khdiar 2018). Although, *P. versiformis* cannot be separated from *P. quercina* and *P. ohioensis* based on ITS1, their detection is doubtful as these species have not been reported within Australia (Paap et al. 2017c, Jung et al. 2018). *Phytophthora versiformis* was associated with marri and was first detected on the Geraldton Sandplain from the roots of this tree species (Paap et al. 2017c, Croeser et al. 2018). However, *P. versiformis* appears to be weakly pathogenic to native hosts and not a major predisposing factor to canker disease experienced by marri (Paap et al. 2017a, Croeser et al. 2018). *Phytophthora versiformis* is probably native to kwongan vegetation; however, it may have been distributed widely through the study region over the past century from another plant community in WA. Conversely, current evidence suggests *P. elongata* may be introduced into the survey region. *Phytophthora elongata* was recently recognised as a distinct species, and it is hypothesised to have been introduced to the Jarrah forest of WA due to a clonal population structure (Rea et al. 2010). This species has primarily been detected in Australia (eastern states and WA), but has been identified in a North American nursery and South African vegetation (Bienapfl and Balci

2014, Bose et al. 2018). *Phytophthora elongata* was detected more often along dispersal pathways and was infrequent in natural vegetation compared to *P. versiformis* complex. The distribution and frequency of detection support the hypothesis that *P. elongata* was introduced into WA, and was likely transmitted into the survey region from a source within the state.

*Phytophthora* species previously hypothesised to be native to kwongan plant communities, such as *P. arenaria*, *P. constricta*, and members of clade 6a were absent or rarely detected in natural vegetation. This result suggests these *Phytophthora* species may not be native to dry shrubland and woodland communities; however, water gaining sites were not extensively surveyed in this study and these species may be localised within wetter niche environments (Burgess et al. 2018c). *Phytophthora arenaria* and *P. boodjera* have been identified as damping-off pathogens and may not have been detected due to the sampling of mature plant species (Chapter 2, Simamora et al. 2017). The absence of previously detected *Phytophthora* species on the Geraldton Sandplain make the status and subsequent management of these pathogens ambiguous.

Traditional baiting and isolation methods were not used to detect *Phytophthora* due to the high probability of false negative results and the time intensive procedure (Hüberli et al. 2000, Davison and Tay 2005, O'Brien et al. 2009). High throughput sequencing (HTS) from roots detects the greatest number of *Phytophthora* species, many of which are not frequently isolated using traditional baiting methods (Khaliq et al. 2018). Traditional baiting methods increase the accuracy of identifications through the sequencing of living *Phytophthora* isolates. Molecular detection methods can identify persistent DNA from deceased *Phytophthora* in soil and plant tissue leading to false positives (Nielsen et al. 2007, Kunadiya et al. 2019). However, extracting DNA from cleaned rhizosphere roots decreases the probability of detecting dead organisms and identifies *Phytophthora* species that are infecting host plants. This method of *Phytophthora* spp. detection is likely the most accurate as it reduces the likelihood of both false negatives and positives associated with traditional baiting and persistent DNA, respectively.

This study sets a baseline for the native *Phytophthora* species that should typically be detected within dry kwongan and Banksia woodland plant communities. Protected areas on the Geraldton Sandplain bioregion remain relatively free from introduced *Phytophthora*

species, despite the high abundance and richness detected by Khdiar (2018) in urban nature reserves and green amenity spaces in the nearest major city. The remote location of the surveyed protected areas and long dry summers likely make anthropogenic dispersal and the establishment of soil-borne plant pathogens difficult in kwongan plant communities. Hygiene and management practices are still necessary within the region as *Phytophthora* species appear to spread easily into the highly susceptible plant community once established on edges (Wills 1993, Shearer et al. 2004). The low species richness and abundance of *Phytophthora* species detected compared to previous studies highlighted sampling collection and processing procedures need to be reviewed to determine their impact on the monitoring of plant disease and metabarcoding surveys. Detections of *P. arenaria*, *P. cooljarloo*, *P. kwongonina*, *P. pseudorosacearum* and *P. rosacearum* in dry native vegetation should be treated with caution until their native range can be confirmed. *Phytophthora versiformis* appears to be native to the region and detections of the species in natural vegetation do not need to be managed as an infestation.

# Chapter 4: Damping-off within post-mining ecological restoration and the influence of fungicide seed coats on seedling emergence and survival


## Abstract

Broadcast seeding is a key technique for returning plant species to post-mining ecological restoration; however, the practice is inefficient and expensive. Soil-borne damping-off pathogens reduce seedling emergence and survival and may impact the efficiency of broadcast seed. Fungicide and arbuscular mycorrhizal fungi seed coatings, novel technologies in ecological restoration, were applied to the seed of native plant species and sown into the restoration of a hyper-diverse Mediterranean plant community. Metalaxyl-M and Fludioxonil fungicides applied as film layer seed coatings improved the seedling emergence of five kwongan and Banksia woodland plant species by 5–18%. Common damping-off genera, *Pythium*, *Fusarium* and *Rhizoctonia* were isolated from restoration topsoil and seedling roots. The effect of fungicides varied over time, indicating damping-off pathogens may be more virulent in late winter and biodegradation of fungicides may occur. The commercial arbuscular mycorrhizal fungi seed coating had no effect on seedling emergence and survival. These results suggest damping-off pathogens are active in the topsoil of ecological restoration and are responsible for a proportion of broadcast seed losses. The management and study of damping-off pathogens may further increase the efficiency of broadcast seed and improve ecological restoration outcomes.


## Introduction

Post-mining ecological restoration is a regulatory requirement in Australia and the practice aims to return vegetation that reflects the floristic diversity and function of natural plant communities. Broadcast seeding is a commonly practiced method of returning plant species to ecological restoration projects. The seed is harvested from adjacent native sites, stored, treated with necessary germination stimulants and sown into the topsoil (Koch 2007a). The efficiency of broadcast seeding can be as low as 2–7% (Rokich et al. 2002, Hallett et al. 2014)

and contribute 1% of total germinable seed (Bellairs and Bell 1993). The cost of broadcast seeding can be expensive due to the time taken to collect a sufficient quantity of seed for large scale restoration projects. Despite the efficiency and financial cost, the method substantially increases the species richness of restoration sites compared to independent return of topsoil (Bellairs and Bell 1993, Rokich et al. 2002, Koch 2007b). Broadcast seeding allows the re-introduction of plant species that are serotinous (or bradysporus), and require environmental triggers which are absent from the restoration site (Koch 2007b). Fire is an integral process in the reproduction of many plant species in Mediterranean-type ecosystems (Bell et al. 1993), and can be simulated through picking and releasing seeds from fruits, heat shock and smoke water treatments (Sweedman and Merritt 2006).

Plant species with soil-stored seed that do not require specific environmental triggers can be returned through topsoil transfer. The process of topsoil transfer re-introduces seed and the microbial community back to restoration sites (Rivera et al. 2014). Topsoil will often be stripped to a depth of 150 mm from a donor site before it is mined, stockpiled for six months or longer, transferred, and then spread over the restoration site (Koch 2007a). The microbial community is important in ecological restoration as it contains beneficial mutualists (Neuenkamp et al. 2018). Mycorrhizal fungi and nitrogen-fixing bacteria improve the health and survival of host seedlings (Van Der Heijden et al. 2008, Jung et al. 2012). The microbial community is disturbed by the initial stockpiling and distribution of topsoil (Harris et al. 1989, Williamson and Johnson 1990), but microbial biomass and arbuscular mycorrhizal fungi (AMF) communities slowly re-establish after these events (Jasper et al. 1987, Frouz et al. 2013, Birnbaum et al. 2017).

Soil-borne plant pathogens can severely impact the emergence and survival of seedlings in managed and unmanaged environments (Comita et al. 2014, Lamichhane et al. 2017). Damping-off pathogens affect the emergence and survival of seedlings by causing the rapid decay of the seed, radicle, hypocotyl and root tissue (Tainter and Baker 1996). The most common damping-off pathogens are from the oomycete genera *Pythium* and *Phytophthora*, and the fungal genera *Fusarium* and *Rhizoctonia* (Tainter and Baker 1996). Native damping-off plant pathogens are a mechanism for maintaining the diversity of natural plant communities (Bever et al. 2015). Despite the focus on natural plant communities in the literature, few studies have investigated the impact of plant pathogens on ecological

restoration. For example, reviews by Kardol and Wardle (2010), Macdonald et al. (2015) and Perring et al. (2015) identify very few studies that explore the role of plant pathogens in ecological restoration. Soil-borne plant pathogenic fungi have been isolated in young and old post-mining forest restoration sites (Nováková 2001). Genera commonly regarded as damping-off pathogens have survival spores and structures (Ayers and Lumsden 1975, Sitton and Cook 1981, Crone et al. 2013, Jung et al. 2013, Ritchie et al. 2013), allowing them to persist long term in the soil and plant material of stockpiles. Given that seedling emergence appears to be the critical life stage in determining the outcome of restoration projects (James et al. 2011), and the efficiency of broadcast seed is low (Bellairs and Bell 1993), damping-off plant pathogens may be responsible for pre- and post-emergent seedling mortality.

Seed coating technologies are an understudied management strategy for improving emergence and seedling health in ecological restoration (Pedrini et al. 2017). Nutrients, protectants and symbiotic microorganisms can be applied to the surface of seed before sowing through pelleting, encrusting or thin film layers (Taylor et al. 1998). Fungicide coatings have primarily been applied to agronomic seeds and can improve germination and emergence (Sharma et al. 2015, Pedrini et al. 2017). Fungicides have been successful in controlling seed-borne pathogenic fungi for species used in desert restoration (Derbel et al. 2010). Other seed coat technologies such as crust penetrative seed agglomerations (Madsen et al. 2012), seed predator protective clays (Overdyck et al. 2013), and herbicide protective layers (Madsen et al. 2014) have demonstrated the ability to improve seedling establishment and health in restoration. Small or no effect was observed when the seed of North American shrub and grass species were treated with biochar (Williams et al. 2016), or when polymer seed coatings were applied to Banksia woodland plant species (Turner et al. 2006) in ecological restoration.

The post-mining restoration of kwongan and Banksia woodland plant communities of the southwest Australia provide an opportunity to test seed coat treatments. Broadcast seeds are an important but often inefficient method of plant species return in these environments (Bellairs and Bell 1993, Rokich et al. 2002). Putatively native *Phytophthora* species are frequently detected from natural and disturbed kwongan and Banksia woodland plant communities (Rea et al. 2011, Burgess et al. 2018c), and the genus is associated with

damping-off in this ecosystem (Chapter 2). However; the abundance, distribution, and diversity of other damping-off soil-borne plant pathogens in natural and disturbed kwongan and Banksia woodland plant communities are not known. Additionally, topsoil stockpiling disturbance disrupts AMF in kwongan plant communities, contributing to lower levels of root colonisation (Birnbaum et al. 2017). Applying fungicides or mycorrhizal spores to the seed coat are a potential solution for reducing damping-off and increasing mycorrhizal availability for native seedlings in restoration. Improving the emergence and survival of broadcast seed in post-mining restoration will reduce the financial cost, pressures on native plant community harvest populations (Nevill et al. 2018) and improve community composition similarity to reference sites (Herath et al. 2009a).

This study aimed to determine the potential benefit of applying fungicide film coat layers or AMF inoculum to broadcast seed used in post-mining restoration. The use of different fungicides will additionally reflect the activity of the various groups of damping-off pathogens. The study aimed to answer the following specific questions:

1. Do fungicide film coat layers and beneficial AMF inoculum applied to seed improve the emergence and survival of seedlings in post-mining restoration?
2. What effect do seed coat treatments have on the emergence and survival of seedlings over time?
3. Do the effects of fungicide seed coats vary, indicating the activity of different groups of damping-off pathogens?

## Methods

### Site location and description

Plots (*n* = 36) were established within the Tronox Ltd Cooljarloo mineral sand operation in 2017 and 2018, and Iluka Resources Ltd Eneabba mineral sand operation in 2017 (Figure 4.1A–C). The Cooljarloo (30.65°S, 115.45°E) and Eneabba (29.875°S, 115.285°E) sites are located 150km and 250km north of Perth, Western Australia (WA), respectively (Figure 4.1D). The Cooljarloo site is bisected by the border of the Geraldton Sandplains and Swan Coast Plain Australian biogeographical regions; however, plots were situated on the Perth

sub-region of the latter (DEE, 2018). Eneabba lies within the Lesueur Sandplains sub-region of the Geraldton Sandplain biogeographical region (DEE, 2018).

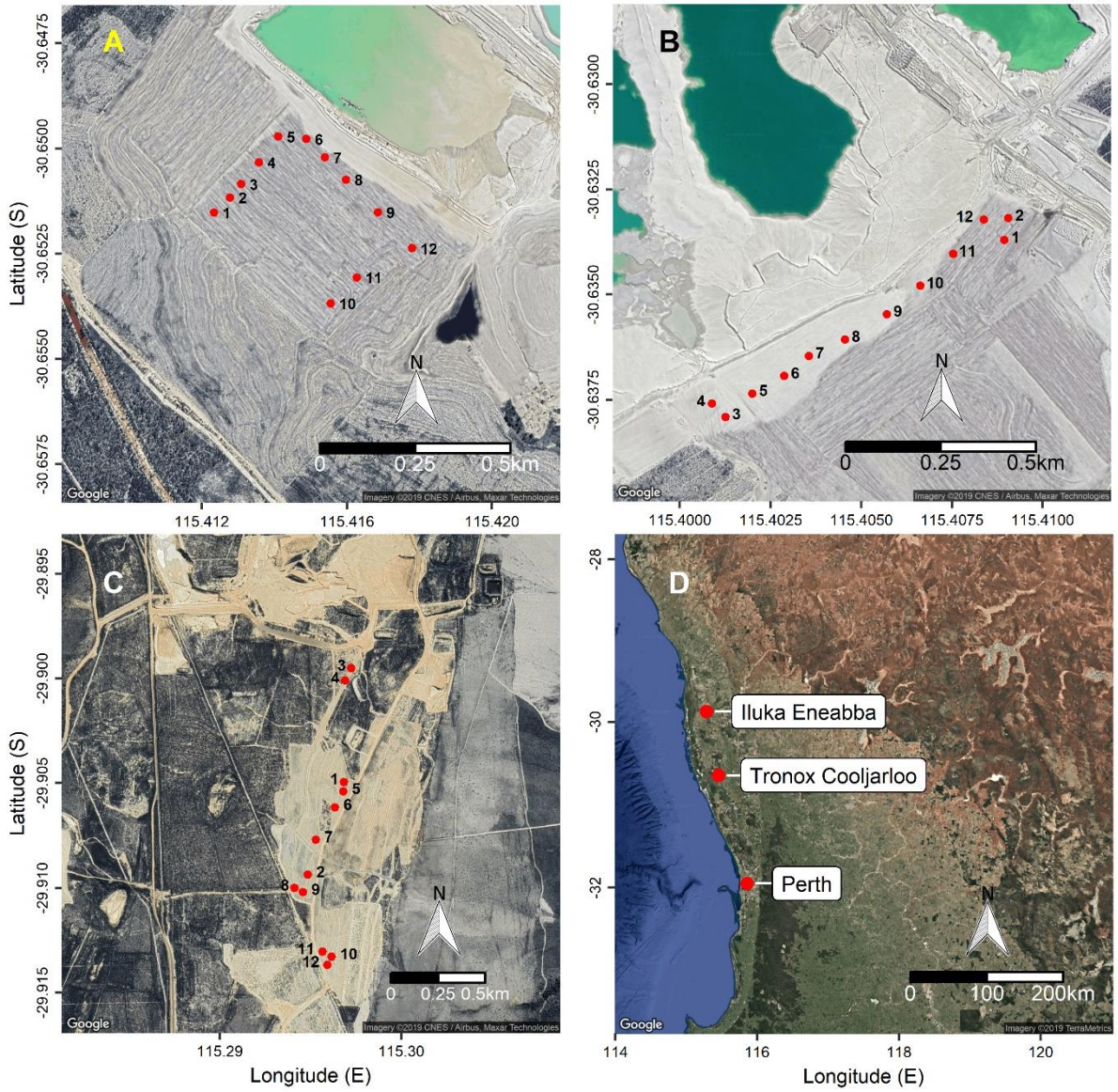


**Figure 4.1:** Plot distributions at sites **A.** Cooljarloo 2017, **B.** Cooljarloo 2018, **C.** Eneabba 2017, and **D.** site locations in WA relative to the capital city, Perth.

Cooljarloo and Eneabba experience a Mediterranean climate, which is characterised by a prolonged period of drought over the summer and rainfall during the winter months. Kwongan plant communities in the region can receive between 450 mm and 625 mm of mean annual rainfall (Mucina et al. 2014). On average (1968–2018) Cooljarloo and Eneabba received annual rainfalls of 559 mm and 489 mm, respectively (Bureau of Meteorology

2019, DPIRD, 2019). The Cooljarloo site is situated between the Lancelin and Badgingarra weather stations and rainfall data were extracted from both weather stations and averaged. After plot establishment, Cooljarloo received an estimated 286 mm of rainfall between June and August 2017 and 292 mm over the same period in 2018 (Bureau of Meteorology 2019). Eneabba received 280 mm between June and August 2017 (DPIRD, 2019).

Pre-mining, Cooljarloo has kwongan and Banksia woodland plant communities. These plant communities are defined by their high species richness and diversity (Lamont et al. 1984, Hopper and Gioia 2004, Mucina et al. 2014). Classification groupings indicate four Wet Heath and two Banksia woodland communities present at Cooljarloo (Tsakalos et al. 2019). Banksia woodland and kwongan plant communities at Cooljarloo were identifiable by the high abundance of proteaceous and myrtaceous genera, respectively. The pre-mining vegetation at the Eneabba site was a mosaic of common subclassifications of kwongan vegetation, and these communities were described as 1A, 3A, 4A, and 6B by Tsakalos et al. (2018).

All experimental plots were located within post-mining restoration. All restoration activities were completed by the mining companies between February and April 2017 and 2018, prior to plot establishment. At Cooljarloo in 2017 and 2018 plots were established in rehabilitated mining pits (Figure 4.1A–B). At these sites, 50 mm of six-month-old topsoil was laid above a 200 mm layer of topsoil that had been stockpiled for less than 10 years. Turf Special fertilizer produced by CSBP Ltd containing trace elements, nitrogen (N), phosphorus (P), and potassium (K) at 12.8-2.3-6.2 was added at 40 kg/ha to Cooljarloo restoration sites. Plots at Eneabba were dispersed across a more heterogeneous restoration site (Figure 4.1C). Three plots were in a mining pit that was being rehabilitated for a second time and two were placed at a site that was previously a haul road. Topsoil was derived from previously rehabilitated kwongan plant communities established in the late 1980s, stockpiled for ten years before being spread to a depth of 100 mm. The Eneabba restoration site was amended with a low phosphorus fertiliser (NPK 18-1-11) at 50 kg/ha that contained additional trace elements produced by Summit Fertilisers.

### Species selection

Plant species selected and analysed in the experiment were predominantly from the Fabaceae and Proteaceae (Table 4.1). The plant species chosen were in the native broadcast

seed mix at both sites. In 2018, some plant species were removed from the experiment and replaced due to the availability or viability of seed (Table 4.1). The seed used was sourced in the immediate locality of each mine site by either the mining companies or through seed suppliers. The seed supplied by each site was not combined as to adhere to the local provenance requirements in restoration areas. Plant species received appropriate germination treatments, as recommended by Sweedman and Merritt (2006), prior to the application of fungicides (Table 4.1).

**Table 4.1:** The plant species selected and the number and location of plots containing seed.

| | | | | Site | | |
|---|---|---|---|---|---|---|
| Family | Species | Germination treatment[1] | Nutrient Acquisition[2] | Eneabba 2017 | Cooljarloo 2017 | Cooljarloo 2018 |
| Fabaceae | *Acacia pulchella* subsp. *pulchella* | HW, S | AM | 12 | 12 | 12 |
| Ericaceae | *Astroloma xerophyllum* | Smk | ERM | | | 10 |
| Proteaceae | *Banksia attenuata* | None | NMCR | 12 | 11 | 12 |
| Proteaceae | *Banksia candolleana* | None | NMCR | 12 | 12 | |
| Proteaceae | *Banksia menziesii* | None | NMCR | 10 | 11 | 12 |
| Proteaceae | *Banksia prionotes* | None | NMCR | | | 12 |
| Fabaceae | *Bossiaea eriocarpa* | HW, S | AM/NMCR | | | 12 |
| Fabaceae | *Daviesia podophylla* | HW, S | AM/ECM | 12 | 11 | 10 |
| Myrtaceae | *Eremaea beaufortioides* var. *beaufortioides* | None | AM/ECM | 12 | 12 | |
| Myrtaceae | *Eucalyptus macrocarpa* subsp. *elachantha* | None | ECM/AM | | | 12 |
| Myrtaceae | *Eucalyptus todtiana* | None | ECM/AM | 12 | 12 | 7 |
| Fabaceae | *Gastrolobium capitatum* | HW, S | ECM | | | 12 |
| Proteaceae | *Hakea costata* | None | NMCR | 11 | 12 | 12 |
| Proteaceae | *Hakea incrassata* | None | NMCR | 11 | 10 | |
| Proteaceae | *Hakea trifurcata* | None | NMCR | 11 | 12 | 10 |
| Fabaceae | *Kennedia prostrata* | HW, S | AM/NMCR | 8 | 9 | 9 |
| Iridaceae | *Patersonia occidentalis* | Smk | AM | | | 12 |
| Asphodelaceae | *Xanthorrhoea preissii* | Smk | AM | 4 | 10 | 11 |

[1] Germination treatments abbreviated as HW, hot water; S, scarify; and Smk, smoke water.
[2] Nutrient acquisition strategy from Zemunik et al. (2015). AM, arbuscular mycorrhizal fungi; ECM, ectomycorrhizal fungi; ERM, Ericoid mycorrhizal; and NMCR, non-mycorrhizal cluster roots.

## Plot establishment

Plant species received four seed coat treatments in 2017, (1) Apron® XL 350 ES, (2) Maxim® XL, (3) a combination of Apron XL 350 ES and Maxim XL, and (4) a negative control. Apron XL 350 ES and Maxim XL are liquid fungicides produced by Syngenta®. Apron's active constituent is Metalaxyl-M at 350 g/l and controls *Pythium* and *Phytophthora* rot. Maxim contains two active constituents, 25 g/l of Fludioxonil and 10 g/l of Metalaxyl-M and controls *Pythium*, *Fusarium* and *Rhizoctonia* diseases. Directions on the product labels were followed when selecting the application method and rate. The Apron and Maxim fungicides

were applied as film coating layers at rates of 2 ml/kg and 3 ml/kg of seed, respectively. In 2018, the combined fungicide treatment was removed and replaced with a commercial vesicular arbuscular mycorrhizal fungi inoculum. Maxx produced by MycoApply® was applied as a dust seed coat at 100 g/kg of seed.

Plots were placed at least 25 m away from the restoration site's edges (Figure 4.1). Each plot was 6.5 m × 2 m and contained four 1 m × 1 m treatment quadrats that were separated by 0.5 m. The seed of each plant species was sown in lines separated by 25 mm in each treatment quadrat, and was covered by 5–10 mm of topsoil. A treatment quadrat contained 75 seed of each plant species with the same seed coat treatment. The order of the treatment quadrats and rows of plant species were randomised. The perimeter of the plot was fenced with 1.2 m high galvanised wire netting to exclude large seed and seedling predators. Throughout the experiment, seedlings that emerged from the soil seed bank were removed from treatment quadrats if it did not disturb the roots of the selected plant species.

Plots were established and seed sown 18–21 April 2017 at Eneabba. Plots were established at Cooljarloo 9–11 May 2017 and 30 May–1 June 2018. Seedling counts were completed three times between late July and September at Eneabba in 2017. At Cooljarloo plots were monitored four times between mid-July and September 2017, and five times between July and October 2018. The number of alive and dead seedlings present in each treatment quadrat were counted during each monitoring visit. A standard photograph was taken from above each treatment quadrat to compare to previous monitoring visits. These photographs were used to confirm seedling numbers and identify perished seedlings between trips. Post-summer monitoring occurred the year following plot establishment in June 2018 and May 2019 after summer drought and moderate rainfalls. In 2018, seedlings were thinned in October so each treatment quadrat contained a maximum of seven individuals of each plant species. The remaining seedlings represented the average health and condition of the species in the treatment quadrat. Plots were thinned to avoid seedling deaths related to high seedling densities.

Dead seedlings and non-emergent seeds were not removed from within treatment quadrats to avoid disturbing the remaining seedlings and seed. Soil samples were collected from around the edge of each plot in 2017 and 2018 during establishment. Soil was baited as

outlined by O'Brien et al. (2009) and leaf tissue was plated onto NARPH and amended NARPH agar plates to isolate any *Phytophthora* and *Pythium* species, respectively (Hüberli et al. 2000). For the detection of *Pythium* spp., hymexazol was removed from a set of NARPH plates due to the sensitivity of this genus to the fungicide (Kato et al. 1990). At Cooljarloo in 2018, in addition to baiting, the remaining seeds that were not used in the experiment were sown in rows in between the treatment quadrats. The plant species sown and harvested in these rows were *A. pulchella*, *B. attenuata*, *B. eriocarpa*, *G. capitatum*, *H. trifurcata* and *X. preissii*. Seedlings were harvested on 5$^{th}$ of September 2018, 9 weeks after the first emergent seedlings, and roots and stems were cleaned with sterile water, dried and then plated onto regular and hymexazol removed NARPH, malachite green agar (MGA), and ½ potato dextrose agar (PDA) agar plates for the isolation of *Phytophthora*, *Pythium*, *Fusarium* (Leslie et al. 2006) and *Rhizoctonia* (Nontachaiyapoom et al. 2010) species, respectively. The MGA and ½ PDA agar plates per 1 L of deionised water, contained antibiotics, 100 mg ampicillin, 1 ml nystatin and 10 mg rifampicin. When isolates were cleaned, they were grown on ½ PDA agar plates without antibiotics for seven days and identified to the species level through amplification and sequencing of the ITS gene region for *Pythium* species as described previously (Burgess et al. 2018c) and for *Fusarium* and *Rhizoctonia* species as described previously (Burgess et al. 2018a).

### Statistical analysis

Plant species with sufficient seedling emergence and survival data were analysed and reported. For individual plant species at each site, the mean proportion of the control was subtracted from the proportion of seedlings in each treatment. The difference between proportions was an absolute effect size and used as a response variable in the analysis. Seedling emergence and survival were a proportion of the total seed sown. Outliers were removed from the dataset. The site and seed coat treatment were interaction terms; however, site was removed from the model as it caused heteroskedasticity. The sites were then analysed separately and collectively without an interaction term. The data sets were analysed with an analysis of variance (ANOVA) with seed coat treatment as the predictive variable and plot as the random intercept. The data sets were first analysed with a binomial generalised linear mixed model (GLMM) before assumption validation procedures

determined that the analysis poorly fit the data (Zuur et al. 2010). The mixed effects ANOVA models met assumptions and fitted the data better than the binomial GLMMs.

Additionally, a two-way mixed effects ANOVA with an interaction between treatment and monitoring time was performed on plant species that had significant response to seed coats. The proportion of emergent seedlings out of the available non-emergent seed was calculated for each treatment at the first two monitoring visits. The mean proportion of the control at each time was subtracted from the proportion of emergent seedlings in each treatment. The absolute effect size of the available non-emergent seed at each monitoring visit was used as the response variable.

Tukey adjusted *P*-values below an alpha of 0.05 were determined to be significant for all analyses. The analysis was performed in R (R Core Team 2018), using the car (Fox and Weisberg 2019), lme4 (Bates et al. 2014), effects (Fox 2003), and emmeans packages (Lenth 2018).

## Results

### Seedling emergence

There was sufficient seedling emergence data for 14 of 18 plant species at one or more sites to be statistically analysed. Individual and combined site analyses were performed for eight plant species where there was sufficient data from two or more sites. Fungicide seed coats protected five plant species from pre-emergent damping-off. The seedling emergence of *B. attenuata, B. candolleana*, *Eucalyptus macrocarpa* subsp*. elachantha, E. todtiana*, and *K. prostrata* were significantly ($P < 0.05$) improved by fungicide seed coats relative to the control.

The Apron seed coat consistently increased the seedling emergence of *B. attenuata* at all sites (Table 4.2). The Apron fungicide improved *B. attenuata* seedling emergence by 7.2% on average in comparison to the control treatments (Figure 4.2). Unlike the other plant species, the Maxim seed coat for *B. candolleana* increased the seedling emergence over the Apron treatments at Cooljarloo in 2017 (Table 4.2). This result was not observed for *B. candolleana* at the Eneabba site in 2017. On average, Maxim improved the seedling emergence of *B. candolleana* relative to the control by 8.6% (Figure 4.2). The seedling emergence of

*K. prostrata*, sown at all three sites, was improved by Apron and the combined fungicide seed coats over the controls at Cooljarloo in 2017 by 8.8% and 9.7%, respectively (Table **4.2**). Averaged across the sites, Apron and the combined fungicide seed coats improved the seedling emergence of *K. prostrata* by 4.2% and 4.9%, respectively (Figure **4.2**). Apron and Maxim seed coats increased the emergence of *Eucalyptus macrocarpa* subsp*. elachantha* in comparison to the control treatment by 13.9% and 15.4%, respectively. Similarly, the Apron seed coat improved *E. todtiana* seedling emergence by 18.7% in comparison to the control at Cooljarloo in 2018 (Table 4.2). The results could not be repeated for *E. macrocarpa* subsp*. elachantha* as it was sown at one site, and *E. todtiana* due to poor seed viability in 2017.

The seedling emergence of the remaining nine plant species was unaffected by fungicide or AMF seed coat treatments at individual sites or averages across experiments. *Acacia pulchella* subsp. *pulchella*, *Bossiaea eriocarpa*, *H. costata* and *H. trifurcata* displayed some positive trends associated with fungicide treatments (Table 4.2, Figure 4.2). Negligible differences between seed coat treatments were found for *B. menziesii*, *Daviesia podophylla*, *Gastrolobium capitatim*, *H. incrassata* and *Xanthorrhoea preissii*. The seedling emergence of plant species with AMF mutualist relationships, *A. pulchella*, *B. eriocarpa*, *G. capitatum*, *K. prostrata* and *X. preissii*, were unaffected by the AMF seed coat treatment (Table 4.2).

**Table 4.2:** The plant species analysed and the mean percentage of the total emergent seedlings in each of the fungicide and arbuscular mycorrhizal fungi treatments. Letters represent homogenous subsets generated from Tukey *post-hoc* adjusted *P*-values ($P < 0.05$), and bold results were significant. The number of plots used in the statistical analyses after outliers were removed is displayed.

| | | | Treatment[1] | | | | |
|---|---|---|---|---|---|---|---|
| Plant species | Site and year | Plots | Control | Apron | Maxim | A+M | AMF |
| *Acacia pulchella* | Eneabba 2017 | 12 | 47.1 a | 36.3 a | 44.0 a | 42.7 a | |
| | Cooljarloo 2017 | 12 | 64.1 a | 70.0 a | 70.1 a | 67.9 a | |
| | Cooljarloo 2018 | 12 | 38.6 a | 53.3 a | 52.2 a | | 48.1 a |
| *Banksia attenuata* | Eneabba 2017 | 6 | 30.2 a | 42.4 a | 43.1 a | 41.6 a | |
| | Cooljarloo 2017 | 11 | 11.3 a | 17.1 a | 13.6 a | 16.4 a | |
| | Cooljarloo 2018 | 12 | 34.8 a | 40.7 a | 36.6 a | | 39.6 a |
| *Banksia candolleana* | Eneabba 2017 | 6 | 21.6 a | 32.9 a | 31.6 a | 27.1 a | |
| | Cooljarloo 2017 | 12 | **34.2 ab** | **27.8 a** | **42.2 b** | **34.7 ab** | |
| *Banksia menziesii* | Eneabba 2017 | 6 | 18.0 a | 12.2 a | 18.2 a | 11.1 a | |
| | Cooljarloo 2017 | 11 | 21.5 a | 26.3 a | 23.8 a | 27.6 a | |
| | Cooljarloo 2018 | 12 | 12.1 a | 13.7 a | 12.1 a | | 8.6 a |
| *Bossiaea eriocarpa* | Cooljarloo 2018 | 12 | 11.4 a | 17.7 a | 14.8 a | | 13.7 a |
| *Daviesia podophylla* | Eneabba 2017 | 12 | 45.4 a | 48.5 a | 52.5 a | 49.9 a | |
| *Eucalyptus macrocarpa* | Cooljarloo 2018 | 12 | **12.0 a** | **25.9 b** | **27.4 b** | | **12.0 a** |
| *Eucalyptus todtiana* | Cooljarloo 2018 | 7 | **25.1 a** | **43.8 b** | **35.8 ab** | | **25.9 a** |
| *Gastrolobium capitatum* | Cooljarloo 2018 | 12 | 23.2 a | 31.1 a | 20.1 a | | 27.4 a |
| *Hakea costata* | Eneabba 2017 | 9 | 36.1 a | 51.1 a | 49.2 a | 39.4 a | |
| | Cooljarloo 2017 | 12 | 8.2 a | 8.7 a | 9.0 a | 10.1 a | |
| *Hakea incrassata* | Eneabba 2017 | 7 | 76.2 a | | 65.3 a | 65.7 a | |
| *Hakea trifurcata* | Eneabba 2017 | 10 | 38.7 a | | 50.8 a | 49.3 a | |
| | Cooljarloo 2017 | 12 | 28.8 a | | 33.1 a | 30.3 a | |
| | Cooljarloo 2018 | 10 | 30.8 a | 32.4 a | 31.2 a | | 30.8 a |
| *Kennedia prostrata* | Eneabba 2017 | 8 | 20.2 a | 21.5 a | 18.0 a | 20.0 a | |
| | Cooljarloo 2017 | 9 | **19.6 a** | **28.4 b** | **25.2 ab** | **29.3 b** | |
| | Cooljarloo 2018 | 9 | 6.8 a | 9.2 a | 10.8 a | | 9.6 a |
| *Xanthorrhoea preissii* | Cooljarloo 2017 | 10 | 49.1 a | 45.6 a | 39.5 a | 47.2 a | |
| | Cooljarloo 2018 | 10 | 48.8 a | 51.3 a | 51.0 a | | 49.9 a |

[1] Treatment names were abbreviated to Apron, Apron XL; Maxim, Maxim XL; A+M, Apron XL and Maxim XL; AMF, MycoApply Maxx.

## Seedling survival

The seedling survival of plant species over winter and early spring was consistent with the observed emergence trends. There was a significantly ($P < 0.05$) greater number of seedlings in fungicide treatments compared to control treatments for five plant species (Table 4.3, Figure 4.2). However, seedling mortality was similar between the seed coat and control treatments, and indicated fungicides and AMF inoculum did not protect against post-emergence damping-off. The greater numbers of *B. attenuata*, *B. candolleana*, *E. macrocarpa* subsp*. elachantha*, *E. todtiana* and *K. prostrata* surviving seedlings in fungicide treatments were primarily the result of improved emergence. Post-emergent seedling deaths above 5% were uncommon. A comparatively large number of *A. pulchella*,

*B. attenuata*, *E. macrocarpa* and *E. todtiana* seedling deaths were recorded at Cooljarloo in 2018 in comparison to other plant species across the three sites.

**Table 4.3:** The mean percentage of surviving seedlings in October of the same year that seeds were sown and treated with fungicides and arbuscular mycorrhizal fungi. Letters represent homogenous subsets generated from the seedling survival statistical analysis and Tukey *post-hoc* adjusted *P*-values ($P < 0.05$), and bold results were significant. The mean percentage of seedling deaths, and post-emergence damping-off, are displayed in brackets.

| | | Treatment[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant species | Site and year | Control | | Apron | | Maxim | | A+M | | AMF | |
| *Acacia pulchella* | Eneabba 2017 | 45.3 a | (-1.8) | 35.7 a | (-0.6) | 42.4 a | (-1.6) | 42.0 a | (-0.7) | | |
| | Cooljarloo 2017 | 60.6 a | (-3.5) | 65.7 a | (-4.3) | 65.3 a | (-4.8) | 61.9 a | (-6.0) | | |
| | Cooljarloo 2018 | 30.6 a | (-8.0) | 47.2 a | (-6.1) | 39.7 a | (-12.5) | | | 36.9 a | (-11.2) |
| *Banksia attenuata* | Eneabba 2017 | 26.2 a | (-4.0) | 39.8 a | (-2.6) | 39.6 a | (-3.5) | 40.0 a | (-1.6) | | |
| | Cooljarloo 2017 | **9.7 a** | (-1.6) | **16.5 b** | (-0.6) | **13.0 ab** | (-0.6) | **15.3 ab** | (-1.1) | | |
| | Cooljarloo 2018 | 29.2 a | (-5.6) | 32.4 a | (-8.3) | 25.9 a | (-10.7) | | | 29.6 a | (-10.0) |
| *Banksia candolleana* | Eneabba 2017 | 19.8 a | (-1.8) | 30.2 a | (-2.7) | 29.6 a | (-2.0) | 25.1 a | (-2.0) | | |
| | Cooljarloo 2017 | **30.9 ab** | (-3.3) | **25.4 a** | (-2.4) | **40.1 b** | (-2.1) | **32.3 ab** | (-2.4) | | |
| *Banksia menziesii* | Eneabba 2017 | 14.4 a | (-3.6) | 9.3 a | (-2.9) | 15.1 a | (-3.1) | 8.7 a | (-2.4) | | |
| | Cooljarloo 2017 | 19.0 a | (-2.5) | 22.7 a | (-3.6) | 22.1 a | (-1.7) | 25.8 a | (-1.8) | | |
| | Cooljarloo 2018 | 10.2 a | (-1.9) | 11.9 a | (-1.8) | 10.4 a | (-1.7) | | | 5.9 a | (-2.7) |
| *Bossiaea eriocarpa* | Cooljarloo 2018 | 8.2 a | (-3.2) | 13.0 a | (-4.7) | 8.8 a | (-6.0) | | | 10.0 a | (-3.7) |
| *Daviesia podophylla* | Eneabba 2017 | 41.1 a | (-4.3) | 44.6 a | (-3.9) | 49.7 a | (-2.8) | 46.3 a | (-3.6) | | |
| *Eucalyptus macrocarpa* | Cooljarloo 2018 | **6.4 a** | (-5.6) | **16.9 b** | (-9.0) | **16.3 b** | (-11.1) | | | **7.4 a** | (-4.6) |
| *Eucalyptus todtiana* | Cooljarloo 2018 | **19.0 a** | (-6.1) | **37.3 b** | (-6.5) | **31.2 ab** | (-4.6) | | | **19.6 a** | (-6.3) |
| *Gastrolobium capitatum* | Cooljarloo 2018 | 20.3 a | (-2.9) | 25.4 a | (-5.7) | 18.1 a | (-2.0) | | | 25.8 a | (-1.6) |
| *Hakea costata* | Eneabba 2017 | 34.1 a | (-2.0) | 49.5 a | (-1.6) | 45.9 a | (-3.3) | 36.7 a | (-2.7) | | |
| | Cooljarloo 2017 | 6.3 a | (-1.9) | 7.4 a | (-1.3) | 6.4 a | (-2.6) | 8.7 a | (-1.4) | | |
| *Hakea incrassata* | Eneabba 2017 | 75.8 a | (-0.4) | | | 64.0 a | (-1.3) | 64.6 a | (-1.1) | | |
| *Hakea trifurcata* | Eneabba 2017 | 34.2 a | (-4.5) | | | 47.5 a | (-3.3) | 46.8 a | (-2.5) | | |
| | Cooljarloo 2017 | 26.7 a | (-2.1) | | | 32.0 a | (-1.1) | 28.0 a | (-2.3) | | |
| | Cooljarloo 2018 | 27.2 a | (-3.6) | 28.7 a | (-3.7) | 26.7 a | (-4.5) | | | 25.6 a | (-5.2) |
| *Kennedia prostrata* | Eneabba 2017 | 19.5 a | (-0.7) | 21.2 a | (-0.3) | 17.3 a | (-0.7) | 19.5 a | (-0.5) | | |
| | Cooljarloo 2017 | **19.3 a** | (-0.3) | **27.9 b** | (-0.5) | **24.6 ab** | (-0.6) | **29.0 b** | (-0.3) | | |
| | Cooljarloo 2018 | 6.7 a | (-0.1) | 8.7 a | (-0.5) | 10.1 a | (-0.7) | | | 9.0 a | (-0.6) |
| *Xanthorrhoea preissii* | Cooljarloo 2017 | 47.1 a | (-2.0) | 43.0 a | (-2.6) | 38.5 a | (-1.0) | 46.3 a | (-0.9) | | |
| | Cooljarloo 2018 | 47.3 a | (-1.5) | 50.4 a | (-0.9) | 50.5 a | (-0.5) | | | 46.7 a | (-3.2) |

[1] Treatment names shortened or abbreviated to Apron, Apron XL; Maxim, Maxim XL; A+M, Apron XL and Maxim XL; AMF, MycoApply Maxx.

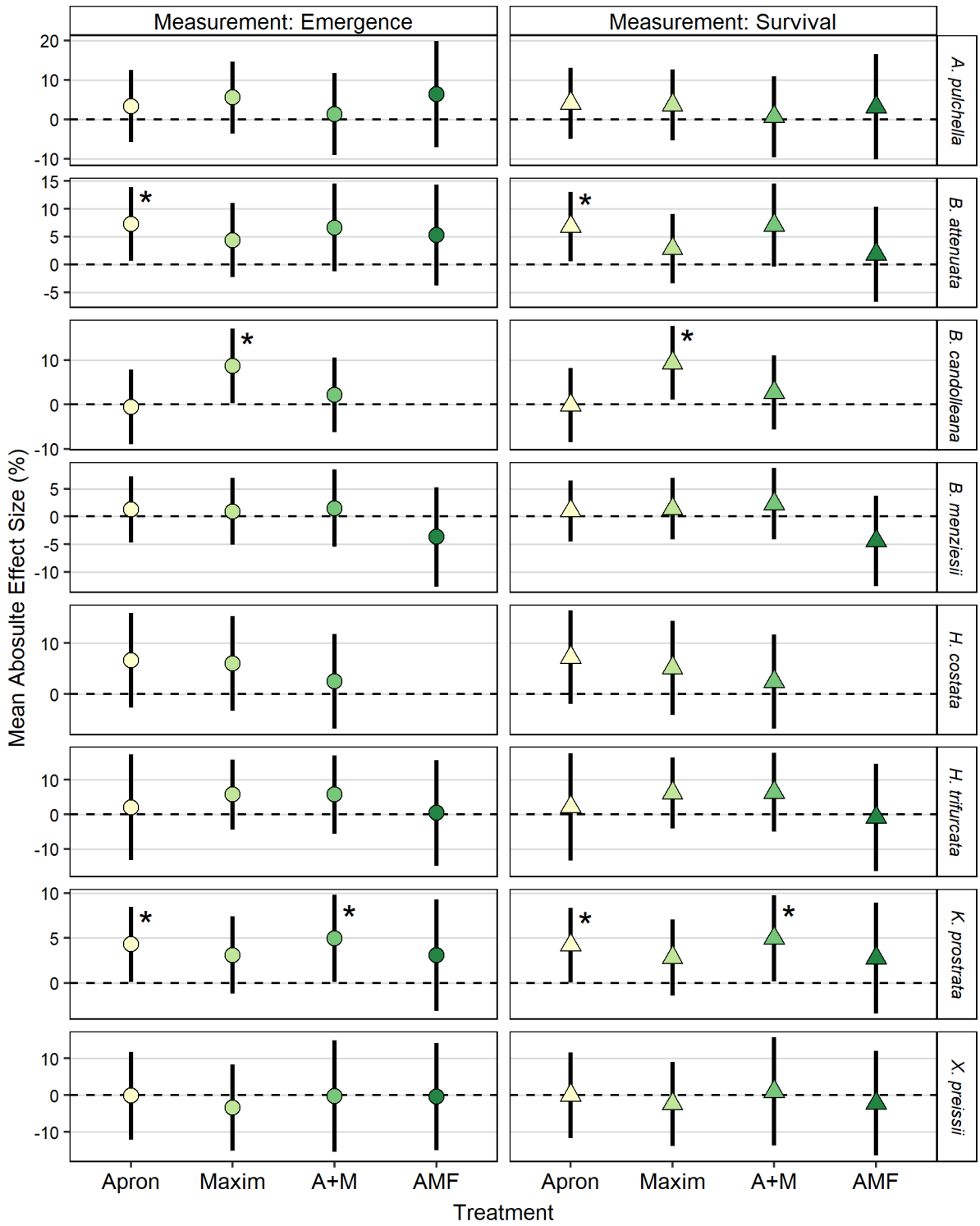


**Figure 4.2:** The mean absolute effect size of fungicide and AMF seed coats on seedling emergence and survival in comparison to the control treatment, for plant species sown at multiple sites. The mean absolute effect size and 95% confidence intervals were extracted from linear mixed effects models. Statistical significance is indicated when the 95% confidence intervals do not intersect zero (emphasized by an asterisk). Treatment names are abbreviated to Apron, Apron XL; Maxim, Maxim XL; A+M, combined Apron XL and Maxim XL; AMF, MycoApply Maxx.

### Seedling emergence over time

Plots were first monitored 20–33 days after the first major rainfall events of the winter (Table 4.4). First rainfall events occurred on the 21 June 2017, and 5 June 2018. Plots were established closer to the completion of rehabilitation works by the mining companies in late April and early May in 2017, and were first monitored a substantial period of time after seeds were sown into the topsoil in comparison to Cooljarloo in 2018 (Table 4.4). The plots were first monitored between 35 and 97 days after sowing (Table 4.4). The timing of the first rainfall event and the late plot establishment in 2018 resulted in different monitoring times. The Cooljarloo site in 2018 received more rainfall between the establishment of the plots and the monitoring visits relative to Eneabba and Cooljarloo in 2017 (Table 4.4).

**Table 4.4:** The period of time and rainfall that occurred between the monitoring visits and the establishment of plots, or the first major winter rainfall event (21/06/2017 and 05/06/2018) at the restoration sites.

| | Monitoring visit 1 | | | Monitoring visit 2 | | |
|---|---|---|---|---|---|---|
| Site and sow year | Post-Establishment (days) | First winter rainfall event (days) | Rainfall (mm) | Post-Establishment (days) | First winter rainfall event (days) | Rainfall (mm) |
| Eneabba 2017 | 97 | 33 | 100.4 | 119 | 55 | 190.2 |
| Cooljarloo 2017 | 63 | 20 | 97.1 | 85 | 42 | 189.2 |
| Cooljarloo 2018 | 35 | 29 | 142.4 | 49 | 43 | 226.7 |

Differences in seedling emergence between seed coat treatments were greatest in mid-July. Seedling emergence primarily occurred before the first monitoring visit to the sites, and had concluded by the third monitoring visit in early to mid-August. The plant species that experienced significantly greater seedling emergence when treated with fungicide seed coats were analysed to determine if the effect of the treatments changed over time (Figure 4.3). The protection provided to pre-emergent seedlings by fungicide treatments significantly ($P < 0.05$) varied over time for *B. attenuata, B. candolleana* and *K. prostrata* at Cooljarloo in 2017, and *E. macrocarpa* subsp. *elachantha* and *E. todtiana* at Cooljarloo in 2018.

*Banksia attenuata* seedling emergence improved relative to the control when treated with Apron and Maxim seed coats in mid-July at Cooljarloo in 2017; however, there was no affect in August (Figure 4.3). As a proportion of available non-emergent seed, the seedling

emergence of *B. attenuata* treated with Apron and Maxim was greater in mid-July compared to August. The seedling emergence of *B. candolleana* and *K. prostrata* treated with Maxim and Apron seed coats, respectively, followed the same trends relative to the control and time as *B. attenuata* (Figure 4.3). The difference between the seedling emergence of *B. candolleana* treated with Apron and Maxim was not consistent during the experiment (Table 4.2; Figure 4.3). The seedling emergence of *B. candolleana* treated with Apron decreased between mid-July and August, and there was no difference between Apron and Maxim treatments in mid-July (Figure 4.3).

Seed coat treatments did not affect *E. macrocarpa* subsp. *elachantha* and *E. todtiana* seedling emergence in early July at Cooljarloo in 2018 (Figure 4.3). *Eucalyptus todtiana* seedling emergence was improved by the Apron seed coat compared to the control in mid-July (Figure 4.3). Additionally, the Apron and Maxim seed coats improved the seedling emergence of *E. macrocarpa* subsp. *elachantha* relative to the control in mid-July (Figure 4.3). The emergence of *E. macrocarpa* subsp. *elachantha* treated with Apron was greater in mid-July compared to the first monitoring visit in early July. At Cooljarloo in 2018 variation between seed coat treatments disappeared by August due to negligible seedling germination.

There were no emergent seedlings in early July at Eneabba in 2017; therefore, monitoring was adjusted to later dates. The seedling emergence of Maxim treated *B. attenuata* was greater than the control in late July at Eneabba in 2017. There was no difference between *B. attenuata* fungicide seed coat treatments and the control in mid-August. There was no substantial difference between the seedling emergence of *B. candolleana* and *K. prostrata* treated with fungicide seed coats and the control at either of the monitoring visits. Fungicide seed coats had no impact on seedling emergence at Eneabba in 2017, and this affect was consistent over time.

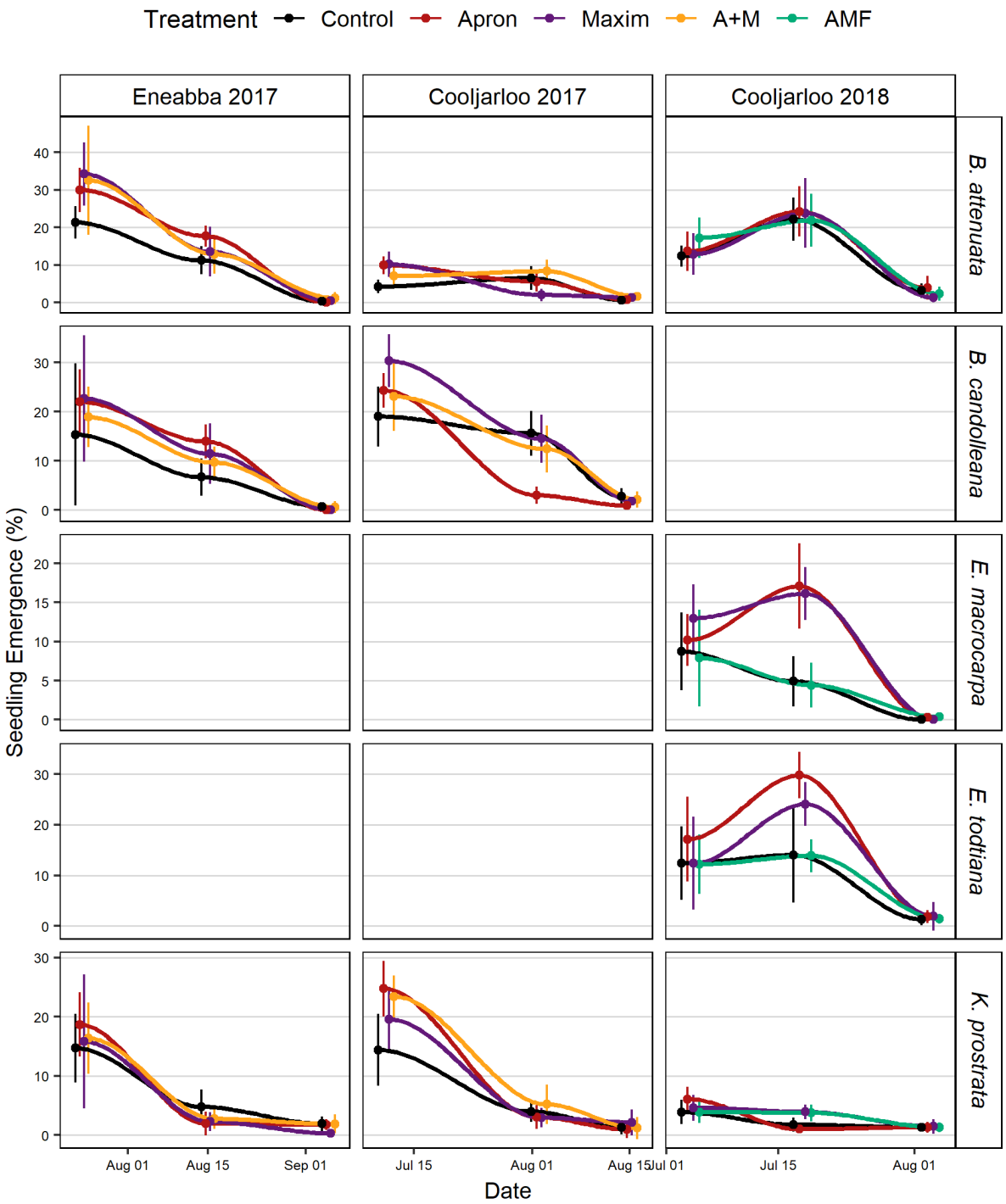


**Figure 4.3:** The seedling emergence as the percentage of the available non-emergent seed at the first three monitoring visits. Plant species included were those with a significant response to a seed coat treatment. Error bars represent the raw 95% confidence intervals. Blank panels are present when species were not included in a trial or were not statistically analysed.

### Post-summer seedling survival

All plant species sown in 2017 and analysed experienced seedling deaths over the summer period. Fungicide and AMF seed coats did not increase the number of surviving seedlings. There were a larger number of *Kennedia prostrata* seedlings surviving in plots after the summer period in comparison to the control (Table 4.5). *Kennedia prostrata* post-summer seedling survival was consistent with emergence and pre-summer survival trends (Table 4.2, Table 4.3), indicating Apron and the combined fungicide seed coats had no effect. Three plant species, *B. attenuata*, *B. menziesii* and *X. preissii*, experienced extremely low seedling survival and were not statistically analysed or reported. Additionally, *B. candolleana* and *H. costata* results are not reported due to strong violations of test assumptions. Additionally, the seedling survival of plant species sown at Cooljarloo in 2018 over the summer period were unaffected by seed coat fungicide and AMF treatments (Table 4.6).

**Table 4.5:** The mean percentage of seedling survival in June 2018 for plant species sown at sites in 2017. Letters represent homogenous subsets generated from the post-summer seedling survival statistical analysis and Tukey *post-hoc* adjusted *P*-values ($P < 0.05$), and bold results were significant. The mean percentage of summer seedling deaths is displayed in brackets.

| | | Treatment[1] | | | |
|---|---|---|---|---|---|
| Plant species | Site and sow year | Control | Apron | Maxim | A+M |
| *Acacia pulchella* | Eneabba 2017 | 28.3 a (-17.0) | 23.3 a (-12.4) | 27.3 a (-15.1) | 26.3 a (-16.4) |
| | Cooljarloo 2017 | 25.8 a (-34.8) | 29.9 a (-35.8) | 26.6 a (-38.7) | 32.6 a (-35.3) |
| *Daviesia podophylla* | Eneabba 2017 | 24.1 a (-17.0) | 28.6 a (-16.0) | 28.9 a (-20.8) | 29.0 a (-17.3) |
| *Hakea incrassata* | Eneabba 2017 | 47.1 a (-28.7) | | 39.9 a (-24.1) | 40.7 a (-25.0) |
| *Hakea trifurcata* | Eneabba 2017 | 23.1 a (-11.1) | | 32.8 a (-14.7) | 31.7 a (-15.1) |
| | Cooljarloo 2017 | 16.4 a (-10.3) | | 18.0 a (-14.0) | 17.1 a (-10.9) |
| *Kennedia prostrata* | Eneabba 2017 | 11.4 a (-8.1) | 11.5 a (-9.7) | 9.0 a (-8.3) | 10.3 a (-9.2) |
| | Cooljarloo 2017 | **4.7 a** (-14.6) | **9.6 b** (-18.3) | **6.6 ab** (-18.0) | **9.2 b** (-19.8) |

[1] Treatment names shortened or abbreviated to Apron, Apron XL; Maxim, Maxim XL; A+M, Apron XL and Maxim XL.

**Table 4.6:** The mean percentage of seedling survival in May 2019 for plant species sown at Cooljarloo in 2018. Seedlings were thinned prior to the summer period and the percentages were calculated based on the number of seedlings that remained in the plot. Letters represent homogenous subsets generated from the post-summer seedling survival statistical analysis and Tukey *post-hoc* adjusted *P*-values ($P < 0.05$).

| | | Treatment[1] | | | |
|---|---|---|---|---|---|
| Plant species | Site and sow year | Control | Apron | Maxim | AMF |
| *Acacia pulchella* | Cooljarloo 2018 | 40.7 a | 42.9 a | 33.3 a | 39.9 a |
| *Banksia attenuata* | Cooljarloo 2018 | 69.0 a | 75.0 a | 67.9 a | 67.9 a |
| *Banksia menziesii* | Cooljarloo 2018 | 42.0 a | 48.0 a | 33.8 a | 51.1 a |
| *Bossiaea eriocarpa* | Cooljarloo 2018 | 55.6 a | 79.5 a | 42.9 a | 71.2 a |
| *Eucalyptus macrocarpa* | Cooljarloo 2018 | 58.1 a | 70.1 a | 77.4 a | 67.7 a |
| *Eucalyptus todtiana* | Cooljarloo 2018 | 83.7 a | 75.5 a | 93.9 a | 79.6 a |
| *Gastrolobium capitatum* | Cooljarloo 2018 | 76.2 a | 66.6 a | 65.3 a | 83.3 a |
| *Hakea trifurcata* | Cooljarloo 2018 | 91.4 a | 94.0 a | 85.7 a | 91.4 a |
| *Kennedia prostrata* | Cooljarloo 2018 | 59.2 a | 83.4 a | 70.6 a | 70.9 a |
| *Xanthorrhoea preissii* | Cooljarloo 2018 | 52.0 a | 54.5 a | 54.5 a | 57.1 a |

[1] Treatment names shortened or abbreviated to Apron, Apron XL; Maxim, Maxim XL; AMF, MycoApply Maxx.

## Data validation

The data for several plant species sown into three plots had to be removed from the analysis at Eneabba in 2017. These three plots were located in a post-mining pit that was restored for a second time and removed from the dataset as they were deemed to be outliers due to substantially lower seedling emergence compared to other plots. The data from three additional plots were removed from the analysis for *B. attenuata*, *B. candolleana* and *B. menziesii* from the Eneabba site due to herbivory from emus (*Dromaius novaehollandiae*). *Hakea incrassata* and *H. trifurcata* had Apron treatments removed from the Eneabba and Cooljarloo in 2017 site data due to spoilt seed caused by an issue with the fungicide application. The majority of individual and combined models for each plant species met the assumptions of the analysis. Some models showed small to moderate violations of the normality assumption which were tested with Q-Q plots of the model residuals, histograms and Shapiro-Wilk tests. It was not possible to analyse seedling deaths due to violations of test assumptions.

## Isolation of pathogens

Three *Pythium* spp. were recovered from Eneabba and Cooljarloo in 2017 through baiting techniques. *Py. irregulare* and *Py. cryptoirregulare* were recovered from all plots at both sites in 2017. *Py. mamilatum* was isolated in all plots at Cooljarloo in 2017; however, it was

not recovered at Eneabba in 2017. *Pythium irregulare* was detected through soil baiting within 11 or the 12 plots at Cooljarloo in 2018. *Fusarium oxysporum*, and *Py.* aff. cederbergense were isolated from seedlings within all plots at Cooljarloo in 2018, respectively. Three isolates of *Rhizoctonia* were found but not identified due to contamination.

## Discussion

Fungicides applied as a seed coat increased the seedling emergence and survival of five of 14 native plant species in post-mining restoration. Both Apron and Maxim fungicide seed coats improved seedling emergence, while commercial AMF treatments had no effect. In addition, the study highlighted that the impact of fungicide seed coat treatments changed over time. Damping-off pathogens were present in post-mining ecological restoration and appear to have a small to moderate impact on the efficiency of broadcast seeding in situ.

Fungicide seed coat treatments are a novel technology for improving the outcomes of broadcast seed in restoration ecology (Pedrini et al. 2017). The present study indicates that seed coat treatments increased the seedling emergence of individual plant species by 5–18% in the field. This technology is practical given germination stimulation treatments are applied to seed prior to sowing in post-mining restoration (Koch 2007a). Financially, the fungicide seed coat treatments may reduce the cost of expensive broadcast seed, such as *B. attenuata* and *B. candolleana*. Assuming *B. attenuata* and *B. candolleana* seed costs approximately $1,500 per kilogram, seeds are applied at 330 g/ha and 40 hectares are rehabilitated annually, the effective fungicide treatments would save $1320 and $1720 for each plant species per year. These treatments may help reduce pressure on natural harvest populations of plant species (Nevill et al. 2018).

Seed coat treatments have seldomly been applied to broadcast seed used in ecological restoration of ecosystems within the southwest of Australia. Compared to polymer seed coatings evaluated by Turner et al. (2006) in Banksia woodland restoration, fungicide seed coats improved emergence to a similar extent. However, fungicide seed coats improved the emergence of more plant species. Fungicide seed coat treatments and drenches have controlled damping-off diseases for many agricultural and silvicultural plant species (Rhodes

and Myers 1989, Munkvold and O'Mara 2002, Linderman et al. 2008, Leisso et al. 2009). The impact of fungicide seed coats on the emergence of agricultural and silvicultural plant species has generally been larger than the plant species tested in this study, likely the result of increased pathogen inoculum due to monocultures and supportive abiotic conditions (Lamichhane et al. 2017).

The commercial AMF treatment did not improve seedling emergence or survival of any plant species. Commercial arbuscular mycorrhizal treatments used in restoration projects were found to be less effective than inoculum sourced from reference systems (Maltz and Treseder 2015). MycoApply AMF products have not improved ecological restoration outcomes in other in situ experiments (Aprahamian et al. 2016, Emam 2016, Perkins and Bennett 2018). The use of additive beneficial microbial treatments in ecological restoration may need to be developed from the local microflora. Kwongan and Banksia woodland top soil contains native AMF propagules (Birnbaum et al. 2017), given these commercial treatments do not appear to improve seedling survival they may not be worth applying within restoration as they can produce negative outcomes (Tarbell and Koske 2007, Koch et al. 2011). It should be noted that the efficacy of these mycorrhizal products are influenced by propagule viability, and overcoming these logistical challenges may lead to improved restoration outcomes.

Fungicide seed coat treatments did not consistently improve seedling emergence throughout the winter period. Differentiation between fungicide seed coats and control treatments was greatest in mid-July. The negligible difference between seed coat treatments in early July at Cooljarloo in 2018 suggested there is a lag time associated with the build-up of inoculum or the activity of damping-off pathogens. Damping-off pathogens are more virulent in soils with higher moisture content (Lamichhane et al. 2017), and seed and root exudation (Nelson 1991). The lag time may be the result of lower initial soil moisture, responding to exudates and the disruption associated with topsoil stockpiling and spreading. The lack of differentiation between seed coat treatments in August at Cooljarloo and Eneabba in 2017 indicated the fungicides lost their ability to prevent damping-off over time. The degradation rate of Metalaxyl-M (Apron) and Fludioxonil (Maxim) in soils is dependent on abiotic and biotic factors (Sukul and Spiteller 2001, Pung 2002, Komárek et al. 2010). Metalaxyl-M had a half-life of 70 days and leached to a mean depth of 18 cm after

800 mm of rainfall and irrigation over 142 days in similar Western Australian soils (Kookana et al. 1995). Fludioxonil has low solubility and mobility (Komárek et al. 2010), and the dissipation half-life from wheat seeds has been found to be as low as 21 days in biologically active soils (NRA 2000). Fludioxonil can often take a longer period of time to degrade in a variety of soils (Komárek et al. 2010). The smaller impact of fungicide seed coats at Eneabba and Cooljarloo in August 2017 may be due to the degradation of the Metalaxyl-M and Fludioxonil between plot establishment and seedling emergence. Additionally, leaching can impact on the persistence of Metalaxyl-M. The persistence of seed coat treatment efficacy was not the primary focus of this study, the timing of monitoring visits was not regular, and fungicide levels were not measured. Fungicide seed coat treatments may only be effective when damping-off pathogens are most active and before degradation.

Seed coat treatments did not reduce post-emergent and summer seedling mortality. Pathogens may cause post-emergent damping-off for several weeks until root tissue hardens (Tainter and Baker 1996, Agrios 2005), and reduce the health of the remaining plants (Huang and Erickson 2007). Metalaxyl-M and Fludioxonil seed treatments used in inoculated trials can provide small to moderate improvements in seedling survival for agricultural and silvicultural plant species for short periods after seedling emergence (Rhodes and Myers 1989, Howell 2007, Linderman et al. 2008, Thakur et al. 2011). In native seedling damping-off experiments, *Py. irregulare* which was present at Cooljarloo in 2017 and 2018 and Eneabba, did not caused substantial levels of post-emergence damping-off (Chapter 2). It is possible that damping-off pathogens present in post-mining restoration did not decrease the post-emergence seedling survival or fungicide degradation and leaching lead to less protection post-emergence.

The ability of fungicide seed coat treatments to increase the emergence of seedlings for several plant species indicates damping-off pathogens were present in the period directly after topsoil return. Both fungicide treatments, Apron XL for *Pythium* spp. and Maxim XL for *Fusarium* and *Rhizoctonia* spp., were found to improve seedling emergence for one or more plant species. *Pythium*, *Fusarium* and *Rhizoctonia* species were isolated and widely distributed within the post-mining ecological restoration sites studied, and these genera are frequently associated with damping-off in agricultural crops (Lamichhane et al. 2017). A range of soil-borne plant pathogens have previously been found in ecological restoration

after topsoil return (Nováková 2001). *Pythium irregulare*, *Py. cryptoirregulare*, and *Py*. *mamilatum* isolated in post-mining restoration at Cooljarloo and Eneabba have been identified as virulent damping-off pathogens in other studies (Mwanza and Kellas 1987, Matoba et al. 2008, Bahramisharif et al. 2013, Abreo et al. 2017). In particular, *Py. irregulare* in glasshouse trials decreased seedling emergence by 50–90% across a broad host range of Banksia woodland and kwongan plant species (Chapter 2). It was likely that these *Pythium*, *Fusarium* and *Rhizoctonia* soil-borne damping-off pathogens were responsible for the loss of seedlings in post-mining ecological restoration.

Damping-off pathogens may not be particularly active or have abundant inoculum given that the effect size of fungicide treatments was moderate for significant treatments. The environmental conditions may not have been as conducive to damping-off pathogens (Lamichhane et al. 2017), due to the low winter rainfall and temperatures at the sites. Birnbaum et al. (2017) and Frouz et al. (2013) found that microorganisms and the microbial community are initially disrupted by both the disturbance and stockpiling of topsoils. It is possible that there is less inoculum of damping-off pathogens within post-mining ecological restoration due to topsoil disturbance and abiotic conditions. Pathogenicity trials of *Fusarium*, *Pythium* and *Rhizoctonia* species isolated from restoration topsoil at different inoculum levels and environmental conditions are required to confirm this hypothesis.

Apron and Maxim fungicides applied as seed coats may only improve the seedling emergence of some native kwongan and Banksia woodland plant species. Fungicide seed coat treatments can improve seedling emergence by 5–18%; however, results may be variable. Rokich et al. (2002) reported the efficiency of broadcast seed was 7% in comparable Banksia woodlands restoration, pre-emergent damping-off may only account for a relatively small proportion of the total seed loss. Broadcast seed treated with fungicides should be sown as close to winter rainfall as possible to minimize the potential degradation of Metalaxyl and Fludioxonil over time. Species of *Pythium*, *Fusarium* and *Rhizoctonia* were isolated from the restoration sites, and the impact of both fungicides indicated different groups of damping-off pathogens are active in topsoil. Therefore, multiple fungicides should be applied as seed coats to protect against different groups of damping-off pathogens. Fungicide seed coats may be more effective when used in ecological restoration projects in climates that experience more conducive warmer and

wetter conditions to damping-off pathogens. Protectants may provide improved seedling emergence when applied at higher rates, within pelleting layers or when treated seeds are sown closer to germination trigger events to prevent degradation or leaching.

# Chapter 5: Plant-soil feedback through damping-off and oomycete associations with plant species and host age in a diverse Mediterranean shrubland


## Abstract

Plant-soil feedbacks mediated by the microbial community can be an important interaction leading to coexistence between plant species and the maintenance of diversity. These interactions have recently been examined in Mediterranean plant communities but the underlying mechanisms causing plant-soil feedback are difficult to identify through common experiment designs. The emergence and survival of seedlings was monitored in conspecific and heterospecific soils for five plant species with contrasting nutrient acquisition strategies from hyper-diverse kwongan plant communities. Additionally, the oomycetes associated with seedlings and mature plants for each species were identified and related to plant-soil feedback observed. Pre- and post-emergent damping-off occurred and caused *Jacksonia floribunda* and *Xanthorrhoea* sp. Lesueur to experience negative plant-soil feedback in conspecific soils. There was little evidence to suggest the presence or abundance of oomycetes influenced seedling emergence and survival; however, year, plant species and host age were significant predictors of the oomycete community detected. Seedlings promoted their own oomycete community without the influence of mature plant species, an indication adult density or distance may have little direct effect on the microbial community producing feedback amongst kwongan seedlings. Plant-soil feedback driven by damping-off may promote coexistence and the oomycete associations with mature plant species provided evidence for previously hypothesised nutrient acquisition trade-offs that can maintain diversity in this Mediterranean shrubland. Interactions between the microbial community and plants are quite possibly a force promoting diversity in Mediterranean shrublands and molecular tools help distinguish between previously ambiguous mechanisms driving plant-soil feedback.

## Introduction

The interactions between plant species and soil microbes play an important component in shaping the structure and diversity of plant communities. The Janzen-Connell (J-C) hypothesis describes the local accumulation of specific natural enemies, such as soil-borne plant pathogens in close proximity to adult plants that reduce the dominance of conspecific seedlings through damping-off and allow heterospecific seedlings to compete for space (Janzen 1970, Connell 1971). The J-C effect represents negative density or distance dependant plant-soil feedback (PSF), and the detrimental impact of plant pathogens present in conspecific soils decreases with lower seedling densities or increased distance from the mature plant (Bever et al. 2012). Negative PSF creates a series of unfavourable "home" and favourable "away" recruitment sites beneath conspecific and heterospecific plant species, respectively.

Plant pathogens, such as 'fungus-like' oomycetes, *Fusarium* and *Rhizoctonia* have been isolated from the seedling rhizosphere or roots and identified as the mechanism responsible for the J-C effect or PSF (Bever et al. 1997, Mills and Bever 1998, Packer and Clay 2000, 2003, Ampt et al. 2019). However, the net effect of the pathogens or microbial community has most frequently been identified through applications of biocides (e.g. Bell et al. 2006) or soil sterilisation (e.g. Packer and Clay 2000). Ampt et al. (2019) found many of experiments in species-rich grasslands determined the role of the below-ground microbial community without identifying the primary soil-borne plant pathogens that caused feedback through isolation and Koch's postulates. There has typically been less focus on the below-ground microbial agents driving feedback in natural plant communities due to the vast taxonomic and functional diversity present (Bever et al. 2012, Bever et al. 2015). Furthermore, the effects of below-ground microbial communities may be hidden compared to more visible impacts of above-ground pathogens (Ampt et al. 2019). The lack of an understanding of the key microbial agents driving PSF creates uncertainty regarding the specific mechanisms that help maintain the diversity of plant communities.

The adult and seedlings may both contribute to the abundance of pathogens driving the J-C effect as root exudations alter the composition of the below-ground microbial community (Broeckling et al. 2008). Negative distance-dependent mortality unaffected by seedling density may indicate adults provide a reservoir of pathogen inoculum for seedlings (Packer

and Clay 2003, Reinhart and Clay 2009, Xu et al. 2015). However, it is difficult to determine the difference between negative feedback caused by seedling densities or distance as seed density is highest beneath the adult plant (Freckleton and Lewis 2006, Reinhart and Clay 2009). Unfavourable environmental conditions, such as poor light underneath the conspecific adult may interact with seedling pathogens to cause negative distance dependant seedling mortality (McCarthy-Neumann and Ibáñez 2013). Xu et al. (2015) explain how distance dependent patterns may follow years of density dependent mortality after previous recruitment periods increased pathogen inoculum close to the adult; or density dependent pre-emergent damping-off may occur leading to the observation of seedling mortality reflecting a distance-dependent response. The role of seedlings and adult conspecifics in directly "culturing" a community of pathogens responsible for negative PSF in many studies is unclear.

Seed and seedlings may be responsible for "culturing" pathogens driving negative PSF in most situations. The microbial community associated with the host's rhizosphere and roots changes over the development of a plant species (Marschner et al. 2002, Houlden et al. 2008). The composition of the rhizosphere microbial community and the presence of specific fungi can change between early and later life stages (Cavaglieri et al. 2009, Chaparro et al. 2014). Successional shifts in the microbial community are likely a response to different exudates released by the roots (Marschner et al. 2002). Additionally, the susceptibility of plant tissues to pathogens can vary over the lifetime of a host (Panter and Jones 2002, Develey-Rivière and Galiana 2007). The oomycete damping-off pathogens *Phytophthora* and *Pythium* become less virulent as seedlings age (Martin and Loper 1999, Simamora et al. 2017). *Pythium* fails to break down suberin and lignin present in mature woody roots (Agrios 2005). Oomycetes may persist in the rhizosphere or plant tissue without causing disease to susceptible hosts as they can produce long lived survival spores that germinate when triggered by environmental or biological conditions (Martin and Loper 1999, Crone et al. 2013, Jung et al. 2013). The abundance of pathogens responsible for damping-off disease driving PSF may not be influenced directly by the adult conspecific plants given the shifts in the microbial community and susceptibility of hosts with age.

Kwongan plant communities are hyper-diverse Mediterranean type shrubland located in southwest Australia. The diversity and richness of plant species and functional traits, such as

nutrient acquisition strategies are greatest in old weathered phosphorus impoverished soils (Laliberté et al. 2014, Zemunik et al. 2015). Plant species with non-mycorrhizal cluster roots (NMCR) are the most effective at extracting P from these soils. However, despite this completive advantage NMCR do not dominate the plant community (Laliberté et al. 2015, Lambers et al. 2018). Plant species with NMCR experience negative PSF when grown in a mix of soils collected from plant species with the same nutrient acquisition strategy (Teste et al. 2017). Additionally, putatively native *Phytophthora* species can equalise the competitive ability of plant species with NMCR when grown with ectomycorrhizal plant species in inoculated glasshouse trials (Albornoz et al. 2016). Plant species with mutualist arbuscular and ectomycorrhizal fungal associations may be less effective at collecting soil resources but provide protection from oomycete and fungal root pathogens that may negatively impact NMCR, leading to coexistence between these functional traits (Laliberté et al. 2015, Lambers et al. 2018). Interactions between plant species of contrasting nutrient acquisition strategies has been linked to coexistence (Laliberté et al. 2015, Lambers et al. 2018). Plant species with superior root structures for extracting specific nutrients from deficient soils may increase the availability of those nutrients for neighbouring plant species with different nutrient acquisition strategies leading to coexistence (Muler et al. 2014, Teste et al. 2014, Teste et al. 2015). Seedlings of NMCR plant species *Banksia* and *Hakea* in kwongan plant communities have displayed negative density-dependent mortality in post-fire microsites (Lamont et al. 1993). Competition for soil moisture was identified as a key driver of negative density dependent PSF in litter microsites by Lamont et al. (1993); however, the role of plant pathogens was never assessed.

Interactions between plant species and the below-ground microbial community may play an important role in shaping the structure and diversity of kwongan vegetation. Previous research in this Mediterranean shrubland has indicated seedlings and functional trait groups experience negative density-dependent mortality and both negative and positive PSF, respectively (Lamont et al. 1993, Teste et al. 2017). Interactions between plant species may also lead to co-existence though nutrient facilitation and exchange (Lambers et al. 2018). The role of pathogens driving PSF has not been examined from the earliest plant developmental stages for kwongan species and few studies have focused on pathogen mediated diversity outside tropical forest, temperate forests and grasslands (Comita et al.

2014, Bever et al. 2015). Pre-emergent damping-off by the microbial community can be responsible for PSF (Miller et al. 2019) despite less evidence to support their impact within the literature (Comita et al. 2014). Putatively native oomycetes have been detected in kwongan plant communities (Chapter 3, Burgess et al. 2018). These oomycetes have been hypothesised to drive negative PSF for hosts with NMCR (Lambers et al. 2018), and can be virulent damping-off pathogens (Chapter 2). Detecting the oomycetes associated with plant species may identify the mechanism driving PSF in seedlings and the functional trait groups found by Teste et al. (2017). Additionally, determining the oomycete communities associated with different plant developmental stages may provide new insight into the role of the adults and seedlings in promoting a community of pathogens within the rhizosphere. Two experiments and a metabarcoding survey were designed to answer four questions,

1) Do conspecific and heterospecific soils and the oomycete community drive plant-soil feedback through the pre- and post-emergent damping-off of kwongan plant species?
2) Are pre- and post-emergent damping-off constant in conspecific and heterospecific soils in the presence of a second plant species?
3) Do plant species affect the oomycete alpha diversity and community?
4) Are oomycete communities age-specific?

## Methods

Rhizosphere soils samples were collected from a natural kwongan plant community to test the level of pre- and post-emergent damping-off of five plant species in soil sources from the same (conspecific) and different plant species (heterospecific) in a glasshouse experiment. The second experiment included two plant species sown together to test if this interaction changed levels of pre- and post-emergent damping-off. The oomycete communities associated with the roots of mature plant species sampled in the field and seedlings harvested from conspecific soils in the glasshouse were identified through

metabarcoding. The oomycete community was additionally used as a predictor of damping-off in the first glasshouse experiment.

### Plant species selection

Five plant species common within kwongan plant communities were selected for the experiments (Table 5.1). Each plant species has a different nutrient acquisition strategy. *Banksia attenuata* and *Hakea lissocarpha* have different non-mycorrhizal cluster root structures (Shane and Lambers 2005), and the genera have displayed different responses to soil-borne root pathogens (Chapter 2). The seed for the five selected plant species was purchased from Nindethana Seed Company (https://www.nindethana.net.au/), and had been collected from the Geraldton Sandplain Bioregion. The seeds of *J. floribunda* and *X.* sp. Lesueur were treated with hot water, and smoke water germination stimulants, respectively (Sweedman and Merritt 2006).

**Table 5.1:** The plant species selected for the metabarcoding and glasshouse experiments together with information on their growth form and nutrient acquisition strategies.

| Family | Plant species | Growth Form | Nutrient acquisition strategy |
|---|---|---|---|
| Asphodelaceae | *Xanthorrhoea* sp. Lesueur[1] | Tree-like monocot | Arbuscular mycorrhizal |
| Fabaceae | *Jacksonia floribunda* | Woody shrub | N-fixing, arbuscular and ectomycorrhizal |
| Myrtaceae | *Eucalyptus todtiana* | Tree | Ectomycorrhizal |
| Proteaceae | *Banksia attenuata* | Tree and woody shrub | Non-mycorrhizal cluster root (compound) |
| Proteaceae | *Hakea lissocarpha* | Woody shrub | Non-mycorrhizal cluster root (simple) |

[1] Previously identified in the area as *Xanthorrhoea preissii* prior to reclassification.

### Study area and site location

The collection of soil samples occurred within Mt Lesueur National Park, Western Australia (Figure 5.1A). The site was located in stage six of the Jurien Bay chronosequence, characterised by old, strongly weathered and phosphorus deficient soils where species and functional diversity are the greatest (Turner and Laliberté 2015, Zemunik et al. 2015, 2016). The site was selected because it contained the five plant species in close proximity (Figure 5.1A). The Department of Conservation, Biodiversity and Attractions (DCBA) approved a

permit for the collection of samples from protected areas under their management (SW019089). Kwongan vegetation with a mosaic of a low *Banksia* and *Eucalyptus* overstorey was present at the site (Figure 5.1B). The overstory contained a mix of *B. attenuata*, *B. menziesii* and *E. todtiana*. *Adenathos cygnorum*, *B. telmatiaea, Conospermum* spp., *Eremaea asterocarpa, Hibbertia* spp., *Isopogon* spp., and *Jacksonia floribunda* comprised the shrub layers.

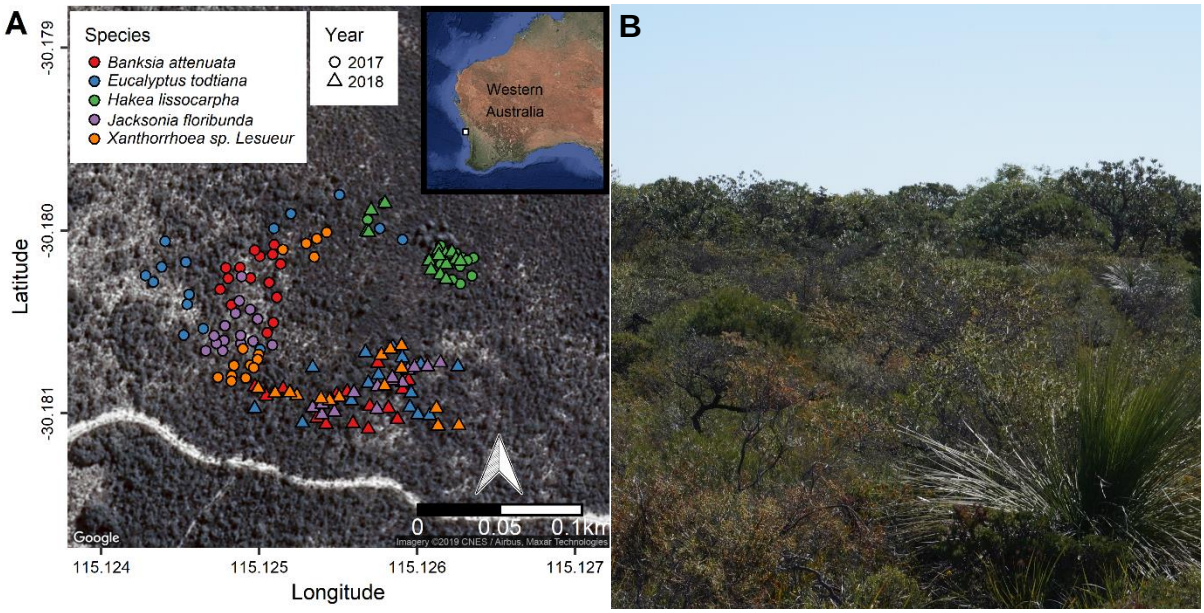


**Figure 5.1: A.** The location of the sampled plant species in 2017 and 2018 at the site located at Mt Lesueur National Park, Western Australia. **B.** The kwongan vegetation sampled at the site.

## Sample collection

Rhizosphere samples were collected in early October 2017 and repeated in late September 2018. The sample collection and processing were consistent between 2017 and 2018; however, an adjacent area of the site was sampled in 2018 for all plant species except *H. lissocarpha* as it was not located elsewhere at the site (Figure 5.1A). The protocol describes how samples were collected in each year.

Each of the five plant species had 15 individuals sampled ($n$ = 75). An individual plant was sampled if it was healthy (showing no signs of dieback or leaf chlorosis), not within 5 m of another sampled individual, and where possible was not growing within 1.25 m (radius) of a different plant species. Small plant species growing at the base of a sampled individual were removed and soil and roots were not collected where roots intermingled. Leaf litter was

removed from the surface, and the sample was collected from the base of the plant (0–1 m) to a depth of 30 cm from each of the cardinal points. The samples collected from the cardinal points of an individual plant were combined in the field.

Two distinct samples were collected from the rhizosphere of the 75 individuals, soil and roots (3 kg for Experiment 1 and 9 kg for Experiment 2) for the glasshouse experiments, and fine roots (100 g) for high throughput sequencing (HTS). Glasshouse samples were stored in insulated boxes (20°C), and then upright and open in the glasshouse before potting within seven days. The individual glasshouse samples were not homogenised based on plant species and were kept independently to allow the oomycete communities to be included as variables in the statistical analysis of Experiment 1.

### Glasshouse Experiment 1

The five plant species were sown into conspecific and heterospecific soils in 2017 and repeated in 2018. The rhizosphere soil and root sample collected from an individual plant were placed into five 0.55 L, small free-draining rectangular pots (95 mm × 85 mm × 95 mm), weighing 0.48 kg when filled, from Garden City Plastics (https://www.gardencityplastics.com/). The bottom of each pot was covered in plastic to prevent it from draining freely and leaching nutrients. For each year, each plant species was sown into 15 replications of conspecific and four heterospecific soils ($n$ = 75), and each pot contained 20 seeds. Seeds were covered by 2–5 mm of soil. Seedling emergence and deaths were monitored over 130 days after the experiment was established in late October 2017 and early October 2018. Seedling emergence and death accumulation curves were monitored to determine when germination and post-emergence damping-off ceased. Wooden toothpick markers were placed in the pots beside a seedling post-emergence and helped to indicate when a new germination or death had occurred. Pots were watered to 70% of field capacity three times a week to keep soil moist and consistent throughout the experiment. The minimum and maximum temperature were measured during the experiment. The mean daily minimum and maximum temperatures in the evaporatively cooled glasshouse were 18.5°C and 34.6°C in 2017 and 15.5°C and 30.7°C in 2018. The roots of each plant species that survived the experiment in conspecific soils were harvested for high throughput sequencing.

## Glasshouse Experiment 2

Plant species of contrasting nutrient acquisition strategies can interact in various ways, the emergence and survival of two plant species sharing the same soil was tested in 2017. The 15 replicates of rhizosphere soil and root samples collected from each plant species were placed in four 1.55 L (140 mm diameter) large round pots (*n* = 60) purchased from Garden City Plastics. The bottom of each pot was covered in plastic to prevent it from draining freely and leaching nutrients. For each of the 15 replicates, the plant species was sown into the four pots containing conspecific soils with one of the other four plant species. This experiment was not fully factorial as one of the plant species was always sown into a conspecific soil. The pots contained the same soils and seed density as Experiment 1, allowing seedlings sown alone into conspecific and heterospecific soils to be compared to the various sharing arrangements. Each pot had 38 seeds sown, 19 seed from both the conspecific and heterospecific plant species. The seed of both species was evenly distributed within the pot using a consistent pattern marked out by a tool and then covered by 2–5 mm of soil. The experiments were located in the same glasshouse and experienced the same temperature conditions. Experiment 2 was monitored and watered in the same way as Experiment 1, but was not repeated in 2018.

## Metabarcoding

The roots of mature plants in the field and harvested seedlings from conspecific soils after glasshouse Experiment 1 in 2017 and 2018 were collected and processed using identical equipment and protocols to that described in Chapter 3. The same methodology, kit and location for the eDNA extractions were used in this experiment as was described in Chapter 3.

The RPS10 mitochondrial gene region (~450 bp) was amplified by a nested PCR. The first PCR used primers PVP9-F1 and PVP9-R1. The first round of PCR tubes contained 12.5 µl of PCR buffer KAPA HiFi HotStart ReadyMix (KAPA Biosystems), 8 µl of PCR grade water, 1 µM of each primer and 2.5 µl of genomic DNA. The first PCR cycling conditions were 1) 94°C for two minutes, 2) 94°C for 30 seconds, 35 cycles at 55°C for 45 seconds followed by 72°C for 1 minute, 3) 72°C for 5 minutes, and 4) and holding at 14°C. A second nested PCR used RSP10 F and RSP10 R primers with Illumina MiSeq EXP000 adapter sequences attached to the 5’ end. The second round of PCR tubes contained 2.5 µl of PCR product. PCR cycling

conditions were 1) 94°C for 2 minutes, 2) 35 cycles of 95°C for 20 seconds, 60°C for 25 seconds and 72°C for 1 minute before 3) a final 72°C for 7 minutes and 4) holding at 4°C. The first and second round of PCR was conducted in triplicate and then combined based on intensity of bands on 2% agarose gels. Extraction and PCR controls were run to test for contamination.

Amplicon library preparation was performed according to recommended protocols (Illumina Demonstrated Protocol: 16S Metagenomic Sequencing Library Preparation). Uniquely indexed libraries were pooled for the sequencing run, which was performed on an Illumina MiSeq using 500-cycle V2 chemistry (250 bp paired-end reads) following the manufacturer's recommendations. The merging and clustering of zero-radius operational taxonomic units (ZOTUs) was performed as described in Chapter 3; however, forward and reverse end reads were trimmed by 17 bp after the oomycete sequences were merged. The generated ZOTUs were matched against a database containing described and designated but undescribed oomycete taxa previously sequenced with the primers. A phylogenetic analysis of the *Pythium* phylotypes was performed using confirmed sequences of species with Geneious tree builder. *Pythium* phylotypes corresponding to new or not previously sequenced species were labelled as *Pythium* sp. followed by the clade letter based on Robideau et al. (2011). *Pythium* phylotypes that did not closely match a species were placed within a clade and *Salisapilia* were numerically labelled. Phylotypes that were oomycetes but did not match a known genus were labelled "Unknown Oomycete" followed by a number. The identified oomycetes are considered phylotypes due to their detection through sequencing compared to the collection of living isolates.

### Statistical analyses

Binomial generalised linear mixed effect models (GLMM) tested the impact of the soil source and oomycete community variables on the emergence and survival of seedlings. The total emergent and surviving seedlings, out of the seed sown, were binary response variables in models for each plant species. Replicate and observation (i.e. pot) level random factors were included in all models. Observation level random effects were used to control for overdispersion (Bates et al. 2014). Dependent variables included soil source (the plant species), trial (2017 and 2018), and indices reflecting the oomycete community. Oomycete community indices were calculated for both mature plants and harvested seedlings,

including a hierarchical clustering of oomycete communities into groups, the presence of at least one oomycete phylotype, the number of oomycete phylotypes and the number of oomycete reads. The hierarchical clusters and the number of detected phylotypes were removed from models as they were unbalanced and highly collinear with oomycete presence. Two models analysed the seedling emergence and survival of each plant species. The first model included all observations, and the dependent variables soil source, trial and mature plant oomycete community indices. The second model only contained observations with seedling oomycete community indices as they were absent from some replicates because there were no remaining seedlings to harvest for metabarcoding. The strength of PSF within conspecific soils in relation to heterospecific soils was calculated by taking the natural logarithm of the odds ratio (Brinkman et al. 2010). The 'glmer' function from the lme4 R package was used to analyse the results with the "bobyqa" optimiser (Bates et al. 2014). An alpha of 0.05 was set to determine a statistically significant result. The emmeans R package was used for *post-hoc* analyses (Lenth 2018), and fixed effects were extracted from models for graphing with the effects package (Fox and Hong 2009). Data exploration and model validation were conducted as described in Zuur et al. (2010) and Zuur and Ieno (2016).

A binomial GLMM tested the effect of sharing soil with a second plant species on the emergence and survival of seedlings for the analysis of glasshouse Experiment 2. Plant species were analysed separately with two dependent variables, soil source and the sharing arrangement. The emergence and survival of seedlings sown alone into each soil in Experiment 1 were included in the sharing arrangement variable as seeds were sown at the same density and were comparable. The analysis was not fully factorial as seeds were not sown into soil heterospecific to both plant species. The sharing arrangement was separated by soil source in *post-hoc* analyses to compare the emergence and survival of plant species sown alone and together under the same conditions. The replicate, individual plant sampled and observation level random factors were included in all models. The same functions, packages and protocols were followed in the analysis as glasshouse Experiment 1.

Generalised linear models (GLM) tested the influenced of the plant species (Table 1), year of sampling (2017 vs 2018) and the plant age (mature field plant vs harvested glasshouse seedling) on the number of oomycete phylotypes or alpha diversity. The number of

oomycete phylotypes was analysed in two separate models. The plant species covariate was removed from the first alpha diversity model. The second alpha diversity model included plant species; however, observations were removed from the analysis if a three-way interaction grouping had zero oomycete detections, the model could not accurately calculate standard errors due to a lack of variance within these categories. The first model was run using a negative binomial GLM to correct for overdispersion and the second with a Poisson distribution using the stats base package in R (R Core Team 2018). Additionally, a separate Poisson model was run to determine if oomycetes associated with mature plants influenced the number of oomycetes detected from seedlings. The seedling model included the plant species, year of sampling and oomycetes presence from the corresponding mature individual in the field as dependent variables. The same functions, packages and protocols were followed as described in the previous analyses.

Statistical analyses were performed to determine if the plant species, age and year of sampling influenced the oomycete community detected. Mature plants and seedlings were first separately analysed to determine if plant species, year of collection and the interaction of covariates affected the detected oomycete community. The third model analysed all observations with the previous covariates, in addition to plant age and its interactions. Samples with at least one detection were included in the analyses. Datasets containing the presence and abundance (number of reads) of oomycetes produced dissimilarity matrices with the Jaccard and Bray-Curtis indices, respectively. The dissimilarity matrices were each used in a permutational multivariate analysis of variance (permanova) run with 9999 permutations, performed with the 'adonis' function in the vegan R package (Oksanen et al. 2018). A Bonferroni *P*-adjusted *post-hoc* test was then used to compare the statistically significant ($P \leq 0.05$) categorical terms with two or more levels with the 'pairwise.adonis' function from the pairwiseAdonis R package (Martinez Arbizu 2017). The 'betadisper' and 'permutest' functions in vegan tested the assumption of homogeneity of multivariate dispersions between categorical levels. The oomycete communities were graphically represented through unconstrained ordination, non-metric multidimensional scaling (NMDS) from the 'metaMDS' function in the vegan R package (Oksanen et al. 2018). The NMDS coordinates for each sample were generated using both Jaccard (presence) and Bray-Curtis (abundance) indices in two dimensions. A dummy oomycete species was added to

each sample due to denuded assemblages preventing NMDS convergence (Clarke et al. 2006). All NMDS coordinates generated using the Jaccard index were randomly jittered by 0.1 along the x and y axes to display the abundance of points plotted at the same location representing identical communities. The NMDS coordinates were extracted and plotted using the GGplot2 R package (Wickham 2016). Ellipses were drawn into plots to display the extent of the oomycete community with 95% confidence intervals for the experiment year and plant age using the 'stat_ellipse' function. Polygons were produced using the function 'chull' in the grDevices base R package (R Core Team 2018) to highlight the area occupied by each plant species.

## Results

### Glasshouse Experiment 1

Soil source contributed to statistically significant changes in the emergence and survival of seedlings for three of the five plant species (Table S5.1, Figure 5.3), and caused negative feedback in conspecific soils for *J. floribunda* and *X.* sp. Lesueur (Figure S5.1). *Banksia attenuata* seedling survival in 2018 was improved by 16.5% and 13.3% in *E. todtiana* soils compared to other heterospecific sources *H. lissocarpha* and *J. floribunda*, respectively (Figure 5.3A). However, this trend was not present in 2017, or when the trials were combined and doesn't indicate a plant-soil feedback (Figure 5.3A). The survival of *J. floribunda* was reduced by 9% in conspecific soil when the trials were combined (Figure 5.3B), despite experiencing no negative effects on seedling emergence in the same conspecific soils (Figure 5.3B). Post-emergent damping-off caused the negative conspecific feedback experienced by *J. floribunda*. In 2017 and combined trials, the seedling emergence and survival of *J. floribunda* was also worse in *B. attenuata* soils compared to *H. lissocarpha* and *X.* sp. Lesueur (Figure 5.3B). In 2018 and the combined trial analyses, the emergence and survival of *X.* sp. Lesueur was higher within *B. attenuata* soils by 13–18% in comparison to conspecific soils (Figure 5.3C). The negative conspecific feedback experienced by *X.* sp. Lesueur was driven primarily by pre-emergent damping-off as a decrease in seedling survival did not substantially contribute to the different between soil sources. Furthermore, *X.* sp. Lesueur seedling emergence and survival experienced negative feedback in *H. lissocarpha* and *J. floribunda* soils when averaged across both trials, respectively (Figure

5.3C). *Hakea lissocarpha* was the only plant species not influenced by either the source of the soil or the oomycete communities associated with mature plants or seedlings (Table S5.1; Table S5.2).

There was a statistically significant correlation between the presence of oomycetes detected in the roots of harvested seedlings with reduced emergence of *E. todtiana* and survival of *J. floribunda* in 2018 (Table S5.2). The presence of oomycetes decreased the emergence of *E. todtiana* and survival of *J. floribunda* by 10% and 12%, respectively. However, the overall presence of oomycetes did not affect *J. floribunda*, and it was only possible to analyse the data from 2018 for *E. todtiana*. The presence and the abundance of oomycetes reads associated with mature plants did not have a statistically significant influence on the emergence and survival of any plant species (Table S5.2).

Seed viability was similar between trials allowing trials to be combined. *Eucalyptus todtiana* seed viability was poor in 2017 and was removed from the analysis. Seedling emergence and survival observations were removed from the analyses for the seedling oomycete community because 20 of the 150 replicates across the two trials had no living seedlings in conspecific soils for metabarcoding. The replicates removed from the analysis came from *E. todtiana* (11), *J. floribunda* (8) and *B. attenuata* (1) soils.

### Glasshouse Experiment 2

The sharing arrangement did not affect seedling emergence. There were statistically significant improvements in the survival of *H. lissocarpha* and *J. floribunda* when sown with another plant species in a heterospecific soil (Figure 5.2A–B). *Hakea lissocarpha* seedling survival was significantly improved when sown with *J. floribunda* and into *J. floribunda* soil (Figure 5.2A). The survival of *H. lissocarpha* seedlings was 16.8% or 1.97 times higher than when it was sown alone in soils sourced from *J. floribunda*, which had no significant negative effect on seedling survival compared to the other four soil sources. The seedling survival of *J. floribunda* was significantly improved by 7.7% or 2.26 times higher when sown with *B. attenuata* and into *B. attenuata* soil (Figure 5.2B). Soil sourced from *B. attenuata* had a significant negative effect on the emergence and survival of *J. floribunda* seedlings when they were sown alone (Figure 5.2B). The survival *of X.* sp. Lesueur and *B. attenuata* was not affected by the various sharing arrangements (data not shown). *Eucalyptus todtiana* was not

analysed and its sharing arrangements were removed from the analyses of the other four plant species due to poor emergence in 2017.

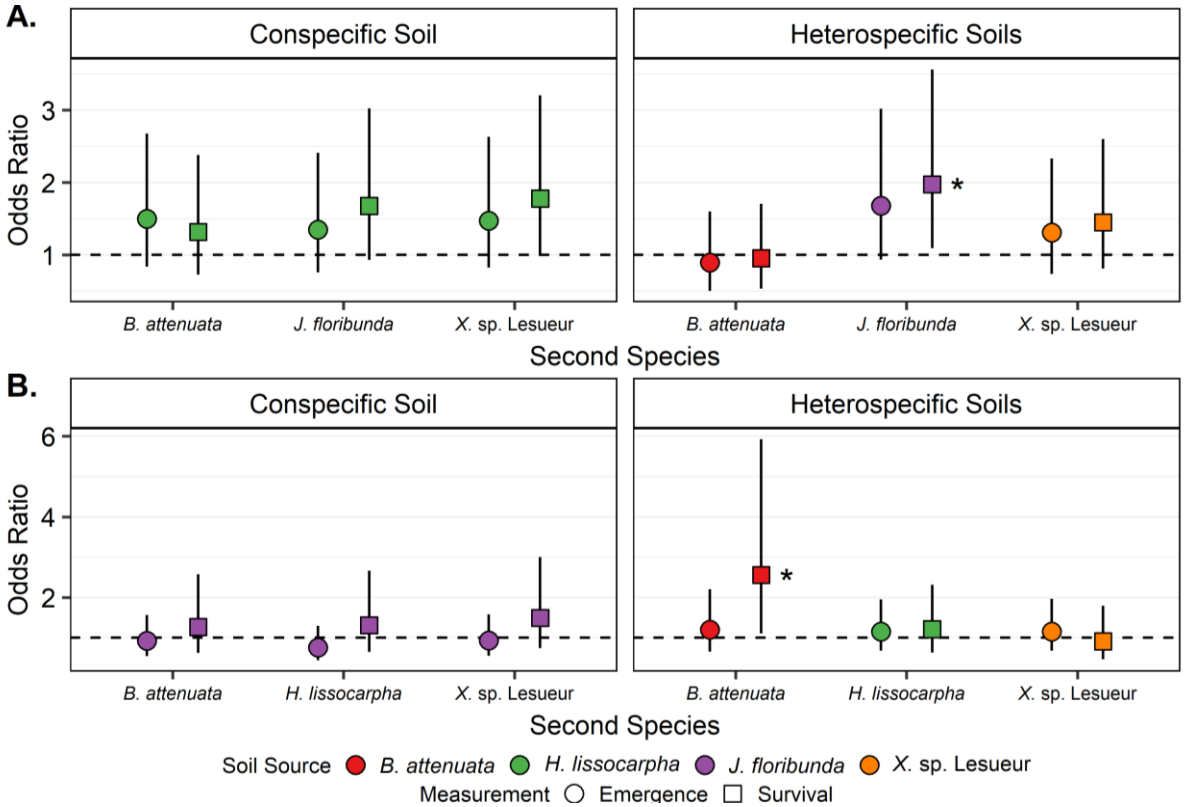


**Figure 5.2:** The effect of a second plant species and soil source on the emergence and survival of **A.** *Hakea lissocarpha*, and **B.** *Jacksonia floribunda* compared to when they were sown alone. Plant species were sown into their own soil (conspecific) and the corresponding second plant species' (heterospecific) soils. The odds ratio (OR) can be interpreted as a positive (OR > 1) or negative (OR < 1) effect of the second plant species on the seedling emergence and survival. The 95% confidence intervals that do not intersect an odds ratio of one (dashed lined) were statistically significant and marked with an asterisk.

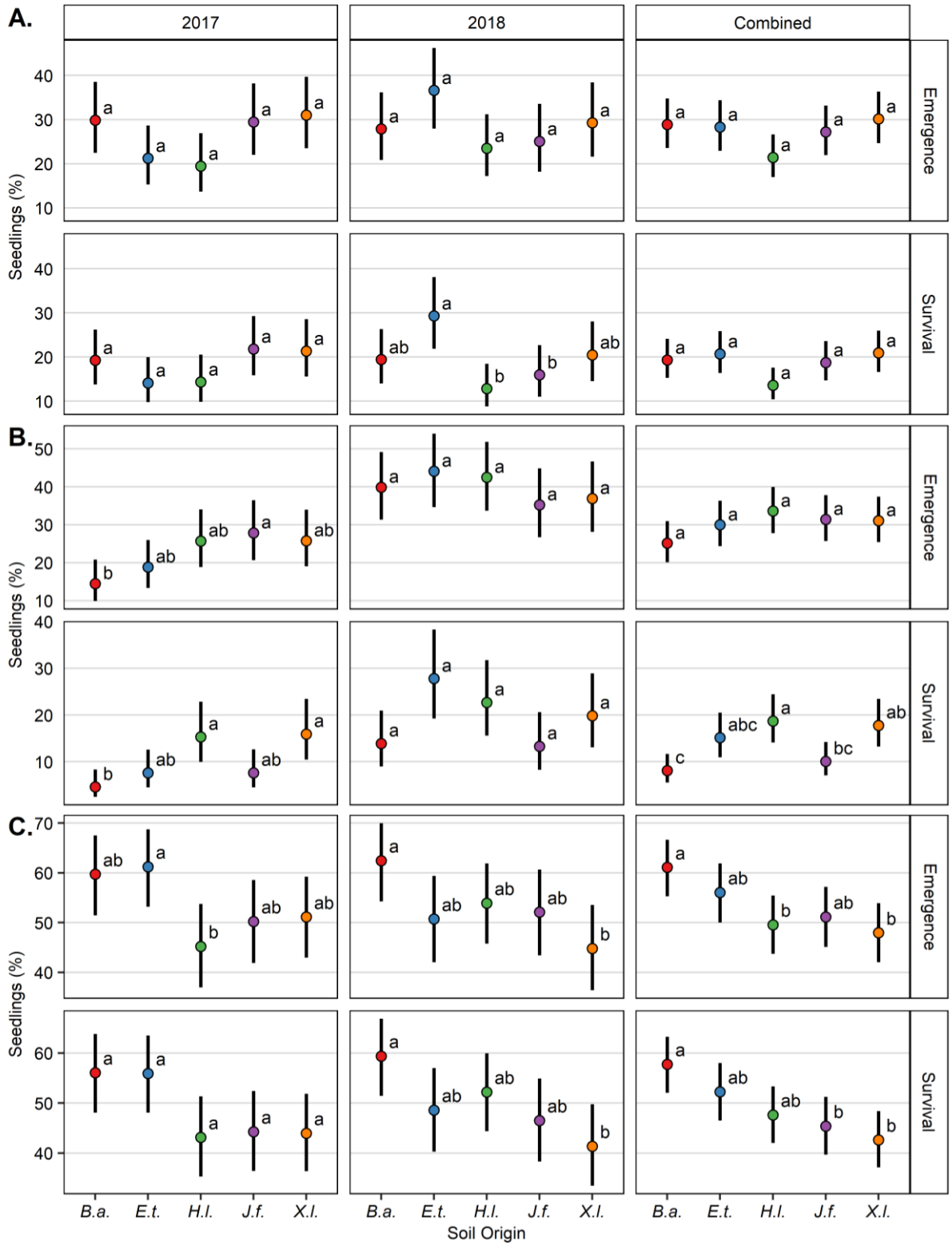


**Figure 5.3:**The mean seedling emergence and survival of plant species in Experiment 1, **A.** *Banksia attenuata*; **B.** *Jacksonia floribunda*; and **C.** *Xanthorrhoea* sp. Lesueur in 2017 and 2018, and the combined analysis of the 2017 and 2018 trials. *Eucalyptus todtiana* and *H. lissocarpha* are not shown. Seedlings of each species were sown into soils originally sourced from *B. attenuata*, *Eucalyptus todtiana*, *Hakea lissocarpha*, *J. floribunda* and *X.* sp. Lesueur. Letters represent homogenous subsets determined through a *post-hoc* analysis ($P \leq 0.05$).

## Metabarcoding

A total of 703,538 paired reads were obtained from the HTS, and 48% of wells produced good quality reads. The merge successfully paired 63% of reads (441,882) with a mean length of 476 bp, and a mean alignment length of 26 bp. No alignment was found for 23% of paired reads because they were too short (<75 bp). Oomycetes accounted for 44% of merged pairs, and on average 2,637 reads were detected in each sample.

The metabarcoding survey detected a total of 19 distinct oomycete phylotypes (Table 5.2). Three oomycete phylotypes were matched with *Phytophthora* species, *P. arenaria*, *P.* sp. kununarra and *P. quercetorum*. Seven *Pythium*, of which five were placed into clades, and two *Salisapilia* spp. phylotypes were identified to the genus level. The remaining seven oomycete phylotypes could not be matched with a genus and species. *Phytophthora arenaria* and *P.* sp. kununarra have previously been detected in Western Australia; however, the other 16 phylotypes were either new detections or absent in the library database.

Oomycetes were detected in 74 samples (26%), 32 (21%) from mature plants from the field and 42 (32%) from harvested seedlings in conspecific soil from the glasshouse. On average, 1.94 and 1.12 oomycete phylotypes were detected within positive samples from mature plants and harvested seedlings, respectively. A total of 15 oomycetes phylotypes were identified in the roots of mature plants and 8 from harvested seedlings. *Phytophthora arenaria* (33 detections), *Pythium* sp. 1 (18), and *P.* sp. kununarra (10) were the most commonly detected phylotypes (Table 5.2). Six oomycete phylotypes, *Py*. sp. 2, *Py.* sp. H, *Salisapilla* sp. 2, and unknown oomycetes 1, 2, and 5 were detected once. The most commonly detected oomycetes were *Pythium* sp. 1 (14) amongst mature plants and *P. arenaria* (32) from harvested glasshouse seedlings (Table 5.2). Only four oomycete phylotypes were detected from the roots of mature plants and seedlings (Table 5.2). *Pythium* sp. G and *Py*. sp. J were uncommon phylotypes regardless of host age, whereas *P. arenaria* and *Py*. sp. 1 were predominantly associated with the roots of seedlings and mature plants, respectively.

**Table 5.2:** The oomycete phylotypes and the number of detections from mature plant and seedling roots in 2017 and 2018. The number of samples collected and the total detections are displayed at the top and bottom of the table, respectively.

| | Detections | | | | |
|---|---|---|---|---|---|
| | | Mature plants | | Seedlings | |
| Oomycete Phylotype | Total *n* = 280 | 2017 *n* = 75 | 2018 *n* = 75 | 2017 *n* = 63 | 2018 *n* = 67 |
| *Phytophthora arenaria* | 33 | 0 | 1 | 24 | 8 |
| *P.* sp. kununarra | 10 | 10 | 0 | 0 | 0 |
| *P. quercetorum* | 3 | 3 | 0 | 0 | 0 |
| *Pythium* sp. 1 | 18 | 12 | 2 | 3 | 1 |
| *Py.* sp. 2 | 1 | 0 | 0 | 0 | 1 |
| *Py.* sp. B | 2 | 0 | 0 | 2 | 0 |
| *Py.* sp. D | 7 | 6 | 1 | 0 | 0 |
| *Py.* sp. G | 4 | 3 | 0 | 1 | 0 |
| *Py.* sp. H | 1 | 0 | 1 | 0 | 0 |
| *Py.* sp. J | 3 | 0 | 2 | 1 | 0 |
| *Salisapilia* sp. 1 | 3 | 3 | 0 | 0 | 0 |
| *Salisapilia* sp. 2 | 1 | 1 | 0 | 0 | 0 |
| Unknown oomycete 1 | 1 | 1 | 0 | 0 | 0 |
| Unknown oomycete 2 | 2 | 0 | 0 | 2 | 0 |
| Unknown oomycete 3 | 4 | 4 | 0 | 0 | 0 |
| Unknown oomycete 4 | 1 | 0 | 1 | 0 | 0 |
| Unknown oomycete 5 | 4 | 0 | 0 | 0 | 4 |
| Unknown oomycete 6 | 7 | 7 | 0 | 0 | 0 |
| Unknown oomycete 7 | 4 | 4 | 0 | 0 | 0 |
| Total | 109 | 54 | 8 | 33 | 14 |

## Oomycete alpha diversity

Plant species and the year of sampling had a statistically significant effect on the oomycete alpha diversity associated with the roots of mature plants. On average a greater number of oomycete phylotypes were detected in the roots of *H. lissocarpha* compared to *B. attenuata*, *E. todtiana* and *X.* sp. Lesueur in 2017 (Figure 5.4). Oomycete alpha diversity was lower in 2018; on average, 0.5 oomycete phylotypes were detected per individual in 2017 compared to 0.074 in 2018 (Figure 5.5). Specifically, in 2018 the oomycete alpha diversity associated with *H. lissocarpha* was significantly lower and no oomycetes were detected in the roots of *E. todtiana*, *J. floribunda* and *X.* sp. Lesueur (Figure 5.4).

The year samples were collected was the only statistically significant predictor of oomycete alpha diversity associated with the roots of seedlings harvested from conspecific soils in the

glasshouse. The oomycete alpha diversity detected was higher in seedling roots harvested in the 2017 experiment (Figure 5.5F). The oomycete alpha diversity associated with each plant species was relatively similar between and within experiments (Figure 5.4). Although, oomycetes were not detected in the roots of *E. todtiana* in 2018 despite double the sample size collected in 2017.

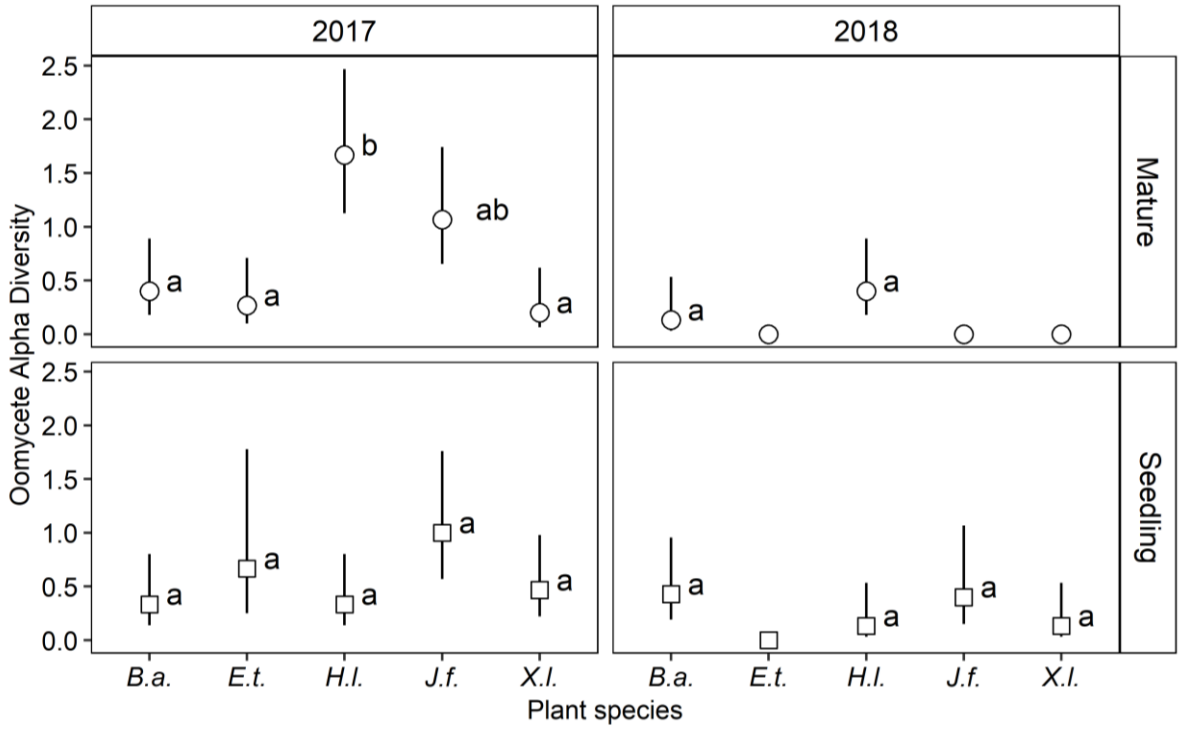


**Figure 5.4:** The oomycete alpha diversity (mean number of oomycete phylotypes) and the 95% confidence intervals associated with the roots of *B. attenuata* (*B.a*), *E. todtiana* (*E.t*), *H. lissocarpha* (*H.l*), *J. floribunda* (*J. f*) and *X.* sp. Lesueur (*X. l*) in 2017 and 2018. Roots were collected from mature plants and seedlings grown in conspecific soils under glasshouse conditions. Letters represent homogenous subsets generated by *post-hoc* analyses ($P \leq 0.05$), plant species without oomycete detections were removed from the analysis and not grouped.

The year samples were collected had a statistically significant effect on the mean number of oomycete phylotypes detected (Figure 5.5F; Table S5.3). Plant age did not influence the oomycete alpha diversity (Table S5.3). Overall, roots collected from mature plants and seedlings grown in conspecific soils had similar oomycete alpha diversities within years (Figure 5.5F; Table S5.3). On average, 0.4 more oomycete phylotypes were detected in each sample in 2017. There was a statistically significant interaction between *H. lissocarpha* and plant age, and in 2017 oomycete alpha diversity was higher in the roots of mature plants

compared to seedlings (Figure 5.5C). Additionally, on average more oomycete phylotypes were detected in the roots of *J. floribunda* and *X.* sp. Lesueur seedlings despite zero detections from their respective mature plants from the field in 2018 (Figure 5.5D–E). The presence of oomycetes in the corresponding mature individual in the field was not a significant (LR $\chi^2$ = 0.213, *P* = 0.644) predictor of the number of oomycete phylotypes detected from seedlings.

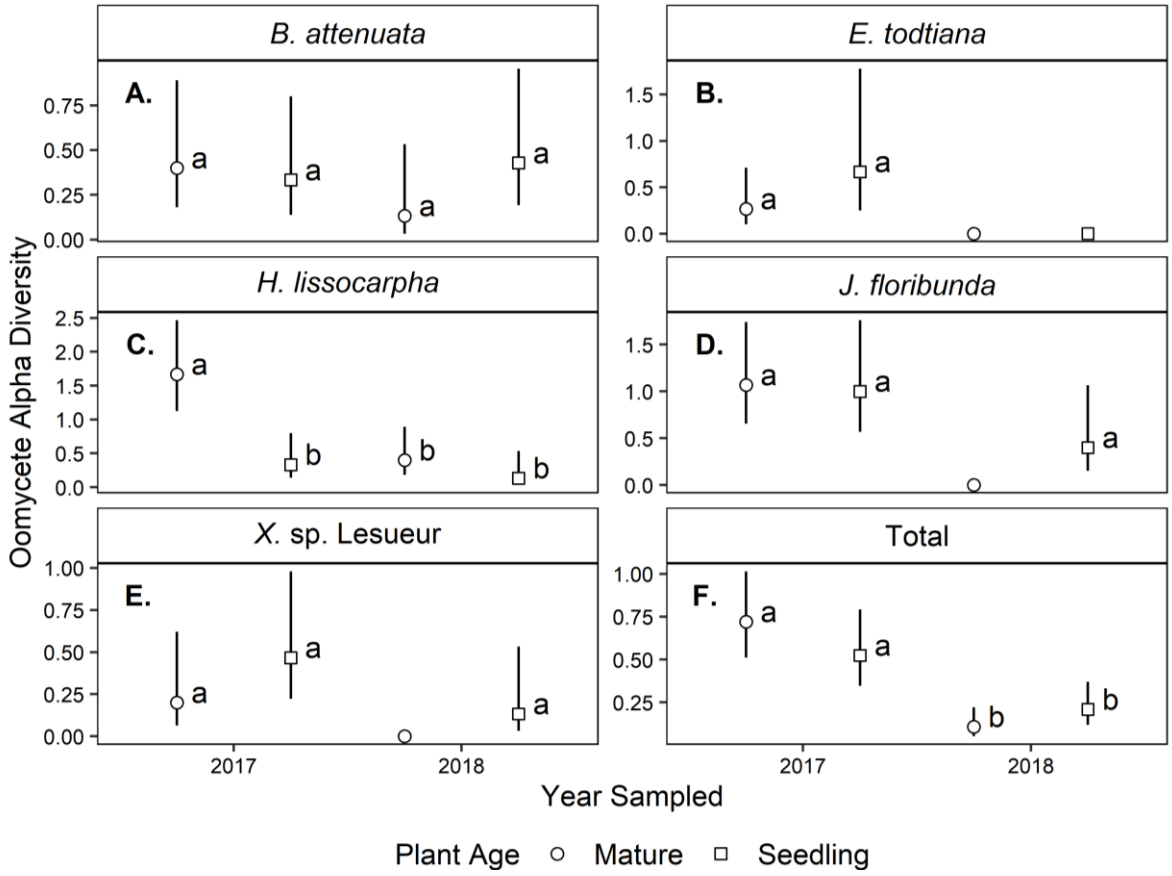


**Figure 5.5:** The oomycete alpha diversity (mean number of oomycete phylotypes) and the 95% confidence intervals associated with the roots of mature plant species in the field and seedlings grown in the same conspecific soils under glasshouse conditions. Oomycete alpha diversity is separated by the year of collection for plant species independently (**A.**–**E**.), and **F.** combined. Letters represent homogenous subsets generated by *post-hoc* analyses (*P* ≤ 0.05), plant species without oomycete detections were removed from the analysis and not grouped.

## Oomycete communities

The year that samples were collected appeared to have little impact on the oomycete communities detected (Figure 5.6A - B). The analyses performed using the presence and abundance dissimilarity indices indicated year explained a small amount of variation in the

oomycete community, despite being a statistically significant term in mature, seedling and global presence models (Table 5.3). Additionally, the oomycete communities associated with individual mature plant species and seedlings did not differ between years.

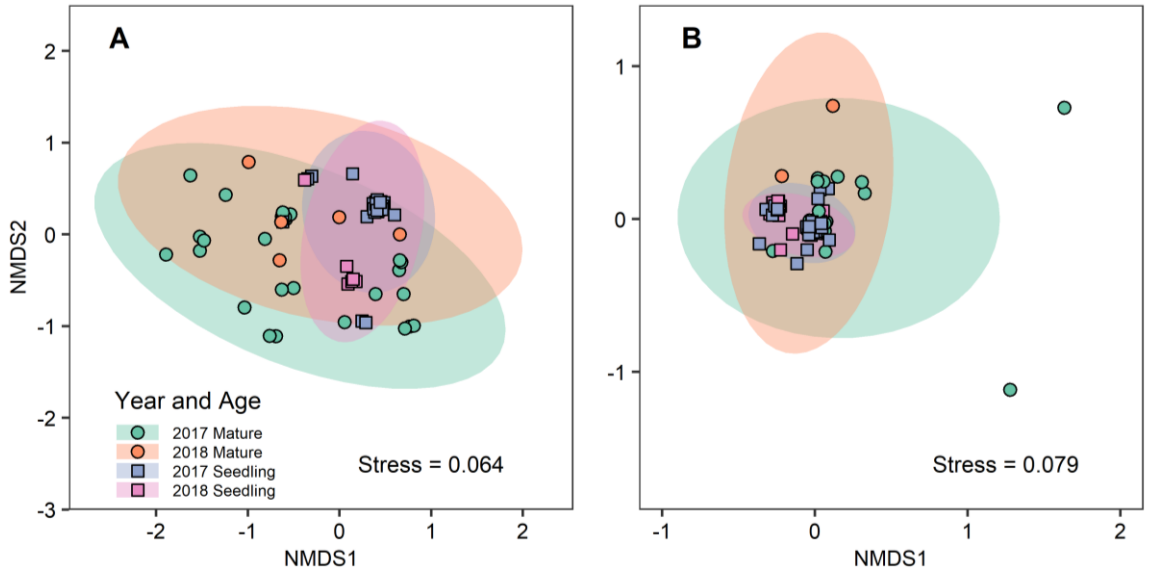


**Figure 5.6:** Non-metric multidimensional scaling ordination of the oomycete communities associated with the year and age of the plant roots were collected. The graphical representations are separated by the dissimilarity index, **A.** Jaccard (presence); **B.** Bray-Curtis (abundance). Small clusters of points in graph **A.** represent the same community of oomycetes as positions were randomly jittered.

Plant age had a statistically significant impact on the oomycete communities. In both presence and abundance analyses, plant age influenced the oomycete community detected (Table 5.3, Figure 5.6A–B). The amount of variation in the oomycete community explained by the abundance model was lower compared to the presence model (Table 5.3, Figure 5.6B). Specifically, the age of the plant species had an impact on the oomycete community associated with *J. floribunda* in the presence analysis. However, the oomycete communities associated with individual plant species did not differ between mature plants and seedlings in the abundance model. There was some overlap between the oomycete phylotypes present within mature plant and seedling roots for several plant species; however, the composition of the oomycete communities was substantially different (Figure 5.7). All plant species had vastly different compositions based on the abundance of oomycete phylotype reads (Figure 5.7).

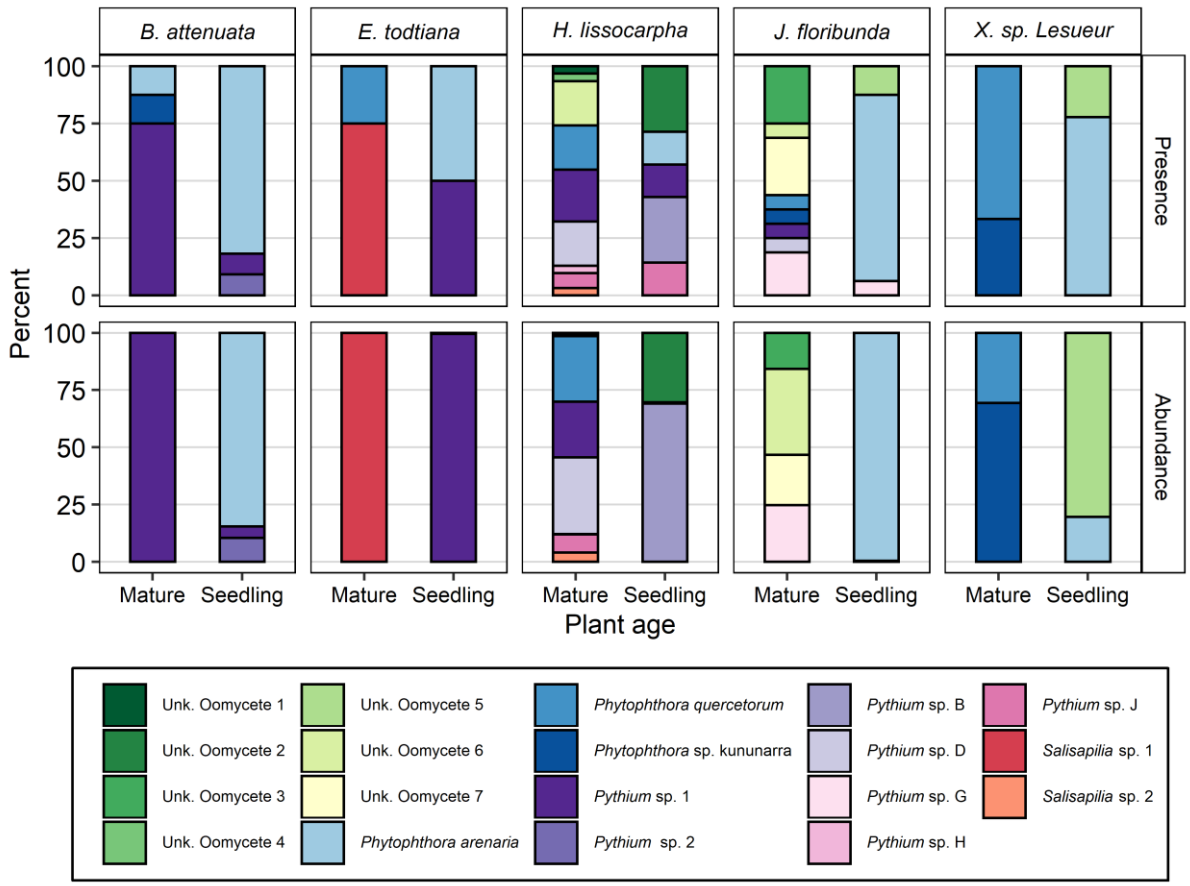


**Figure 5.7:** The composition of the oomycete communities associated with the roots of mature plant species and their seedlings grown in conspecific soils. The two calculations of oomycete composition, presence and abundance are based on the total number of phylotype detections and reads, respectively. The oomycete communities are separated by plant species and plant age.

Plant species explained a larger amount of variation in the oomycete community associated with mature plants and seedlings (Table 5.3). In both presence and abundance analyses of mature plants, the oomycete community of *B. attenuata* was significantly different from *H. lissocarpha* and *J. floribunda* (Figure 5.8A – B). Additionally, the oomycete community associated with mature *H. lissocarpha* plants was also significantly different to *E. todtiana* and *J. floribunda* in the presence analysis (Figure 5.8A). Results varied between analyses of the oomycete community associated with seedlings. The seedling oomycete community associated with *H. lissocarpha* was significantly different from *B. attenuata*, *J. floribunda* and *X.* sp. Lesueur (Figure 5.8C). However, there were no differences amongst plant species when the abundance of oomycetes was analysed (Figure 5.8D).

**Table 5.3:** Permanova results for the global and individual plant age analyses that were separated by mature plants sampled in the field at Lesueur National Park, WA, and seedlings grown and harvested from the same conspecific soils in the glasshouse. The results of a presence analysis using a Jaccard index and abundance analysis using the Bray-Curtis index are displayed. The results display the effect of plant species, sampling year, and plant age and their interaction on the composition of oomycete communities associated with rhizosphere roots. Significant ($P \leq 0.05$) results are presented in bold.

| | | Presence | | | | | Abundance | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Factor | df | SS | MS | F-value | $R^2$ | *P*-value | SS | MS | F-value | $R^2$ | *P*-value |
| Global | | | | | | | | | | | |
| Plant species | 4 | 2.73 | 0.68 | 7.53 | 0.183 | **0.0001** | 3.48 | 0.87 | 2.58 | 0.115 | **0.0001** |
| Year | 1 | 0.49 | 0.49 | 5.36 | 0.033 | **0.0005** | 0.36 | 0.36 | 1.07 | 0.012 | 0.3277 |
| Plant age | 1 | 2.32 | 2.32 | 25.60 | 0.155 | **0.0001** | 1.30 | 1.30 | 3.85 | 0.043 | **0.0004** |
| Plant species: Year | 3 | 1.39 | 0.46 | 5.11 | 0.093 | **0.0001** | 1.98 | 0.66 | 1.95 | 0.065 | **0.0023** |
| Plant species: Plant age | 4 | 2.06 | 0.52 | 5.69 | 0.138 | **0.0001** | 3.04 | 0.76 | 2.25 | 0.100 | **0.0001** |
| Year: Plant age | 1 | 0.24 | 0.24 | 2.60 | 0.016 | **0.0222** | 0.28 | 0.28 | 0.82 | 0.009 | 0.6409 |
| Plant species: Year: Plant age | 1 | 0.46 | 0.46 | 5.05 | 0.031 | **0.0002** | 0.37 | 0.37 | 1.10 | 0.012 | 0.3289 |
| Residuals | 58 | | | | | | | | | | |
| Total | 73 | | | | | | | | | | |
| Plant age - Mature | | | | | | | | | | | |
| Plant species | 4 | 2.90 | 0.72 | 5.25 | 0.400 | **0.0001** | 3.46 | 0.87 | 2.28 | 0.256 | **0.0001** |
| Year | 1 | 0.63 | 0.63 | 4.57 | 0.086 | **0.0003** | 0.34 | 0.34 | 0.90 | 0.025 | 0.5552 |
| Plant species: Year | 1 | 0.33 | 0.32 | 2.36 | 0.044 | **0.0234** | 0.22 | 0.22 | 0.58 | 0.016 | 0.9416 |
| Residuals | 25 | | | | | | | | | | |
| Total | 31 | | | | | | | | | | |
| Plant age - Seedling | | | | | | | | | | | |
| Plant species | 4 | 1.06 | 0.26 | 4.86 | 0.247 | **0.0002** | 2.55 | 0.64 | 2.09 | 0.170 | **0.0089** |
| Year | 1 | 0.39 | 0.39 | 7.08 | 0.090 | **0.0008** | 0.44 | 0.44 | 1.45 | 0.029 | 0.1762 |
| Plant species: Year | 3 | 1.03 | 0.34 | 6.31 | 0.242 | **0.0002** | 1.99 | 0.66 | 2.17 | 0.132 | **0.0073** |
| Residuals | 33 | | | | | | | | | | |
| Total | 41 | | | | | | | | | | |

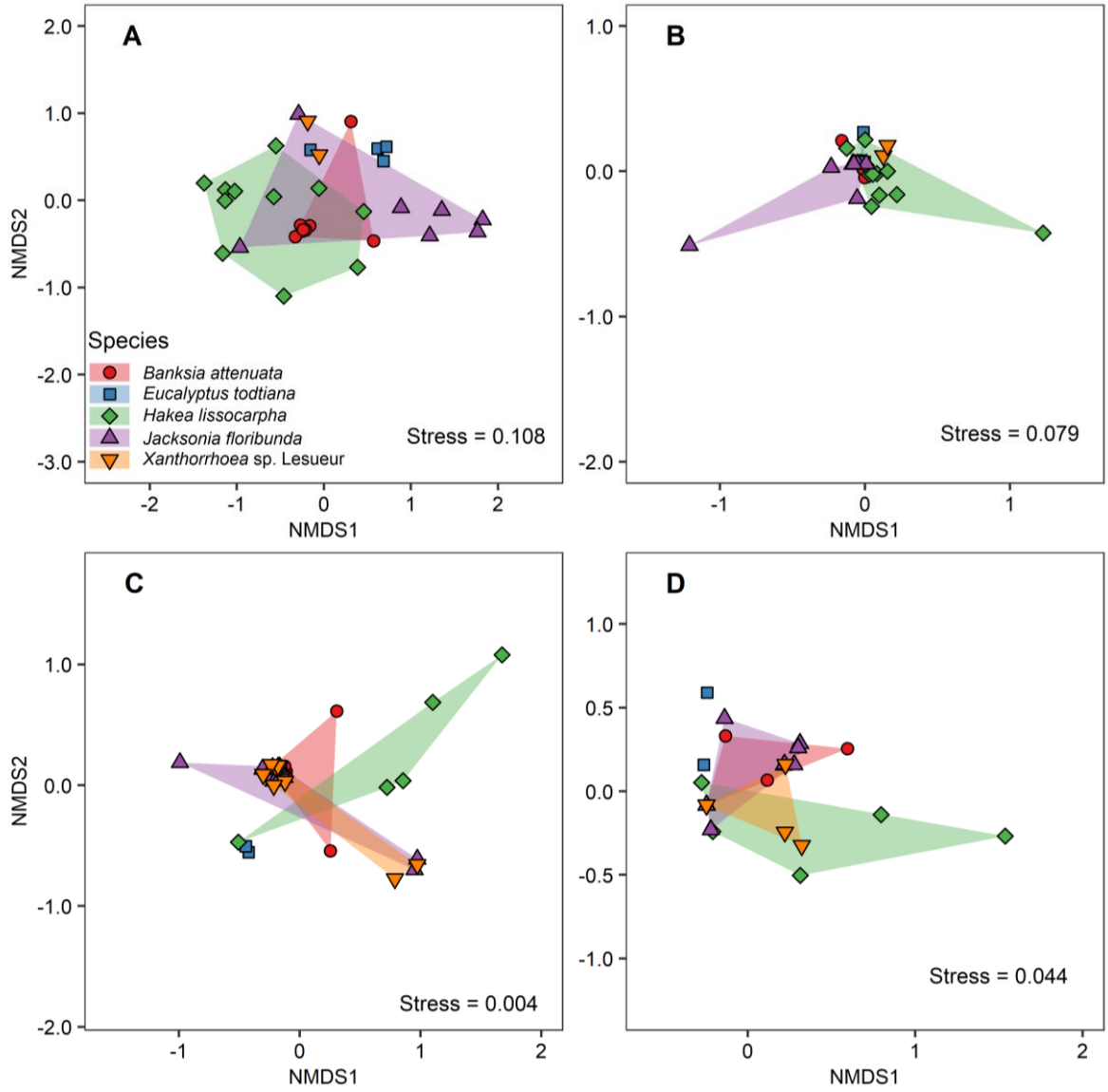


**Figure 5.8:** Non-metric multidimensional scaling ordination of the oomycete communities associated with the roots of mature plant species and their seedlings grown in conspecific soils. The NMDS representations of the oomycete communities are separated by the dissimilarity index and plant age, **A.** Jaccard (presence) mature plants; **B.** Bray-Curtis (abundance) mature plants; **C.** Jaccard (presence) seedlings; and **D.** Bray-Curtis (abundance) seedlings. Small clusters of points in graphs **A** and **C** represent the same community of oomycetes as positions were randomly jittered.

## Discussion

Negative plant-soil feedback occurred in conspecific and heterospecific soils for *J. floribunda* and *X.* sp. Lesueur, two of the five kwongan plant species screened. Pre- and post-emergent damping-off contributed to the feedback observed. The diversity of kwongan plant communities may be influenced by PSF or a Janzen-Connell effect beginning from the pre-

emergent seedling stages. Several PSF studies have been conducted in natural Mediterranean plant communities (Hyatt et al. 2003, Kulmatiski et al. 2008, Miki 2012, Comita et al. 2014), and the results of the current study are consistent with previous findings suggesting these plant communities can be influenced by the microbial community or natural enemies (Steinitz et al. 2011, Bonanomi et al. 2012, Teste et al. 2017, Png et al. 2019). There was little evidence to suggest the addition of a second plant species contributed to substantial changes in pre- and post-emergent seedling mortality. The presence and abundance of oomycetes detected from the roots of either the mature plants or seedlings were not correlated with seedling emergence or survival. However, the oomycete communities detected were significantly influenced by the plant species and host age. This is one of the first studies to link the below ground oomycete communities to PSF through metabarcoding, a novel tool that allows the microbial community to be related to the response of plant species (Merges et al. 2019, Miller et al. 2019). The early PSF and oomycete communities found may influence local diversity of kwongan vegetation and drive changes in pre- and post-emergent seedling mortality in these plant communities.

### Plant-soil feedback through the pre- and post-emergent damping

The primary aim of this study was to determine if conspecific and heterospecific soils, and the oomycete community drive plant-soil feedback through the pre- and post-emergent damping-off of kwongan plant species. Levels of pre- and post-emergent damping-off varied significantly in soils sourced from different plant species and appears to play a role in shaping the abundance of seedlings in kwongan plant communities. Damping-off impacted *X.* sp. Lesueur and *J. floribunda* in conspecific soils causing negative PSF primarily through pre- and post-emergent seedling mortality, respectively. Negative PSF in conspecific soils leads to coexistence between plant species (Bever et al. 2015). Pre-emergent damping-off in a heterospecific soil led to a temporary positive PSF for *J. floribunda* during the earliest developmental stage. Comparatively, seed germination in conspecific soils of seven grassland plant species produced both negative and positive PSF (Miller et al. 2019). A meta-analysis found weaker effects of conspecific soils pre-emergent damping-off compared to seedling mortality; however, a significant negative density-dependent effect on seed was identified across the literature (Comita et al. 2014). Previous studies on kwongan plant species and PSF have typically ignored early plant developmental stages and focused on the

equally important measurement of biomass accumulation (e.g. Albornoz et al. 2016, Teste et al. 2017). Studies that focus on transplanting seedlings in natural environments and the glasshouse (Reinhart and Clay 2009), or observations of seedling distributions in natural environments (Condit et al. 1992, Comita et al. 2010, Johnson et al. 2012) may be missing pre- and post-emergent seedling mortality an important period of shaping plant communities.

The presence and abundance of oomycetes detected from mature plants or harvested conspecific seedlings appeared to have little effect on emergence and survival seedlings in this study. The total effect of oomycete presence and abundance was negligible for four plant species and the significant negative effect displayed by *E. todtiana* was not repeated. Oomycetes have been identified as the natural enemies causing negative PSF in tropical forests, temperate forests and grassland plant communities (Mills and Bever 1998, Packer and Clay 2000, Bell et al. 2006). *Phytophthora arenaria* composed most of the oomycete community in seedlings and caused significant pre- and post-emergent damping-off of *B. attenuata*, *E. todtiana* and *J. floribunda* in pathogenicity trials (Chapter 2). Despite the pathogenicity and detection frequency of *P. arenaria*, few negative effects on seedlings were attributable to the oomycete community. Previous pathogenicity trials may not have reflected natural plant-soil interactions by using excessive *P. arenaria* inoculum levels, isolates collected from diseased mature plants (Rea et al. 2011), and by not including other rhizosphere microbes that directly influence pathogen abundance or seedling health (Bennett et al. 2006). The PSF observed may have been the sum of many different microbial interactions, including mycorrhizal fungi and bacteria (Bever et al. 2015), or other damping-off fungi, such as species of *Fusarium* (Liu et al. 2012b) which were not in the scope of the present study. Without a sterile control treatment, soil properties modified by the mature plant species may have also contributed to PSF (Png et al. 2019). The metabarcoding results may have not entirely represented the oomycete communities of heterospecific seedlings and subsequently were not correlated with seedling emergence and survival. Alternatively, the oomycete community and *P. arenaria* may reduce the growth of plant species during early developmental periods (Albornoz et al. 2016).

### Impact of a second plant species on pre- and post-emergent damping-off

Pre- and post-emergent damping-off was rarely influenced by the presence of a second plant species. The seedling survival of *J. floribunda* and *H. lissocarpha* were each significantly improved in a single heterospecific soil. This result does not provide reliable evidence to suggest interactions between plant species that impact levels post-emergent damping-off are widespread, and there was no indication of any effect on plant species in conspecific soils or pre-emergent mortality. Previous studies have found below ground nutrient facilitation and exchanges between plant species with different nutrient acquisition strategies may contribute to coexistence (Muler et al. 2014, Teste et al. 2014, Teste et al. 2015). Additionally, plant pathogens have promoted coexistence between competing plant species with different nutrient acquisition strategies (Albornoz et al. 2016). The interactions between kwongan plant species in previous studies found changes in the accumulation of biomass, mutualist mycorrhizal root colonisation and nutrient concentrations in tissue, but these may not cause identifiable variation in pre- and post-emergent seedling mortality. Additionally, the seedlings from different plant species may not have had enough time to substantially manipulate the soil microbial community. Plant-soil feedback can be driven by pre- and post-emergent damping-off and studies focusing on mortality during early developmental stages of plants may not need to include interactions between seedlings of different plant species, despite the importance of interactions between mature plants.

### Effect of plant species on oomycete alpha diversity and community composition

The oomycete alpha diversity and community were significantly affected by plant species. Seedling oomycete communities were similar due to the association of *P. arenaria* with three of the five plant species studied. Seedlings have previously been found to be influenced by generalist plant pathogens more frequently than host specialists (Augspurger and Wilkinson 2007, Hersh et al. 2012), and this appears to be the same for the seedlings of kwongan plant species and oomycetes. Conversely, mature plant species had varied oomycete communities and alpha diversity. The mature NMCR plant species, *H. lissocarpha* dominated a separate area of vegetation at the site and had a different oomycete community and significantly higher alpha diversity. Other evenly dispersed plant species at the site had significantly lower oomycete alpha diversity indicating *H. lissocarpha* may be experiencing a negative PSF. Bever et al. (2012) suggest negative PSF would be enhanced by

multiple plant pathogen infections due to a cumulative increase in virulence. Alternatively, variation in abiotic conditions, such as soil moisture at the site may be responsible for changes in the oomycete community.

The year and plant species impacted the detected oomycete community. There were more oomycetes detected from mature plants and seedlings in 2017 compared to 2018. Rainfall recorded at adjacent weather stations in the month leading up to sampling was substantially lower in 2018 compared to 2017 and the long-term averages (BoM 2019, DPIRD 2019). Soil moisture can have a substantial effect on the ability of oomycetes, such as *Phytophthora* to infect roots (Weste and Marks 1987, Rhoades et al. 2003), and may have influenced their detection from mature plants and seedlings if inoculum levels in the soils were low.

### Host age and oomycete communities

Oomycete communities associated with mature plants were significantly different to seedlings. Community analyses identified significant shifts between host ages, particularly in the phylotype presence model. There were clear differences in the composition of oomycete communities between age groups for each plant species. In total, mature plants and seedlings shared four oomycete phylotypes, of which none were either common or abundant in both host age groups. Furthermore, oomycetes detected in the roots of mature plants did not significantly increase the likelihood of detection in seedlings. Adult plant species likely have little or no direct influence on the oomycetes infecting conspecific seedling roots, and host root tissue does not appear to act as a large oomycete inoculum reservoir for the kwongan plant species studied. Oomycetes can remain in the soil through survival structures between years and can quickly propagate to infect seedlings (Martin and Loper 1999, Jung et al. 2013). *Phytophthora* can also remain dormant in asymptomatic host roots (Crone et al. 2013), but this does not appear to be the case for the five adult plants studied. The natural enemies present in conspecific soils of kwongan plant species may be a response to seedling densities if they are manipulated in the same way as oomycetes. These findings are comparable to studies from managed systems that identified the rhizosphere microbial community associated with plant species changed between developmental stages (Houlden et al. 2008, Cavaglieri et al. 2009, Chaparro et al. 2014). Although the oomycete communities were not correlated with seedling performance, metabarcoding adult and

seedling microbial communities could be a robust method to identify the mechanisms responsible for driving PSF as experimental evidence can be ambiguous (Reinhart and Clay 2009, Xu et al. 2015).

### Plant-soil feedback and oomycete communities in kwongan vegetation

The findings of the current study were relatively consistent with previous research and provided evidence to support the hypothesised role of pathogens in kwongan plant communities. The negative conspecific feedback of *J. floribunda* and *X.* sp. Lesueur was previously found by Teste et al. (2017) using homogenised soil mixes. The NMCR plant species *B. attenuata* and *H. lissocarpha* did not experience lower survival in conspecific soils relative to heterospecific soils in the current study. This was not consistent with the feedback identified by Teste et al. (2017), and suggests seedling mortality may not contribute to the hypothesised susceptibility trade-off leading to coexistence between nutrient acquisition strategies (Laliberté et al. 2015, Lambers et al. 2018). However, the oomycete communities associated with mature plant roots supported the hypothesis that NMCR plant species may be more susceptible to root pathogens (Laliberté et al. 2015, Lambers et al. 2018). *Hakea lissocarpha* dominated an area of the site and had the greatest oomycete alpha diversity associated with its roots. The other NMCR plant species, *B. attenuata*, was evenly distributed throughout the site and had significantly lower oomycete alpha diversity compared to *H. lissocarpha* in 2017. Additionally, as hypothesised by Laliberté et al. (2015) and Lambers et al. (2018), the mature plant species with mycorrhizal associations, *E. todtiana* and *X.* sp. Lesueur had the lowest oomycete alpha diversity, implying mycorrhizal fungi protected roots from pathogens. The oomycete communities indicate NMCR plant species may experience negative PSF when they become dominant and mycorrhizal mutualists provide protection from root pathogens leading to a trade-off that promotes coexistence amongst mature plants.

The experiment aimed to identify the changes in seedling emergence and survival in conspecific and heterospecific soils. In the present study, the factorial design identified the role of each heterospecific soil source, a level of detail not within the scope of previous experiments completed with kwongan plant species (Cahill Jr et al. 2017, Teste et al. 2019). Furthermore, a large component of this study focused on detecting the communities of

oomycetes associated with seedlings, homogenising soil sources would have likely skewed these results (Reinhart and Rinella 2016, Rinella and Reinhart 2019). Despite reducing the required resources, PSF experiments conducted with conspecific vs sterilised soil sources, homogenised soils, or pooled heterospecific soils are not necessarily reflective of specific microbial communities associated with a plant species and can lead to problems with the statistical analysis (Reinhart and Rinella 2016, Rinella and Reinhart 2019). The findings of Chapter 3 highlighted several sampling strategies used in past surveys of *Phytophthora*, such as soil bulking lead to higher estimations of species abundance at site levels. The choice to not homogenise soils and avoid bulking root samples for metabarcoding in the present study meant sampling from seedlings in heterospecific soils was not possible due to the considerable resources required to sample all individuals. Despite the higher variability associated with individual soil samples compared to homogenised samples (Kulmatiski 2016), the experiment was adequately replicated and repeated to ensure confidence in the results and to avoid Type II error (Gundale et al. 2017). Sterile conspecific treatments were initially included in the experiment design but were subsequently removed from the statistical analysis as fungal and oomycete damping-off pathogens were isolated from dead seedling roots. Sterile soil treatments would have helped to identify and separate the effect of the microbial community and abiotic effects on the feedback experienced in conspecific soils for each plant species.

Relating the oomycete community to the response of seedlings included assumptions that may have influenced the outcome of the analyses. A survivorship bias was present as seedlings were harvested at the conclusion of the experiment. Subsequently, 19 replicates were removed from analyses due to seedling death that may have been due to virulent pathogens. However, half of these replicates removed were *E. todtiana* in 2017 and their roots were not available to collect due to poor seed viability. Additionally, the roots of dead seedlings were not removed to avoid disturbing the root systems of remaining seedlings and influencing pathogen inoculum levels. Seedling roots were often destroyed by the pathogen before damping-off symptoms became apparent or soon after emergence. Despite many plant species having similar oomycete communities, the indices used in the analysis were not likely to be entirely representative of the oomycete community affecting heterospecific seedlings and represented a selection bias. The microbial community will often change

substantially during the development of a plant. Therefore, the samples collected at 130 days did not contain many new emergent seedlings and may not identify oomycetes affecting seedlings soon during early developmental periods. Seedling roots harvested after the experiment may reflect a temporal bias. It is difficult to quantify the effect of these sampling biases on the outcome of the statistical analyses.

The microbial community may promote coexistence between plant species in kwongan shrublands. Two of the five plant species experienced negative PSF in conspecific soils which was driven by either pre- or post-emergent damping-off. There was little evidence to suggest pre- and post-emergent seedling mortality were influenced by oomycetes or the addition of a second plant species. The oomycete communities detected from mature plant and seedling roots had little in common suggesting adult plants do not provide a reservoir of inoculum. Metabarcoding indicated fewer oomycetes were associated with mature plant species with mycorrhizal associations, and pathogens may respond to the dominance of NMCR plant species and promote negative feedback. This study identified the diversity of kwongan plant communities may be shaped by damping-off and found further evidence for nutrient acquisition strategy trade-offs. Additionally, molecular tools can be employed to determine the microbial community of both adults and seedlings to more accurately identify mechanisms promoting PSF.

# Chapter 6: General discussion

## Major Findings

This study was the first to explore the role of damping-off and the distribution of *Phytophthora* and oomycetes in kwongan plant communities, a diverse Mediterranean shrubland. It was hypothesised damping-off may substantially influence natural and restored kwongan plant communities and be caused by *Phytophthora* species. Experiments identified damping-off was a mechanism promoting co-existence between plant species in natural vegetation and reduced the establishment of seedlings in post-mining ecological restoration. Oomycetes and fungal pathogens were identified directly through isolation or indirectly by the positive effect of fungicides on seedling emergence in restoration. However, while there was clear evidence for pre- and post-emergent damping-off, there was little evidence to suggest *Phytophthora* and other oomycetes were the cause in natural soils. The richness and abundance of *Phytophthora* were low in kwongan plant communities, but evidence strongly suggests *P. versiformis* is native as well as *P. arenaria*. The results of these experiments viewed in the context of one another, provide insight into a mechanism that can influence the abundance of plant species in kwongan plant communities.

The putatively native *Phytophthora arenaria,* introduced *P. cinnamomi,* and *Py. irregulare* were virulent damping-off pathogens with a wide host range of native plant species in glasshouse pathogenicity trials (Chapter 2). *Phytophthora* and *Pythium* damping-off pathogens impacted a substantial number of Fabaceae plant species, as well as Myrtaceae and Proteaceae. The closely related *Phytophthora* species placed into phylogenetic clade 6a are hypothesised to be native to kwongan plant communities, but did not cause substantial levels of damping-off. In a novel functional trait model, plant species slowest to emerge were significantly more susceptible to *P. arenaria*. These results suggest that native and introduced *Phytophthora* may be influencing kwongan plant communities through damping-off, a plant disease that has not been studied thoroughly in natural Mediterranean plant communities.

Putative native and introduced *Phytophthora* species were believed to be abundant and distributed widely throughout kwongan plant communities. However, the current survey for *Phytophthora* species using metabarcoding revealed their richness and abundance in natural kwongan plant communities were exceptionally low (Chapter 3). This key finding was at odds with a previous survey (Burgess et al. 2017b); however, the elimination of sampling biases, by not sampling from symptomatic plants, and potential sources of cross-contamination, suggest the current study has set a reliable baseline for natural kwongan plant communities. The harsh road surface where *Phytophthora* species are most likely to be introduced into an environment, may be a key hurdle in the establishment of introduced *Phytophthora* species given their overall low detection frequency. However, if these pathogens do establish they move easily deep into natural vegetation, well away from roads and other areas of anthropogenic disturbance.

A large proportion of topsoil seedbank and broadcast seed applied to post-mining ecological restoration does not establish in kwongan plant communities (Bellairs and Bell 1993). Fungicide seed coats improved the seedling emergence for five of 14 plant species by 5–18% (Chapter 4). The seedling survival was unaffected by fungicide seed coats and their ability to improve seedling emergence declined with time. Several damping-off pathogens, such as *Pythium*, *Fusarium* and *Rhizoctonia* species were detected in ecological restoration, and topsoil disturbance likely homogenised, diluted and reduced pathogen inoculum. Fungicide seed coats are a novel technology for improving the seedling emergence of plant species susceptible to damping-off.

Native damping-off pathogens can be a mechanism driving negative PSF and help to maintain the diversity of plant communities. Two of the five plant species experienced negative conspecific PSF through pre- or post-emergent damping-off, indicating the co-existence between plant species may be promoted through this mechanism (Chapter 5). A coinciding metabarcoding survey indicated plant species, host age, and the year of sample collection all had a significant influence on either the oomycete alpha diversity or community composition. Additionally, oomycetes detected from the roots of mature plant species and seedlings were not correlated with pre- and post-emergent damping-off. Although, the oomycete communities associated with the mature plant species matched the predicted nutrient acquisition strategy trade-off which may help maintain the diversity of

kwongan plant communities (Laliberté et al. 2015, Lambers et al. 2018). For the first time it was identified the oomycete communities associated with adult plants and seedlings were vastly different, suggesting seedlings promote their own microbial community without the influence of the parent. Identifying the individual microbial communities of seedlings and adults determines the specific processes driving plant-soil feedback, these mechanisms remain ambiguous without the use of metabarcoding analyses.

## Damping-off in kwongan plant communities

Damping-off was identified in post-mining ecological restoration and in soils collected from natural plant communities. *Phytophthora arenaria* and *Py. irregulare* were found to be virulent damping-off pathogens with wide host ranges in the glasshouse pathogenicity trial (Chapter 2) and were detected frequently (Chapter 4, Chapter 5). Statistical analyses indicated the presence and abundance of oomycetes detected from seedling roots, of which *P. arenaria* was approximately two-thirds of all detections, were not significantly correlated with pre- and post-emergent damping-off in natural soils (Chapter 5). The presence of oomycetes in seedling roots was correlated with reduced *E. todtiana* seedling emergence. The impact of oomycetes on *E. todtiana* was substantially lower than observed in glasshouse pathogenicity trials (Chapter 2), and the experiment was not repeated for this plant species (Chapter 5). Despite the detection of *Py. irregulare* from all plots in the 2017 ecological restoration, pre-emergent damping-off was reduced by 7% on average for plant species effected by the oomycete fungicide seed coat treatment (Chapter 4). The complexity of natural and rehabilitated soils compared to sterilised soils used in the glasshouse pathogenicity trial may influence the level of damping-off. The virulence of damping-off pathogens in the glasshouse pathogenicity trial was likely increased by higher levels of inoculum, high soil moisture, warmer temperatures and biologically inactive soils. However, without sterile or pasteurised control treatments in restoration (Chapter 4) and natural soil experiments (Chapter 5) the levels of damping-off may have been higher then observed as there was no baseline comparison.

The correlation between the susceptibility of functional traits and damping-off by multi-host pathogens revealed little about the potential role of damping-off in natural plant communities. Plant species exposed to *P. arenaria* for the longest period before seedling

emergence appeared to be most susceptible in the glasshouse pathogenicity trial (Chapter 2). The transfer of the potential susceptibility of this trait to natural soils was unlikely as oomycetes were not associated with damping-off. Functional traits other than the role of nutrient acquisition strategies may be important in shaping kwongan plant communities. *Jacksonia floribunda* and *X*. sp. Lesueur were the only plant species used in the natural damping-off experiment that store their seed in the soil (Chapter 5), whereas the seed of *B. attenuata*, *H. lissocarpha* and *E. todtiana* remain in the canopy. Conspecific negative feedback caused by pre- and post-emergent damping-off only affected *J. floribunda* and *X*. sp. Lesueur and may indicate the annual input of seed into the soil may promote a community of damping-off pathogens. Studies of natural kwongan seed banks identified the lowest densities of soil stored seed occurred on the most nutrient deficient soils with the highest richness of plant species with canopy storage (Enright et al. 2007). Low soil seedbank density on nutrient deficient sites was not explained by lower seed input or other trade-offs due to nutrient availability (Enright et al. 2007). Given plant species with soil seedbanks experienced damping-off in conspecific soils (Chapter 5) canopy storage may be a strategy or inadvertently avoid exposure to soil-borne damping-off pathogens. The level of damping-off or the abundance of plant pathogens may vary depending on soil nutrients, species richness and the abundance of seedbank functional traits due to the changes described by Enright et al. (2007). Future plant-soil feedback experiments may wish to incorporate seed bank functional traits into analyses to determine their interaction and effect on kwongan plant communities.

Previous studies of spatial distributions of plant species and functional traits in kwongan plant communities does not provide evidence for the Janzen-Connell hypothesis. Plant species regularly display aggregated spatial distributions compared to segregated distributions predicted by the Janzen-Connell hypothesis (Perry et al. 2008, Miller et al. 2010, Perry et al. 2014). Additionally, nearest neighbour relationships in kwongan plant communities are rare and when observed primarily occur between conspecifics (Perry et al. 2009a, Perry et al. 2014). Woody non-resprouting plant species that are dependent on seed to persist at sites are more likely to be spatially aggregated compared to resprouters (Enright et al. 2007, Perry et al. 2013). The level of aggregation between plant species typically declines as nutrient availability decreases and species richness increases (Perry et

al. 2008, Perry et al. 2009b, Perry et al. 2014). Freckleton and Lewis (2006) highlighted that seedling mortality must be overcompensating to prevent the highest levels of recruitment still occurring closest to the parent. For example, damping-off experienced by *X*. sp. Lesueur was greatest in conspecific soils; however, over 40% of the total seed sown emerged and survived (Chapter 5). The negative feedback caused by damping-off in the most diverse kwongan plant communities on nutrient deficient soils studied in Chapter 5 may help prevent an accumulation of soil stored seed leading to these plant species dominating the plant community, and or be a selective force promoting canopy storage. Laliberté et al. (2015) hypothesised monodominant stands of plant species with ectomycorrhizal nutrient acquisition strategies may be a positive plant-soil feedback mechanism that allows less competitive plant species to persist.

Assessing the changes in the seedling emergence and survival of plant species used in ecological natural and restoration topsoil may indicate how soil disturbance can influence damping-off for different plant species. *Xanthorrhoea preissii* was not affected by fungicide seed coat treatments in restoration trials (Chapter 4), but pre-emergent seedling mortality of *X*. sp. Lesueur was the greatest in conspecific natural soils (Chapter 5). This may indicate the correct biocide was not applied to the seedcoat of *X. preissii,* or topsoil disturbance and homogenisation associated with stockpiling and soil transfer during restoration diluted and reduced inoculum levels of damping-off pathogens impacting *X*. sp. Lesueur (Figure 6.1A). However, this hypothesis assumes *Xanthorrhoea* species are comparably susceptible and are affected by the same damping-off pathogens in natural soils based on a strong phylogenetic signal (Gilbert and Webb 2007, Bever et al. 2015). Pre-emergent damping-off experienced by *E. todtiana* was caused by oomycetes in ecological restoration and correlated with the presence of oomycetes in natural soils. The emergence of *E. todtiana* did not differ based on the collection of soils from different plant species, but oomycetes were rarely detected from *E. todtiana* seedlings harvested from conspecific soils. Unlike the *Xanthorrhoea* species, this may indicate plant species like *E. todtiana* that rarely experience damping-off in conspecific soils may be more affected in restoration due to soil homogenisation increasing their likelihood of exposure to damping-off pathogens (Figure 6.1B). Plant species that did not display spatially variable levels of pre- and post-emergent damping-off in natural soils, such as *B. attenuata* experienced significant pre-emergent

damping-off in restoration. Plant species with more evenly distributed damping-off pathogens in natural soils may not be affected by the mixing of topsoil (Figure 6.1C). Damping-off pathogen inoculum may have been reduced due to topsoil disturbance as *B. attenuata* seedling survival decreased by 10% in natural soils compared to 2% in ecological restoration. Topsoil disturbance associated with ecological restoration homogenises and likely reduces damping-off pathogen inoculum, the impact on the emergence and survival of seedlings may be dependent on the spatial distribution of damping-off pathogens in natural soils.

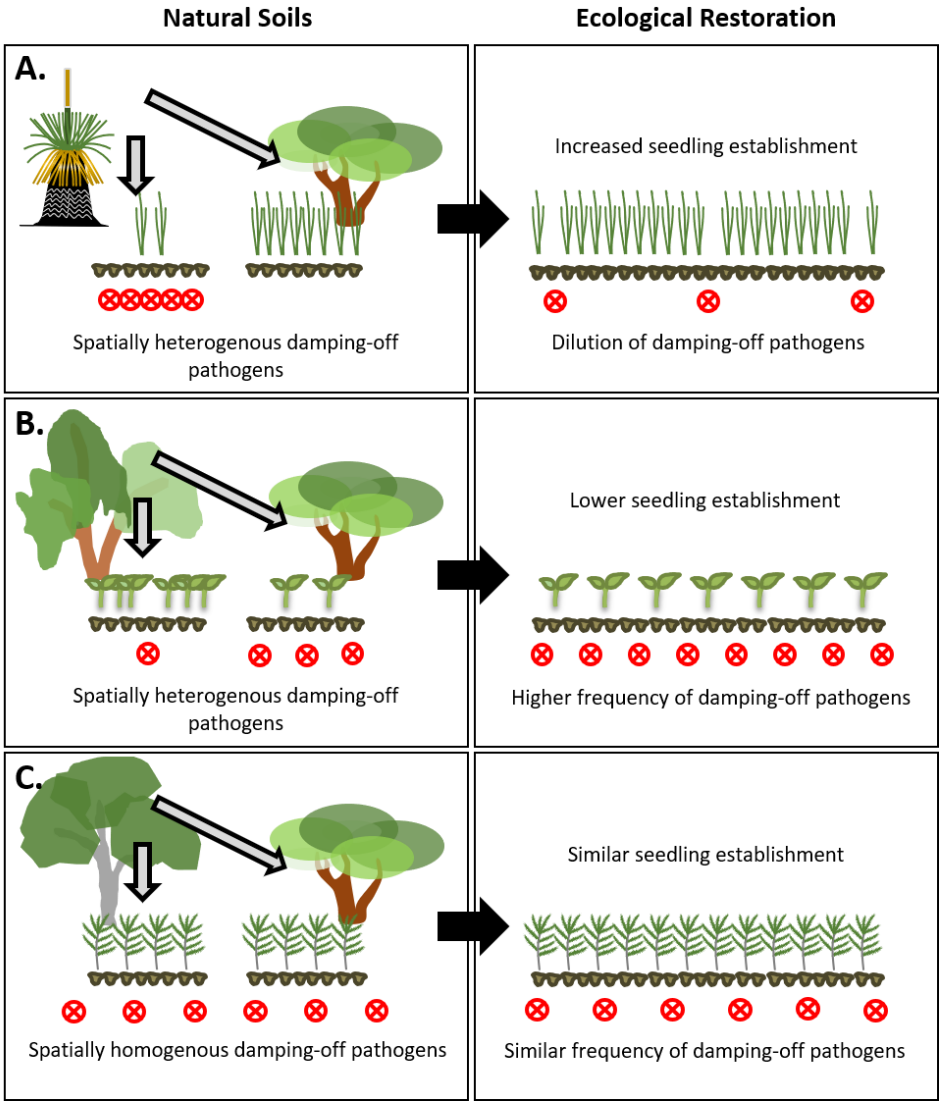


**Figure 6.1:** The hypothesised effect of restoration topsoil disturbance on the spatial distribution of damping-off pathogens and seedling establishment. The three examples are based on observations of **A.** *Xanthorrhoea preissii* and *X.* sp. Lesueur, **B.** *Eucalyptus todtiana* and **C.** *Banksia attenuata* seedling emergence and survival. Examples on the left are based on seedling establishment in conspecific and heterospecific natural soils (Chapter 5) and ecological restoration (Chapter 4). Grey arrows represent dispersal and crosses pathogens.

## *Phytophthora* and oomycetes in kwongan plant communities

The richness and abundance of *Phytophthora* species were low in kwongan plant communities, but the detection of these plant pathogens was influenced by several factors. A total of nine *Phytophthora* phylotypes were detected across both metabarcoding surveys, of which *P. versiformis* and *P. arenaria* were the most common. *Phytophthora versiformis* was detected frequently and appears native to kwongan plant communities (Chapter 3). *Phytophthora arenaria* was abundant amongst seedlings at one site and current evidence suggests the species may also be native (Chapter 5). The detection of *Phytophthora* and other oomycetes appears to be influenced by the plant species and the host's age (Chapter 5). Surprisingly, *P. versiformis* was not detected from mature plant species or seedlings in the natural damping-off experiment (Chapter 5) despite being found in samples collected from different locations within Mt Lesueur National Park (Chapter 3). The absence of *P. versiformis* may be due to the collection of samples from specific plant species of which it may not infect, or a single site and vegetation type that it may not be present within. *Phytophthora arenaria* was associated with seedlings of several plant species, while *P*. sp. kununurra was detected only predominantly from the mature plants of *X.* sp. Lesueur. Rainfall or soil moisture was a key abiotic variable influencing the detection, pathogenicity and potentially the distribution of *Phytophthora*. A reduction of rainfall in the month leading to sample collection in 2018 compared to 2017 levels was likely a key factor in the detection of fewer oomycetes from the roots of mature plant species and seedlings harvested in the glasshouse. Avoiding wetlands and water gaining sites when surveying kwongan vegetation in this study may be a reason for reduced *Phytophthora* species richness found in comparison to previous surveys where wetter sites favoured. These sites are more suited to the survival and aquatic species belonging to clade 6 (Burgess et al. 2018b, Burgess et al. 2018c, Redondo et al. 2018), and several have been detected from kwongan previously. Furthermore, the delay in the effect of fungicide seed coats observed in ecological restoration (Chapter 4) could have been due to the build-up of damping-off pathogen inoculum after the first major winter rainfalls. Biotic and abiotic variables effect the detection of *Phytophthora* from kwongan plant communities.

Species of *Pythium* are as common as *Phytophthora* in kwongan plant communities. Eight *Pythium* phylotypes were detected in the regional metabarcoding survey with *Phytophthora*

specific primers (Chapter 3) and seven with oomycete primers from roots collected from plant species apart of the natural soils damping-off experiment (Chapter 5). The richness of *Pythium* species was greater than that of *Phytophthora* in kwongan plant communities (Chapter 5). *Pythium* species were detected more frequently in the roots of mature plants (18%) compared to seedlings (6.9%) in kwongan plant communities. The age associations of *Pythium* species challenging the assumption they are predominantly damping-off or seedling pathogens (Martin and Loper 1999) and needs to be examined further. Several *Pythium* species were commonly detected in plots established within post-mining ecological restoration through soil baiting and direct isolation. Seven oomycetes were unknown and did not fall within a described genus (Chapter 5). Unknown oomycetes were assumed to be damping-off pathogens for the statistical analyses, but their role in natural kwongan plant communities has not been determined. The unknown oomycetes, *Pythium* and *Phytophthora* phylotypes need to be isolated and pathogenicity tested to determine their potential role in the microbial and plant community (Ampt et al. 2019).

Overall, *Phytophthora* and other oomycetes are not common in kwongan plant communities. The Mediterranean climate and seasonal environmental conditions are unfavourable to introduced and native *Phytophthora* species. The dry environmental conditions throughout most of the year are not conducive to damping-off caused by *Phytophthora* or *Pythium* species. Despite moist and warm glasshouse conditions in the natural soils damping-off experiment (Chapter 5), there was little evidence to suggest the presence and abundance of oomycetes was correlated with damping-off for the plant species tested. Fungal pathogens may be more important than oomycetes. Species of *Fusarium* associated with cereal disease have distributions in areas that receive less than 300 mm of annual average rainfall within Australia (Backhouse and Burgess 1995), much lower than the 450mm of average annual rainfall measured at the driest sampling locations in kwongan plant communities (Chapter 3). Within post-mining ecological restoration, *Fusarium oxysporum* was isolated from seedling roots, and *Fusarium* and *Rhizoctonia* specific fungicides significantly improved seedling emergence for two plant species (Chapter 3). *Fusarium oxysporum* has been isolated from harsh environments, such as the Simpson Desert, and subgroups or formae speciales can be virulent damping-off pathogens (Summerell et al. 2011). Damping-off caused by *Fusarium* and *Rhizoctonia* species is less

dependent on high soil moisture; conversely, oomycetes are reliant on high soil moisture or saturation (Lamichhane et al. 2017). Although some *Pythium* species can be saprophytes (Martin and Loper 1999, Schroeder et al. 2013), many *Fusarium* and *Rhizoctonia* species are saprobes (Keijer 1996, Ogoshi 1996, Summerell et al. 2011). Saprophytes are associated with the depletion of soil seed banks (Wagner and Mitschunas 2008) and increased activity of saprophytic and pathogenic fungi may lead to additional infections of seed. Whereas, oomycetes may need to germinate from resting structures when exudates are present in the soil (Lamichhane et al. 2017). Fungal damping-off pathogens can have greater environmental tolerances, may be more active in soils as saprobes and are not as reliant as oomycetes on high soil moisture to cause damping-off disease; therefore, they may be the more important seedling pathogens in kwongan plant communities.

*Phytophthora arenaria*

*Phytophthora arenaria* was highlighted as a potentially important pathogen in kwongan plant communities by Rea et al. (2011). The ecological role of *P. arenaria* in kwongan plant communities has become clearer. *Phytophthora arenaria* appears to be an age specific, multi-host, damping-off or seedling pathogen of kwongan plant species (Chapter 2, Chapter 5). *Phytophthora arenaria* was not virulent in soils collected from natural plant communities, and oomycetes were not correlated with pre- and post-emergent seedling mortality. Damping-off caused by *P. arenaria* may only occur when soils are saturated for longer periods; alternatively, the species may reduce the growth of seedlings (Albornoz et al. 2016). In the natural plant communities sampled, *P. arenaria* was very rarely associated with mature plants (Chapter 3, Chapter 5). Prior to the results of regional *Phytophthora* and local oomycete metabarcoding surveys (Chapters 3, Chapter 5), *P. arenaria* was assumed to be polyphagous due to its isolation from mature diseased plants and its ability to cause damping-off (Rea et al. 2011, Simamora et al. 2017). Mature plant deaths caused by *P. arenaria* may be rare and only occur when other predisposing factors are present, such as summer rainfall events, waterlogging or anthropogenic disturbance. This matches with the original description of *P. arenaria* diseased mature plants by Rea et al. (2011), and in addition to its infrequent infection of mature plants accounts for the lack of dieback fronts.

*Phytophthora arenaria* is most likely native to kwongan plant communities and perhaps more widely Australia. Despite highlighting issues with the sampling and processing methodologies of previous *Phytophthora* surveys (Chapter 3), *P. arenaria* has been detected frequently in natural plant communities and urban environments in Western Australia and Australia (Barber et al. 2013, Burgess et al. 2017b, Khaliq et al. 2018, Khdiar 2018). Metabarcoding surveys that have regularly detected *P. arenaria* from either collected rhizosphere soil and fine roots (Burgess et al. 2017b, Khdiar 2018), or from seedling roots grown as baits in soils (Khaliq et al. 2018). These methods circumvent the biological filter that appears to be mature plant roots. However, it is difficult to hypothesise how common *P. arenaria* is on the Geraldton Sandplain as it was not detected in the regional *Phytophthora* survey because mature plants were sampled (Chapter 3). *Phytophthora* species once established on roadsides appear to move into the natural plant communities with ease, there is a remote possibility that *P. arenaria* was introduced to the study area where samples were collected for the damping-off experiment in natural soils (Chapter 5). However, surveys of natural and managed plant communities in other countries have not reported detections of *P. arenaria* through baiting or metabarcoding surveys of rhizosphere soils. Desert and semi-arid plant communities located in central Australia are a large physical barrier preventing the natural dispersal of plant pathogens or geneflow between populations (e.g. Hayden et al. 2007). *Phytophthora arenaria* may have been introduced to one side of the continent given the large environmental barrier separating Western Australia and the eastern Australian states. Introduced isolates of *P. arenaria* may have low genetic diversity if there were few introduction events or sources (Dobrowolski et al. 2003, Brar et al. 2018).

*Phytophthora* species closely related to *P. arenaria* in clade 4 have distributions outside and within Australia. *Phytophthora boodjera* has been only detected within Australian natural and urban environments (Burgess et al. 2017b, Simamora et al. 2018), but the species is not known to co-occur with *P. arenaria* suggesting the two species may have different host specialisations (Burgess et al. 2018b). Other closely related species *P. alticola* and *P. quercetorum* have been detected primarily in natural and disturbed vegetation within South Africa (Maseko et al. 2007, Bose et al. 2018) and the USA (Balci et al. 2008, McConnell and Balci 2014), respectively. *Phytophthora quercetorum* was detected through

metabarcoding for the first time from kwongan plant communities (Chapter 5). Collection of isolates is required to confirm the presence of *P. quercetorum* in kwongan plant communities. Phylogenetically these *Phytophthora* species within clade 4 are closely related; however, current evidence suggests they are native to countries separated by large distances. Assessing the variability in isolates from different locations to determine if they have been introduced, and additional studies of *Phytophthora* in natural plant communities is required to determine if there is a single origin of clade 4 species.

## Management of *Phytophthora* and damping-off

The baseline *Phytophthora* communities obtained through the current regional and local metabarcoding surveys (Chapter 3, Chapter 5) indicate *P. versiformis* should be treated as native, and P. arenaria as putatively native to dry kwongan vegetation. A survey of seedlings in the field would help to confirm *P. arenaria* is native to the region. Evidence currently suggests clade 6a *Phytophthora* species are native to niche water gaining kwongan plant communities and further research is required to establish their distribution or associations with specific plant species. However, detections of clade 6a *Phytophthora* should be treated with a level of caution until their status can be confirmed on the Geraldton Sandplains. Despite being native, the nursery industry should treat *P. arenaria* infestations seriously given the host range and virulence of the damping-off pathogen at high inoculum levels. The large financial impact of *P. boodjera* on a nursery highlights how destructive the closely related *P. arenaria* may be in similar conditions (Simamora et al. 2015, Simamora et al. 2018). Detections of *P. arenaria* and *P. versiformis* do not need to be managed as infestations in kwongan plant communities. However, measures should be taken to prevent the movement of *P. arenaria* and *P. versiformis* present within soil and plant material outside of kwongan plant communities to avoid these species becoming invasive elsewhere and potential hybridization (Brasier et al. 1999, Brasier 2000).

The results of the regional *Phytophthora* survey (Chapter 3) comparing natural and disturbed plant communities support the current management strategies for *P. cinnamomi* and other introduced soil-borne plant pathogens (O'Gara et al. 2005a). Given that *Phytophthora* species appear to move deep into natural plant communities, reducing the number of potential infestations is recommended. Road surfaces are particularly harsh

environments for large proportions of the year in kwongan plant communities, the high temperatures of bitumen and gravel roads are rapidly fatal for *Phytophthora*. Sand tracks should aim to remove all vegetation as this may be a haven for any introduced *Phytophthora* until rainfall occurs and they can be spread further from the initial infestation (Crone et al. 2014). Promoting roadside engineering that diverts water into culverts free from hosts, and do not drain freely into vegetation would be recommended (Colquhoun and Kerp 2007). This could prevent the increased run-off from roads in wet conditions spreading *Phytophthora* from road surfaces and anthropogenically disturbed areas into natural vegetation. Given the findings of recent *Phytophthora* surveys in urban environments highlighting the richness and abundance of potential introduced pathogens (Barber et al. 2013, Hulbert et al. 2017, Paap et al. 2017b, Khdiar 2018), implementing management strategies to minimise the risk of introductions is vital. These management strategies include vehicle wash down stations, boot cleaning stations, strategic road and track closures and green bridging. The distance from the main metropolitan centre Perth likely shields kwongan plant communities from introductions; although, the risk of a new infestation only increases with time.

Fungicide seed coats and several current ecological restoration practices may help to manage damping-off. As demonstrated in the ecological restoration experiment (Chapter 4), fungicide seed coats provide low to moderate protection against pre-emergent damping-off pathogens for some plant species. Additionally, fungicides applied to expensive seed provide some financial incentives. For example, seed coats applied to *Banksia attenuata* and *B. candolleana* can save an estimated $1000 – $2000 annually for each plant species, assuming a cost of $1500 /kg of seed, sown at 330 g/ha across 40 ha (Chapter 4). The effect of fungicide seed coats on emergence appeared to be the greatest when seeds were sown shortly before the first period of winter rainfall to avoid biodegradation over time. Oomycete and fungal damping-off pathogens were responsible for damping-off, potentially applying multiple compatible fungicides to the seed of plant species with unknown susceptibility will ensure they are protected. Post-emergent damping-off did not appear to decrease the survival of many seedlings by a large amount, on average 2.9% of control seedlings died. Other chemical control methods, such as granular fungicides applied to the soil during sowing may provide more widespread suppression of post-emergent damping-

off pathogens. Current restoration practices likely assist in reducing the levels of damping-off. The stripping, stockpiling and spread of topsoil reduces microbial biomass and activity (Harris et al. 1989, Williamson and Johnson 1990, Birnbaum et al. 2017). Sowing seed prior to the winter and late autumn rains helps to reduce damping-off by limiting the exposure of seed and seedlings to higher inoculum levels that build up several weeks after the first major winter rainfalls (Chapter 4). The application of fertilizer increases seedling vigour during a period of vulnerability and the greatest exposure to damping-off pathogens (Lamichhane et al. 2017). Other damping-off management practices, such as tillage or ripping have an unknown effect on damping-off as these measures may benefit some pathogens or may create ponding (Lamichhane et al. 2017).

## Future research

Fire is an important disturbance in kwongan plant communities as it triggers the recruitment of most plant species by providing heat shock, smoke, abiotic germination cues and by releasing seed stored in the canopy (Miller and Dixon 2014). The influence of fire on the microbial community in kwongan has not been investigated and may substantially change the interactions observed using soils in the period between fires. Significant shifts in the microbial community and declines in microbial biomass, particularly amongst fungi are common in the upper soil layers directly after wildfires in natural vegetation (Hart et al. 2005, Dooley and Treseder 2012, Holden and Treseder 2013). Changes in the microbial community have been recorded in Mediterranean plant communities (D'Ascoli et al. 2005, Goberna et al. 2012) and Western Australia (Muñoz-Rojas et al. 2016). The activity of plant pathogens may change due to the abiotic conditions (i.e. soil moisture and temperature), fire has as a direct and indirect impact through changes in the plant community on abiotic conditions (Auld and Bradstock 1996). Fewer studies have specifically focused on pathogens post-fire, but the introduced oomycete pathogen *Phytophthora cinnamomi* may become more virulent (Moore et al. 2015). Lamont et al. (1993) identified negative density dependent mortality amongst Proteaceae seedlings, and canopy stored seeds in the post-fire environment may not follow typical seed shadow distribution present in other biomes. Seed dispersal patterns are dependent on reproductive functional traits, such as soil vs canopy stored seed (Enright et al. 2007). Given large shifts post-fire, future studies should

aim to identify if PSF exists and is mediated by the microbial community and abiotic factors during this period.

The root pathogens associated with nutrient acquisition strategies need to be explored further in the period between fires. It was identified in natural soils damping-off experiment (Chapter 5) oomycetes were more frequently associated with NMCR plant species whereas mycorrhizal plant species had the fewest detections and lowest alpha diversity. There was evidence to support a potential negative PSF associated with *H. lissocarpha*; however, an area of lower density was not studied. A thorough study to examine the oomycete, fungal pathogen and mycorrhizal communities associated with different nutrient acquisition strategies, and the abundance of those plant species in the field to determine if the microbial community promotes feedback is recommended.

Oomycetes were not associated with pre- and post-emergent damping-off of most plant species studied, and fungal primers may provide greater information on the microbial community interacting with seedlings and saplings in kwongan plant communities. As discussed previously, fungal damping-off pathogens may be more important in kwongan plant communities as they do not require high soil moisture or flooding to cause disease and they can have greater environmental tolerances. It can often be difficult to assign functional roles to all the phylotypes identified when using fungal primers as they provide large amounts of information on the composition of the fungal community (Ampt et al. 2019). Null model testing was applied by Merges et al. (2019) to identify operational taxonomic units associated with seedling mortality or health. Fungal plant pathogens may be relatively important in kwongan heath under drying and warming conditions predicted to occur due to climate change (Birnbaum et al. 2019). Future studies would be advised to use fungal primers to identify potential correlations with damping-off or plant disease.

The design of *Phytophthora* surveys should be considered carefully to tailor sampling methods to answer specific questions. *Phytophthora* surveys are more frequency conducted in natural plant communities (Hansen et al. 2012). However, surveys of natural vegetation typically collect bulked soil and include sampling biases which may influence the *Phytophthora* species detected. Surveys which aim to identify potential new infestations and introductions should target symptomatic vegetation and sites which are most likely to contain *Phytophthora* (i.e. disturbed roadsides and disease fronts). Alternatively, studies

aimed at identifying native *Phytophthora* should avoid locations which may be conducive to detecting introduced pathogens as this creates ambiguity in the results. The potential impact of *Phytophthora* on the natural plant community may be difficult to estimate if bulked samples are used as abundance cannot be determined. Furthermore, sampling different plant developmental stages may potentially lead to the identification of age specific pathogens. Over 200 plant species and 50 genera were sampled in the regional *Phytophthora* survey (Chapter 3), but kwongan plant communities have an estimated richness of over 2450 plant species (Lamont et al. 1984). Bulk sampling a specific genera and families may be a less resource intensive study to first identify *Phytophthora* associated with uncommon plant species. The regional *Phytophthora* survey did not directly examine the impact of specific sample collection biases and processing procedures on the number or community of the *Phytophthora* detected (Chapter 3). A future study may wish to directly address specific sample collection biases to determine their impact on the *Phytophthora* detected.

## Conclusion

This study investigated damping-off and *Phytophthora* within kwongan plant communities. The abundance and richness of *Phytophthora* species was low, but *Phytophthora arenaria* and *P. versiformis* are likely native. Within natural plant communities damping-off is a mechanism that promotes conspecific negative plant-soil feedback and potentially the co-existence between plant species. *Phytophthora* and other oomycetes are virulent damping-off pathogens; however, there was little evidence to suggest they were responsible for reduced seedling emergence and survival in natural soils. The associations of oomycetes with the roots of mature plant species provided evidence for the hypothesised trade-off between susceptibility to soil-borne pathogens and effective nutrient acquisition that may help maintain diversity within kwongan plant communities. In post-mining ecological restoration, damping-off reduced the number of established seedlings. Fungicide seed coats improved seedling emergence by a low to moderate amount and are a potentially a novel strategy for reducing the impact of damping-off pathogens in restoration. Variation between natural and restored topsoil suggests disturbance during rehabilitation practices may alter the spatial distribution of damping-off pathogens leading to changes seedling emergence and survival. Damping-off and soil-borne plant pathogens are an important

component of the microbial community influencing the structure of kwongan plant communities.

# Supplementary Material

## Chapter 2

**Table S2.1:** Supporting literature for the functional trait classification of plant species. Superscript letters represent the following: [C] sources that conflict with the reported trait, [G] sources that report the functional trait of the genus of specific species of the same genus, and [F] sources that report the functional trait for the family.

| Species | Nutrient Acquisition and N-fixing species | Seed storage | Fire response |
|---|---|---|---|
| *Acacia pulchella* | [1] | [2, 3] | [3, 4] |
| *Bossiaea eriocarpa* | [1, 5] | [2, 3, 6] | [3][C]<br>[4, 6] |
| *Daviesia nudiflora* | [1, 7, 8][CG]<br>[9, 10][G]<br>[11] | [6, 12] | [6, 12] |
| *Gastrolobium spinosum* | [7, 9, 13][G] | [3] | [3, 14] |
| *Gompholobium knightianum* | [4, 8]<br>[9][G] | [4, 15, 16] | [4, 14-16] |
| *Gompholobium tomentosum* | [1, 8, 9, 12] | [4, 12, 16] | [17][C]<br>[4, 12, 16] |
| *Jacksonia floribunda* | [1, 8] | [3, 12] | [3, 12, 17, 18] |
| *Jacksonia sternbergiana* | [1] | [3, 4] | [3, 4] |
| *Kennedia prostrata* | [7-9, 19] | [2, 4, 15] | [4, 15, 16, 20] |
| *Viminaria juncea* | [8, 9, 19, 21] | [3] | [3, 22] |
| *Beaufortia elegans* | [7][F] | [3, 4, 9] | [3, 4, 17, 23] |
| *Calothamnus hirsutus* | [1] | [4, 9][G] | [4, 15][G] |
| *Calytrix flavescens* | [1][G] | [2, 3] | [2, 3] |
| *Eremaea asterocarpa* | [1, 5, 13] | [3] | [3] |
| *Eucalyptus todtiana* | [1, 13] | [3, 12] | [3, 12] |
| *Hypocalymma angustifolium* | [8]<br>[1, 7, 9][G] | [3] | [3] |
| *Leptospermum erubescens* | [7][G] | [3] | [3] |
| *Melaleuca seriata* | [1, 7][G] | [3][G] | [3][G] |
| *Scholtzia laxiflora* | [1][G] | [3, 6] | [3, 6] |
| *Verticordia densiflora* | [1] | [4, 6, 20] | [4, 6]<br>[20, 24][C] |
| *Banksia attenuata* | [1, 12] | [3, 12] | [3, 12, 17] |
| *Banksia telmatiaea* | [25] | [4] | [26] |
| *Conospermum stoechadis* | [1] | [3][G] | [3][G]<br>[27, 28] |
| *Grevillea eriostachya* | [7, 29, 30][G] | [3, 4, 6] | [3, 6, 27] |
| *Grevillea shuttleworthiana* | [7, 29, 30][G] | [3, 6] | [3, 27, 31] |
| *Hakea costata* | [1, 25, 30] | [6, 32] | [6, 31, 32] |
| *Hakea trifurcata* | [1, 25, 30] | [3, 32, 33] | [3, 27, 32, 33] |
| *Lambertia multiflora* | [29, 30][G] | [6][C]<br>[4] | [6, 17, 27] |
| *Petrophile drummondii* | [29, 30][G] | [3, 6] | [3, 6, 27] |
| *Stirlingia latifolia* | [1] | [3, 6] | [3, 6, 17, 27, 31] |

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**Table S2.2:** The mean seedling emergence (left) and mean seedling survival (right) (as a percentage of the negative control) when soils were inoculated with a *Phytophthora* species for the plant species with adequate seed viability in Experiment 1. Isolates of *Phytophthora* species included did not significantly influence the seedling emergence and survival of the plant species.

| | *P. cooljarloo* | | | | *P. kwonganina* | | *P. rosacearum* | | *P. pseudorosacearum* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant species | MUCC770 | | HSA2313 | | IMI 329669 | | HSA2529 | | HSA2530 | |
| *Allocasuarina humilis* | 53 | 52 | 79 | 81 | 60 | 50 | 91 | 67 | 81 | 83 |
| *Banksia attenuata* | 59 | 55 | 105 | 125 | 64 | 79 | 92 | 100 | 69 | 81 |
| *Banksia telmatiaea* | 57 | 59 | 120 | 128 | 101 | 109 | 79 | 79 | 113 | 115 |
| *Calothamnus quadrifidus* | 119 | 127 | 93 | 105 | 104 | 109 | 56 | 68 | 78 | 91 |
| *Eremaea pauciflora* | 170 | 179 | 98 | 97 | 195 | 213 | 170 | 179 | 135 | 142 |
| *Eucalyptus todtiana* | 83 | 90 | 83 | 70 | 175 | 200 | 92 | 110 | 158 | 150 |
| *Gompholobium tomentosum* | 96 | 96 | 96 | 98 | 113 | 115 | 121 | 131 | 129 | 140 |
| *Hypocalymma angustifolium* | 267 | 700 | 33 | 50 | 167 | 400 | 133 | 100 | 0 | 0 |
| *Hakea trifurcata* | 86 | 78 | 105 | 100 | 99 | 97 | 98 | 104 | 91 | 88 |
| *Leptospermum erubescens* | 170 | 213 | 52 | 60 | 148 | 180 | 30 | 40 | 26 | 27 |
| *Melaleuca brevifolia* | 121 | 153 | 21 | 29 | 121 | 141 | 71 | 53 | 96 | 94 |
| *Melaleuca seriata* | 106 | 107 | 109 | 110 | 62 | 50 | 82 | 65 | 103 | 76 |
| *Patersonia occidentalis* | 74 | 74 | 87 | 87 | 84 | 84 | 89 | 89 | 83 | 83 |
| *Xanthorrhoea preissii* | 104 | 94 | 136 | 146 | 93 | 77 | 140 | 142 | 120 | 106 |

**Table S 3.0.1:** Site number, site type, sample point, and location.

| Site | Site Type | Sample Point | Latitude | Longitude |
|---|---|---|---|---|
| 1 | Disturbed | 1 | -30.55746995844 | 115.44440198690 |
| 1 | Disturbed | 2 | -30.55755201727 | 115.44491001405 |
| 1 | Disturbed | 3 | -30.55756601505 | 115.44593797065 |
| 1 | Disturbed | 4 | -30.55755604059 | 115.44649201445 |
| 1 | Disturbed | 5 | -30.55762804113 | 115.44728301466 |
| 1 | Natural | 1 | -30.55937097408 | 115.44551300816 |
| 1 | Natural | 2 | -30.55941103958 | 115.44532902539 |
| 1 | Natural | 3 | -30.55948304012 | 115.44482602738 |
| 1 | Natural | 4 | -30.55965998210 | 115.44450097717 |
| 1 | Natural | 5 | -30.55984999985 | 115.44572599232 |
| 2 | Disturbed | 1 | -30.48133595847 | 115.40850003250 |
| 2 | Disturbed | 2 | -30.48163896427 | 115.40969696827 |
| 2 | Disturbed | 3 | -30.48177802004 | 115.41043097153 |
| 2 | Disturbed | 4 | -30.48203500919 | 115.41164299473 |
| 2 | Disturbed | 5 | -30.48232602887 | 115.41255402379 |
| 2 | Natural | 1 | -30.47863296233 | 115.41187802330 |
| 2 | Natural | 2 | -30.47856003977 | 115.41149899364 |
| 2 | Natural | 3 | -30.47848301008 | 115.41107302532 |
| 2 | Natural | 4 | -30.47829198651 | 115.41167803109 |
| 2 | Natural | 5 | -30.47834898345 | 115.41211598553 |
| 3 | Disturbed | 1 | -30.14153603464 | 115.16618197784 |
| 3 | Disturbed | 2 | -30.14051796868 | 115.16592800617 |
| 3 | Disturbed | 3 | -30.13876397163 | 115.16546398401 |
| 3 | Disturbed | 4 | -30.13753702864 | 115.16517899930 |
| 3 | Disturbed | 5 | -30.14139102772 | 115.16462001018 |
| 3 | Natural | 1 | -30.14173896052 | 115.16419496387 |
| 3 | Natural | 2 | -30.14207297936 | 115.16403201967 |
| 3 | Natural | 3 | -30.13991698623 | 115.16578601673 |
| 3 | Natural | 4 | -30.14242703095 | 115.16373496503 |
| 3 | Natural | 5 | -30.14271000400 | 115.16353103332 |
| 4 | Disturbed | 1 | -30.29709669761 | 115.18404892646 |
| 4 | Disturbed | 2 | -30.29538896866 | 115.18353796564 |
| 4 | Disturbed | 3 | -30.29439303093 | 115.18329103477 |
| 4 | Disturbed | 4 | -30.29264800251 | 115.18417197280 |
| 4 | Disturbed | 5 | -30.29339700937 | 115.18712801859 |
| 4 | Natural | 1 | -30.29911003076 | 115.18198697828 |
| 4 | Natural | 2 | -30.29889796861 | 115.18159403466 |
| 4 | Natural | 3 | -30.29878104106 | 115.18137996085 |
| 4 | Natural | 4 | -30.29852204025 | 115.18091300502 |
| 4 | Natural | 5 | -30.29812700115 | 115.18119698390 |
| 5 | Disturbed | 1 | -30.38535998203 | 115.43140199035 |
| 5 | Disturbed | 2 | -30.38488598540 | 115.43183298782 |
| 5 | Disturbed | 3 | -30.38422498852 | 115.43274200521 |
| 5 | Disturbed | 4 | -30.38392701186 | 115.43323201127 |
| 5 | Disturbed | 5 | -30.38340096362 | 115.43385202065 |
| 5 | Natural | 1 | -30.38696100935 | 115.43451402336 |

| Site | Site Type | Sample Point | Latitude | Longitude |
|---|---|---|---|---|
| 5 | Natural | 2 | -30.38719503209 | 115.43438200839 |
| 5 | Natural | 3 | -30.38749703206 | 115.43443699367 |
| 5 | Natural | 4 | -30.38752896711 | 115.43492096476 |
| 5 | Natural | 5 | -30.38741296157 | 115.43540099636 |
| 6 | Disturbed | 1 | -29.98215498403 | 115.30140101910 |
| 6 | Disturbed | 2 | -29.98298697174 | 115.30243501067 |
| 6 | Disturbed | 3 | -29.98364998028 | 115.30316599645 |
| 6 | Disturbed | 4 | -29.98280902393 | 115.30181198381 |
| 6 | Disturbed | 5 | -29.98336801305 | 115.30237097293 |
| 6 | Natural | 1 | -29.98098302633 | 115.30338996090 |
| 6 | Natural | 2 | -29.98111898080 | 115.30374602415 |
| 6 | Natural | 3 | -29.98106600717 | 115.30418599024 |
| 6 | Natural | 4 | -29.98110003769 | 115.30469502322 |
| 6 | Natural | 5 | -29.98135702685 | 115.30498503707 |
| 7 | Disturbed | 1 | -29.74887396209 | 115.45360799879 |
| 7 | Disturbed | 2 | -29.74823702127 | 115.45469303615 |
| 7 | Disturbed | 3 | -29.74783997051 | 115.45553298667 |
| 7 | Disturbed | 4 | -29.74720998667 | 115.45658801682 |
| 7 | Disturbed | 5 | -29.74657597952 | 115.45776802115 |
| 7 | Natural | 1 | -29.74461100996 | 115.45483603142 |
| 7 | Natural | 2 | -29.74464504048 | 115.45519402251 |
| 7 | Natural | 3 | -29.74418696947 | 115.45542402193 |
| 7 | Natural | 4 | -29.74432501942 | 115.45503904112 |
| 7 | Natural | 5 | -29.74415402859 | 115.45463302173 |
| 8 | Disturbed | 1 | -29.99480101280 | 115.33394300379 |
| 8 | Disturbed | 2 | -29.99569896609 | 115.33409798518 |
| 8 | Disturbed | 3 | -29.99666900374 | 115.33418599516 |
| 8 | Disturbed | 4 | -29.99788203277 | 115.33421097323 |
| 8 | Disturbed | 5 | -29.99924501404 | 115.33397996798 |
| 8 | Natural | 1 | -29.99505096115 | 115.33088696189 |
| 8 | Natural | 2 | -29.99510502443 | 115.33018103801 |
| 8 | Natural | 3 | -29.99535002746 | 115.32985003665 |
| 8 | Natural | 4 | -29.99579804018 | 115.33020802774 |
| 8 | Natural | 5 | -29.99581899494 | 115.33064397052 |
| 9 | Disturbed | 1 | -30.23114402778 | 115.34134103917 |
| 9 | Disturbed | 2 | -30.23159296252 | 115.34125998616 |
| 9 | Disturbed | 3 | -30.23225303739 | 115.34128697589 |
| 9 | Disturbed | 4 | -30.23292602040 | 115.34123903140 |
| 9 | Disturbed | 5 | -30.23361601867 | 115.34121698700 |
| 9 | Natural | 1 | -30.23156303912 | 115.34320601262 |
| 9 | Natural | 2 | -30.23144896142 | 115.34357003868 |
| 9 | Natural | 3 | -30.23101695813 | 115.34340801649 |
| 9 | Natural | 4 | -30.23046400398 | 115.34362100065 |
| 9 | Natural | 5 | -30.23009997793 | 115.34373499453 |
| 10 | Disturbed | 1 | -30.39389301091 | 115.49326797947 |
| 10 | Disturbed | 2 | -30.39392603561 | 115.49293002114 |
| 10 | Disturbed | 3 | -30.39393802173 | 115.49248200841 |
| 10 | Disturbed | 4 | -30.39386400953 | 115.49209602177 |
| 10 | Disturbed | 5 | -30.39378798567 | 115.49171003513 |

| Site | Site Type | Sample Point | Latitude | Longitude |
|---|---|---|---|---|
| 10 | Natural | 1 | -30.39509397000 | 115.49206601456 |
| 10 | Natural | 2 | -30.39522296749 | 115.49179301597 |
| 10 | Natural | 3 | -30.39545497857 | 115.49167097546 |
| 10 | Natural | 4 | -30.39526202716 | 115.49129596911 |
| 10 | Natural | 5 | -30.39495198056 | 115.49119899049 |
| 11 | Disturbed | 1 | -30.52788401954 | 115.20021200180 |
| 11 | Disturbed | 2 | -30.52795501426 | 115.19902001135 |
| 11 | Disturbed | 3 | -30.52782400511 | 115.19807897508 |
| 11 | Disturbed | 4 | -30.52778201178 | 115.19569298252 |
| 11 | Disturbed | 5 | -30.52768402733 | 115.19461096264 |
| 11 | Natural | 1 | -30.53035802208 | 115.19426001236 |
| 11 | Natural | 2 | -30.53059196100 | 115.19398097880 |
| 11 | Natural | 3 | -30.53111197427 | 115.19381199963 |
| 11 | Natural | 4 | -30.53123703226 | 115.19405901432 |
| 11 | Natural | 5 | -30.53139201365 | 115.19419102930 |
| 12 | Disturbed | 1 | -30.56748197414 | 115.10963698849 |
| 12 | Disturbed | 2 | -30.56745003909 | 115.10916399769 |
| 12 | Disturbed | 3 | -30.56739002466 | 115.10866301134 |
| 12 | Disturbed | 4 | -30.56749303825 | 115.11040703394 |
| 12 | Disturbed | 5 | -30.56745096110 | 115.11138402857 |
| 12 | Natural | 1 | -30.56970099919 | 115.11000503786 |
| 12 | Natural | 2 | -30.56994600222 | 115.11035900563 |
| 12 | Natural | 3 | -30.57024800219 | 115.11080701835 |
| 12 | Natural | 4 | -30.57057598606 | 115.11066704057 |
| 12 | Natural | 5 | -30.57086801156 | 115.11015599594 |
| 13 | Disturbed | 1 | -29.87275497057 | 115.09093998000 |
| 13 | Disturbed | 2 | -29.87307398580 | 115.09133602493 |
| 13 | Disturbed | 3 | -29.87330599688 | 115.09159795940 |
| 13 | Disturbed | 4 | -29.87229103222 | 115.09040999226 |
| 13 | Disturbed | 5 | -29.87203798257 | 115.08998502977 |
| 13 | Natural | 1 | -29.87588795833 | 115.09074803442 |
| 13 | Natural | 2 | -29.87586499192 | 115.09030496702 |
| 13 | Natural | 3 | -29.87570003606 | 115.08999802172 |
| 13 | Natural | 4 | -29.87574898638 | 115.08969501592 |
| 13 | Natural | 5 | -29.87569701858 | 115.08922797628 |
| 14 | Disturbed | 1 | -29.88749898970 | 115.06052398123 |
| 14 | Disturbed | 2 | -29.88607599400 | 115.06005903706 |
| 14 | Disturbed | 3 | -29.88427698612 | 115.05971596576 |
| 14 | Disturbed | 4 | -29.88209903240 | 115.05792399868 |
| 14 | Disturbed | 5 | -29.88007798791 | 115.05905597471 |
| 14 | Natural | 1 | -29.88732598722 | 115.05798803642 |
| 14 | Natural | 2 | -29.88710302860 | 115.05758897401 |
| 14 | Natural | 3 | -29.88711300306 | 115.05704498850 |
| 14 | Natural | 4 | -29.88754500635 | 115.05686796270 |
| 14 | Natural | 5 | -29.88792696968 | 115.05681498908 |
| 15 | Disturbed | 1 | -30.39920495823 | 115.15900103375 |
| 15 | Disturbed | 2 | -30.39902097546 | 115.15922801569 |
| 15 | Disturbed | 3 | -30.39995899424 | 115.16003896482 |
| 15 | Disturbed | 4 | -30.40050398558 | 115.16036401503 |

| Site | Site Type | Sample Point | Latitude | Longitude |
|---|---|---|---|---|
| 15 | Disturbed | 5 | -30.40105501190 | 115.15952196904 |
| 15 | Natural | 1 | -30.39924301207 | 115.15529899858 |
| 15 | Natural | 2 | -30.39929497987 | 115.15503304079 |
| 15 | Natural | 3 | -30.39939698763 | 115.15445502475 |
| 15 | Natural | 4 | -30.39957501926 | 115.15425201505 |
| 15 | Natural | 5 | -30.39986796677 | 115.15417900868 |
| 16 | Disturbed | 1 | -30.26195900515 | 115.10116397403 |
| 16 | Disturbed | 2 | -30.26339197531 | 115.10138802230 |
| 16 | Disturbed | 3 | -30.26514303870 | 115.10154501535 |
| 16 | Disturbed | 4 | -30.26619002223 | 115.10191901587 |
| 16 | Disturbed | 5 | -30.26709903963 | 115.10220601223 |
| 16 | Natural | 1 | -30.26178700849 | 115.09957300499 |
| 16 | Natural | 2 | -30.26162800379 | 115.09951299056 |
| 16 | Natural | 3 | -30.26145801879 | 115.09919196367 |
| 16 | Natural | 4 | -30.26146598160 | 115.09892801754 |
| 16 | Natural | 5 | -30.26170897298 | 115.09899197146 |
| 17 | Disturbed | 1 | -30.18761797808 | 115.11033000425 |
| 17 | Disturbed | 2 | -30.18678599037 | 115.11036797427 |
| 17 | Disturbed | 3 | -30.18537397496 | 115.11039001867 |
| 17 | Disturbed | 4 | -30.18477818929 | 115.11041608639 |
| 17 | Disturbed | 5 | -30.18374511972 | 115.11041516438 |
| 17 | Natural | 1 | -30.18699101172 | 115.10622303933 |
| 17 | Natural | 2 | -30.18724800088 | 115.10600795969 |
| 17 | Natural | 3 | -30.18734598532 | 115.10620501824 |
| 17 | Natural | 4 | -30.18771101721 | 115.10635497048 |
| 17 | Natural | 5 | -30.18795995973 | 115.10655404069 |
| 18 | Disturbed | 1 | -30.55951497518 | 115.36832800135 |
| 18 | Disturbed | 2 | -30.55797496811 | 115.36838801578 |
| 18 | Disturbed | 3 | -30.55685103871 | 115.36850796081 |
| 18 | Disturbed | 4 | -30.55662003346 | 115.36734396592 |
| 18 | Disturbed | 5 | -30.55666202679 | 115.36631902680 |
| 18 | Natural | 1 | -30.55904701352 | 115.36648096517 |
| 18 | Natural | 2 | -30.55905899964 | 115.36689603701 |
| 18 | Natural | 3 | -30.55914198048 | 115.36711899564 |
| 18 | Natural | 4 | -30.55942503735 | 115.36711002700 |
| 18 | Natural | 5 | -30.55929302238 | 115.36688203923 |
| 19 | Disturbed | 1 | -30.70360399783 | 115.16594300978 |
| 19 | Disturbed | 2 | -30.70314701647 | 115.16562600620 |
| 19 | Disturbed | 3 | -30.70270302705 | 115.16541696154 |
| 19 | Disturbed | 4 | -30.70424043573 | 115.16619815491 |
| 19 | Disturbed | 5 | -30.70463296026 | 115.16634500585 |
| 19 | Natural | 1 | -30.70266899653 | 115.16904498450 |
| 19 | Natural | 2 | -30.70240898989 | 115.16891003586 |
| 19 | Natural | 3 | -30.70184396580 | 115.16879403032 |
| 19 | Natural | 4 | -30.70175201632 | 115.16940297559 |
| 19 | Natural | 5 | -30.70218997076 | 115.16962199472 |
| 20 | Disturbed | 1 | -30.96652401611 | 115.61673098244 |
| 20 | Disturbed | 2 | -30.96703598276 | 115.61674900353 |
| 20 | Disturbed | 3 | -30.96808296628 | 115.61678403988 |

| Site | Site Type | Sample Point | Latitude | Longitude |
|---|---|---|---|---|
| 20 | Disturbed | 4 | -30.96907697618 | 115.61678596772 |
| 20 | Disturbed | 5 | -30.97036301158 | 115.61680298299 |
| 20 | Natural | 1 | -30.96664697863 | 115.61337101273 |
| 20 | Natural | 2 | -30.96701997332 | 115.61331300996 |
| 20 | Natural | 3 | -30.96758801490 | 115.61341501772 |
| 20 | Natural | 4 | -30.96788096242 | 115.61388096772 |
| 20 | Natural | 5 | -30.96825697459 | 115.61399001628 |

**Table S 3.2:** Site by species matrix with the number of reads for *Phytophthora* species.

| Site | *Phytophthora arenaria* | *Phytophthora cinnamomi* | *Phytophthora* aff. inundata | *Phytophthora elongata* | *Phytophthora citricola* complex | *Phytophthora* sp. nov. 10 | *Phytophthora versiformis* complex |
|---|---|---|---|---|---|---|---|
| 1-1-D | 0 | 0 | 0 | 0 | 0 | 9379 | 0 |
| 1-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-1-N | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| 1-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-1-D | 0 | 0 | 0 | 6 | 0 | 0 | 4 |
| 2-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-5-D | 0 | 0 | 2 | 2 | 0 | 0 | 3 |
| 2-1-N | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| 2-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 1312 |
| 3-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 375 |
| 3-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-1-N | 0 | 827 | 0 | 0 | 0 | 0 | 0 |
| 4-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Site | *Phytophthora arenaria* | *Phytophthora cinnamomi* | *Phytophthora* aff. inundata | *Phytophthora elongata* | *Phytophthora citricola* complex | *Phytophthora* sp. nov. 10 | *Phytophthora versiformis* complex |
|---|---|---|---|---|---|---|---|
| 5-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 8622 |
| 5-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 6-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-3-D | 0 | 0 | 0 | 2 | 2468 | 0 | 1612 |
| 7-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 1915 |
| 7-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 10321 |
| 7-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 8-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 9-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Site | *Phytophthora arenaria* | *Phytophthora cinnamomi* | *Phytophthora* aff. inundata | *Phytophthora elongata* | *Phytophthora citricola* complex | *Phytophthora* sp. nov. 10 | *Phytophthora versiformis* complex |
|---|---|---|---|---|---|---|---|
| 9-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9-3-N | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| 9-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 2211 |
| 9-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 10-1-D | 0 | 0 | 0 | 2236 | 0 | 0 | 0 |
| 10-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10-4-D | 0 | 0 | 0 | 1547 | 0 | 0 | 0 |
| 10-5-D | 0 | 0 | 0 | 1685 | 0 | 0 | 0 |
| 10-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 10-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-4-D | 0 | 2839 | 0 | 0 | 0 | 0 | 0 |
| 11-5-D | 0 | 532 | 0 | 0 | 0 | 0 | 0 |
| 11-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-3-N | 0 | 1047 | 0 | 0 | 0 | 0 | 0 |
| 11-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-1-D | 0 | 0 | 0 | 4195 | 0 | 0 | 4 |
| 12-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| 12-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| 12-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 12-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Site | *Phytophthora arenaria* | *Phytophthora cinnamomi* | *Phytophthora* aff. inundata | *Phytophthora elongata* | *Phytophthora citricola* complex | *Phytophthora* sp. nov. 10 | *Phytophthora versiformis* complex |
|---|---|---|---|---|---|---|---|
| 13-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 13-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-3-D | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-4-D | 3276 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 15-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 15-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 3459 |
| 17-2-D | 0 | 0 | 0 | 0 | 0 | 2556 | 0 |
| 17-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Site | *Phytophthora arenaria* | *Phytophthora cinnamomi* | *Phytophthora* aff. inundata | *Phytophthora elongata* | *Phytophthora citricola* complex | *Phytophthora* sp. nov. 10 | *Phytophthora versiformis* complex |
|---|---|---|---|---|---|---|---|
| 17-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-1-N | 0 | 0 | 8449 | 0 | 0 | 0 | 0 |
| 19-2-N | 0 | 0 | 0 | 8935 | 0 | 0 | 7 |
| 19-3-N | 0 | 0 | 0 | 2 | 0 | 0 | 5 |
| 19-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20-1-D | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 20-2-D | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| 20-3-D | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20-4-D | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 20-5-D | 0 | 0 | 0 | 0 | 0 | 0 | 11670 |
| 20-1-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20-2-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20-3-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20-4-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20-5-N | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Sampling review

The study area was located north of Perth, Western Australia, in kwongan plant communities. The north and south edges of study area were between -29.15°S in the north -31.00°S. The eastern boundary of the survey region ran roughly parallel to the coast, the western boundary, between -29.15°S,115.85°E and -31.00°S, 116.37°E.

The diagnostic data reviewed was submitted to a publicly available online resource, Dieback Information Delivery and Management System (DIDMS) and was collected between 1990 and 2017 (https://didms.gaiaresources.com.au/). *Phytophthora* spp. positive sample location data (*n* = 171) was downloaded directly from DIDMS; however, negative sample locations (*n* = 1264) were provided by Project Dieback as they were not available to the public to download. The positive sample data was primarily the result of traditional baiting techniques (Tsao 1983, O'Brien et al. 2009), generated by the Vegetation Health Service (VHS) and The Centre for *Phytophthora* Science and Management (CPSM) *Phytophthora* diagnostic laboratories in Western Australia. Duplicate entries were removed from the dataset. Only three species, *P. arenaria*, *P. cinnamomi* and *P. multivora* were used in the review as they were most commonly isolated from the study area, representing 94% of positive samples and had a sufficient sample size for statistical analysis (*n* > 20).

The diagnostic point data was placed on top of an aerial image layer, imported using the OpenLayers QGIS plugin (QGIS Development Team 2018). All points were viewed and the surrounding disease pathways (roads, fire breaks, fence lines, boundaries and vehicle tracks) were digitized into a line vector layer using the Bing 2018 Aerial imagery. The nearest vector pathway was then created using the "v.distance" module in the Geographic Resource Analysis Support System 7 (GRASS) QGIS plugin and the length calculated with the field calculator (QGIS Development Team 2018).

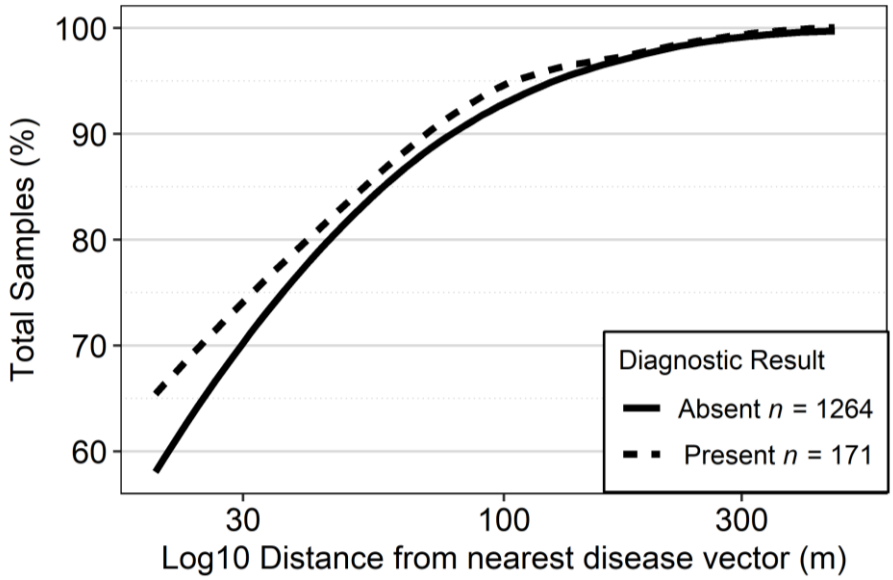


**Figure S3. 1:** The results and percentage of the *Phytophthora* spp. samples collected relative to the nearest disease vector between 1990 and 2017 in the kwongan survey region north of Perth, Western Australia.

**Table S3.3:** The phylotypes and corresponding oomycete species detected in kwongan and Banksia woodland plant communities in Southwest Australia. The count and percentage of detections at sites and sample points for individual *Peronospora* and *Pythium* species are displayed. The number of detections at disturbed and natural subsite sample points is included. The total number of detections at sites and sample points is summarised below.

| | Sites | | Sample points | | | |
|---|---|---|---|---|---|---|
| Oomycete | *n* | % | *n* | % | Disturbed | Natural |
| *Peronospora manshurica* | 2 | 10 | 6 | 3 | 1 | 5 |
| *Peronospora schachtii* | 1 | 5 | 1 | 0.5 | 0 | 1 |
| *Pythium perplexum* | 1 | 5 | 1 | 0.5 | 0 | 1 |
| *Pythium rostratifingens* | 8 | 40 | 13 | 6.5 | 6 | 7 |
| *Pythium rostratum* | 4 | 20 | 4 | 2 | 4 | 0 |
| *Pythium* sp. 1 | 6 | 30 | 10 | 5 | 7 | 3 |
| *Pythium* sp. 2 | 1 | 5 | 1 | 0.5 | 1 | 0 |
| *Pythium* sp. 3 | 3 | 15 | 5 | 2.5 | 3 | 2 |
| *Pythium* sp. 4 | 1 | 5 | 1 | 0.5 | 1 | 0 |
| *Pythium* sp. 5 | 2 | 10 | 5 | 2.5 | 1 | 4 |
| Total | 14 | 70 | 34 | 17 | 24 | 23 |

Survey Comparison

A wide survey of Australian natural vegetation using HTS metabarcoding was performed to fill the gaps in the distribution of *P. cinnamomi* (Burgess et al. 2017a). The results of the HTS

metabarcoding of all *Phytophthora* species were published in Burgess et al. (2017b) and the data made public in the following publication (Burgess et al. 2018b). The results of the survey were extracted from the dataset for 23 sampling sites located within the same survey region as Figure 1. Sampling sites were located in areas where *Phytophthora* species were most likely to be found, such as water gaining sites. Sampling methodology, bulked vs subsamples; sample material, soil vs roots; material processing, grinding vs cutting; and HTS platform, 454 pyrosequencing vs Illumina differed between Burgess et al. (2017b) and the current study.

Estimates if extrapolated species richness were projected across all sites and natural and disturbed subsite categorizations using the specpool function in the vegan R package (Oksanen et al. 2018). The Jackknife 1 index was reported as the Jackknife 2 index did not handle the low observations of species richness. Mean observed alpha ($\bar{\alpha}$) was calculated per site, for all sites and natural and disturbed subcategorizations. Rarefied species richness was not calculated and reported to compare the studies as sites were not subsampled by Burgess et al. (2017b).

**Table S3.4:** The summarised findings of HTS metabarcoding *Phytophthora* surveys in kwongan and Banksia woodland plant communities within the current study region. The total number of samples (N), observed richness (γ), Jackknife 1 species richness estimator (Jack), and mean observed alpha diversity per site ($\bar{\alpha}$) are displayed for all sites and subsite categorisations.

| | | | | | | | Subsite / disturbance categorisation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Disturbed | | | | Natural | | | |
| Survey | Sample | Sites | N | γ | Jack | $\bar{\alpha}$ | N | γ | Jack | $\bar{\alpha}$ | N | γ | Jack | $\bar{\alpha}$ |
| Current survey[1] | Roots | 20 | 200 | 7 | 7.00 | 1.45 | 100 | 7 | 8.98 | 0.95 | 100 | 5 | 6.98 | 0.8 |
| Burgess et al. (2017b) | Soil | 23 | 23 | 27 | 31.78 | 7.61 | 14 | 24 | 30.50 | 6.64 | 9 | 21 | 24.55 | 9.11 |

[1] The data was combined by site to make it more comparable to the Burgess et al. (2017b) survey. Mean alpha diversity was calculated based on the presence of *Phytophthora* species at a site, as opposed to within each sample.

**Table S5.1:** The results of generalised linear mixed effects models testing the influence of soil origin, trial, and the presence and number of reads of oomycete species detected from the roots of mature plants on the emergence and survival of seedlings. The Wald chi-squared, degrees of freedom and *P*-values calculated by a type-III analysis of variance are displayed for each plant species and measurement. Bold terms were statistically significant ($P \leq 0.05$).

| | Emergence | | | Survival | | |
|---|---|---|---|---|---|---|
| Term | $\chi^2$ | Df | $Pr(>\chi^2)$ | $\chi^2$ | Df | $Pr(>\chi^2)$ |
| *Banksia attenuata* | | | | | | |
| Soil origin | 8.69 | 4 | 0.0692 | 6.55 | 4 | 0.1618 |
| Trial | 0.06 | 1 | 0.8060 | 0.05 | 1 | 0.8160 |
| Oomycete reads | 0.60 | 1 | 0.4381 | 0.60 | 1 | 0.4371 |
| Oomycete presence | 0.49 | 1 | 0.4860 | 0.01 | 1 | 0.9199 |
| Soil origin: Trial | 8.60 | 4 | 0.0719 | **12.76** | **4** | **0.0125** |
| Trial: Oomycete presence | 0.08 | 1 | 0.7737 | 0.37 | 1 | 0.5404 |
| *Eucalyptus todtiana* | | | | | | |
| Soil origin | 5.20 | 4 | 0.2675 | 6.55 | 4 | 0.1616 |
| Oomycete reads | 0.57 | 1 | 0.4490 | 1.58 | 1 | 0.2085 |
| Oomycete presence | 3.37 | 1 | 0.0665 | 2.89 | 1 | 0.0892 |
| *Hakea lissocarpha* | | | | | | |
| Soil origin | 5.42 | 4 | 0.2472 | 7.26 | 4 | 0.1229 |
| Trial | 0.41 | 1 | 0.5218 | 0.00 | 1 | 0.9669 |
| Oomycete reads | 0.08 | 1 | 0.7822 | 1.12 | 1 | 0.2903 |
| Oomycete presence | 0.03 | 1 | 0.8530 | 0.08 | 1 | 0.7768 |
| Soil origin: Trial | 5.03 | 4 | 0.2845 | 6.01 | 4 | 0.1984 |
| Trial: Oomycete presence | 0.22 | 1 | 0.6389 | 1.06 | 1 | 0.3033 |
| *Jacksonia floribunda* | | | | | | |
| Soil origin | **10.97** | **4** | **0.0269** | **17.76** | **4** | **0.0014** |
| Trial | **21.00** | **1** | **0.0000** | **9.16** | **1** | **0.0025** |
| Oomycete presence | 1.75 | 1 | 0.1863 | 3.25 | 1 | 0.0714 |
| Soil origin: Trial | **9.78** | **4** | **0.0444** | 8.71 | 4 | 0.0687 |
| Trial: Oomycete presence | 0.13 | 1 | 0.7203 | 0.44 | 1 | 0.5065 |
| *Xanthorrhoea* sp. Lesueur | | | | | | |
| Soil origin | **10.71** | **4** | **0.0300** | **10.86** | **4** | **0.0281** |
| Trial | 0.53 | 1 | 0.4678 | 0.52 | 1 | 0.4709 |
| Oomycete reads | 1.42 | 1 | 0.2336 | 2.20 | 1 | 0.1383 |
| Oomycete presence | 0.31 | 1 | 0.5775 | 1.27 | 1 | 0.2602 |
| Soil origin: Trial | 6.83 | 4 | 0.1449 | 4.64 | 4 | 0.3260 |
| Trial: Oomycete presence | 0.84 | 1 | 0.3601 | 0.29 | 1 | 0.5918 |

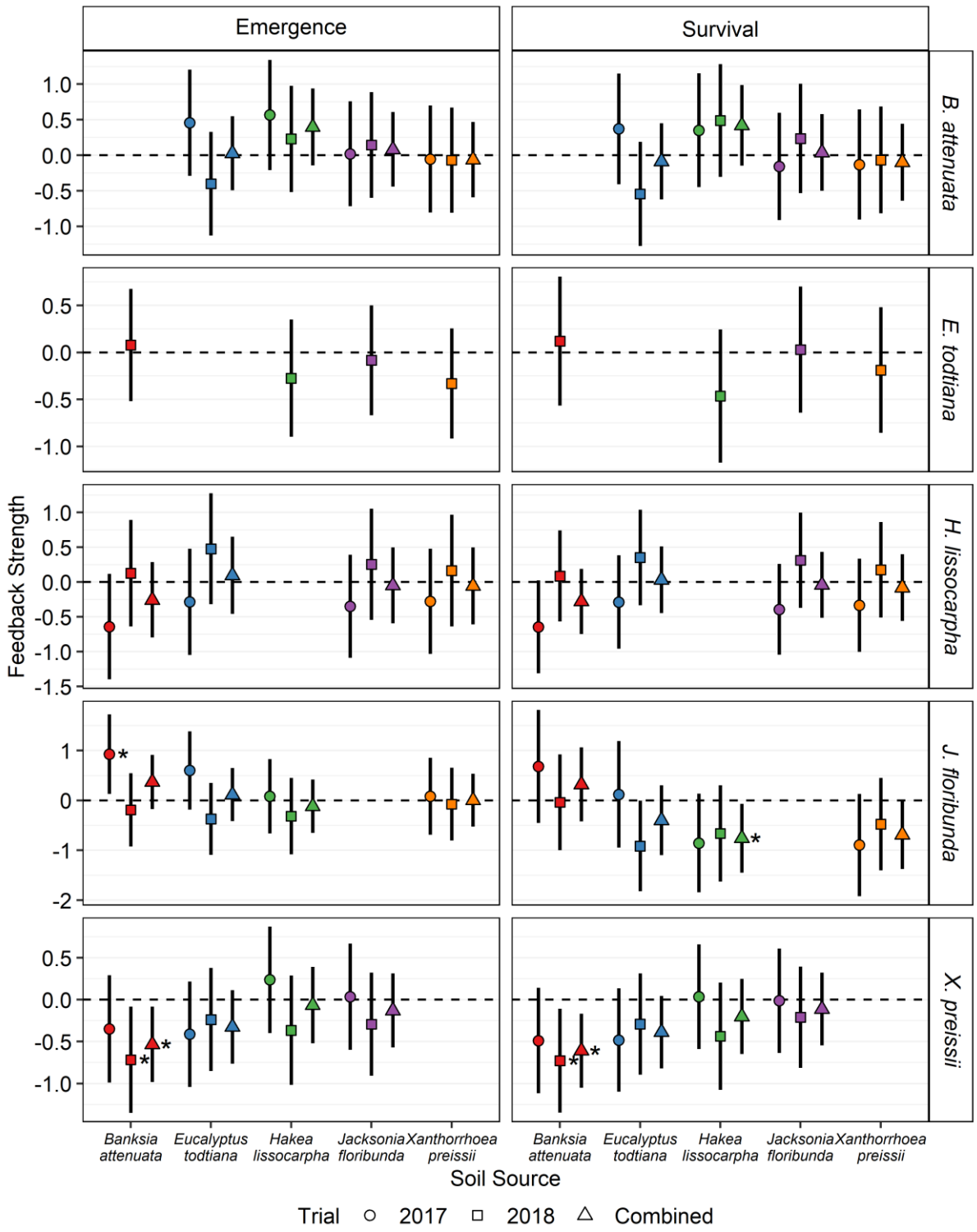


**Figure S5.1:** The seedling emergence and survival plant-soil feedback of five kwongan heath plant species. Each type of feedback was calculated as the logarithm of the odd ratio i.e. ln(conspecific probability/heterospecific probability) and the strength and direction represents the effect of conspecific soils on seedling emergence and survival relative to the heterospecific soil. Error bars representing 95% confidence intervals that do not intersect zero are marked with an asterisk and indicate statistically significant plant-soil feedback.

**Table S5.2:** The results of generalised linear mixed effects models testing the influence of the presence and number of reads of oomycete species detected from the roots of harvested seedlings on the emergence and survival of seedlings. The Wald chi-squared, degrees of freedom and *P*-values calculated by a type-III analysis of variance are displayed for each plant species and measurement. Bold terms were statistically significant ($P \leq 0.05$).

| | Emergence | | | Survival | | |
|---|---|---|---|---|---|---|
| Term | $\chi^2$ | Df | *P*r(>$\chi^2$) | $\chi^2$ | Df | *P*r(>$\chi^2$) |
| *Banksia attenuata* | | | | | | |
| Trial | 3.40 | 1 | 0.0651 | 3.77 | 1 | 0.0523 |
| Oomycete reads | 2.51 | 1 | 0.1134 | 2.01 | 1 | 0.1564 |
| Oomycete presence | 0.08 | 1 | 0.7835 | 0.14 | 1 | 0.7117 |
| Trial: Oomycete presence | 0.69 | 1 | 0.4056 | 1.35 | 1 | 0.2459 |
| *Eucalyptus todtiana* | | | | | | |
| Oomycete reads | 1.72 | 1 | 0.1901 | 0.94 | 1 | 0.3325 |
| Oomycete presence | **3.91** | **1** | **0.0480** | 2.10 | 1 | 0.1472 |
| *Hakea lissocarpha* | | | | | | |
| Trial | **5.60** | **1** | **0.0179** | 1.83 | 1 | 0.1762 |
| Oomycete reads | 1.69 | 1 | 0.1941 | 1.44 | 1 | 0.2305 |
| Oomycete presence | 1.24 | 1 | 0.2663 | 0.23 | 1 | 0.6336 |
| Trial: Oomycete presence | 0.52 | 1 | 0.4720 | 0.06 | 1 | 0.8095 |
| *Jacksonia floribunda* | | | | | | |
| Trial | **30.75** | **1** | **0.0000** | **17.40** | **1** | **0.0000** |
| Oomycete reads | 0.64 | 1 | 0.4246 | 0.60 | 1 | 0.4389 |
| Oomycete presence | 2.21 | 1 | 0.1374 | 0.82 | 1 | 0.3663 |
| Trial: Oomycete presence | 0.91 | 1 | 0.3412 | **4.87** | **1** | **0.0273** |
| *Xanthorrhoea* sp. Lesueur | | | | | | |
| Trial | 0.60 | 1 | 0.4385 | 0.04 | 1 | 0.8475 |
| Oomycete reads | 0.03 | 1 | 0.8555 | 0.06 | 1 | 0.8074 |
| Oomycete presence | 2.41 | 1 | 0.1208 | 2.07 | 1 | 0.1500 |
| Trial: Oomycete presence | **5.56** | **1** | **0.0184** | **4.30** | **1** | **0.0382** |

**Table S5.3:** The results of two generalised linear models testing the influence of sampling year, plant age and plant species on the number of oomycete phylotypes detected. The Likelihood ratio chi-squared, degrees of freedom and *P*-values calculated by a type-III analysis of variance are displayed for Model 1 and 2. Bold terms were statistically significant ($P \leq 0.05$).

| | Model 1 | | | Model 2 | | |
|---|---|---|---|---|---|---|
| Term | LR $\chi^2$ | Df | *P*r(>$\chi^2$) | LR $\chi^2$ | Df | *P*r(>$\chi^2$) |
| Year | **29.13** | **1** | **< 0.0001** | **16.66** | **1** | **< 0.0001** |
| Plant age | 1.37 | 1 | 0.2415 | 2.01 | 1 | 0.1566 |
| Year: Plant age | 3.42 | 1 | 0.0644 | 1.80 | 1 | 0.1791 |
| Plant species | | | | **22.12** | **4** | **0.0002** |
| Plant species: Year | | | | 2.90 | 3 | 0.4071 |
| Plant species: Plant age | | | | **19.55** | **4** | **0.0006** |
| Plant species: Year: Plant age | | | | 0.37 | 1 | 0.5412 |

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