School of Molecular and Life Sciences

Bauxite Mining Restoration with Natural Soils and Residue Sands: Comparison of the Recovery of Soil Ecosystem Function and Ground-dwelling Invertebrate Diversity

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Author's Declaration

To the best of my knowledge and belief, this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university.

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Statement of authors' contributions

Experimental set up, data collection, data analysis and data interpretation for Chapter 2, 3,4 and 5 was done by D. Mihindukulasooriya.

Experimental set up established by Lythe et al. (2017) used for experimental chapter 6. Data collection, data analysis and data interpretation for long term effect of woody debris addition was done by D. Mihindukulasooriya.

Abstract

Human destruction of the natural environment has been identified as a global problem that has triggered the loss of biodiversity. This degradation and loss has altered ecosystem processes and the resilience of ecosystems to environmental changes. Restoration of degraded habitats forms a significant component of conservation efforts. Open cut mining is one activity that can dramatically alter local communities, and successful vascular plant restoration does not necessarily result in restoration of other components of flora and fauna or result in a fully functioning ecosystem. Therefore, restoration studies should focus on improving ecological functions such as nutrient cycling and litter decomposition, seed dispersal and/ or pollination, and assess community composition beyond vegetation to attain fully functioning systems. Within such functioning systems, invertebrates are important ecosystem drivers involved in numerous ecological functions; hence, understanding how restoration activities impact their recovery is vital.

In this thesis, I investigated the re-establishment of soil ecosystem functions and ground-dwelling invertebrate diversity in a chronosequence of restored sites (20-, 10-, five-, and two-year-old sites, with three replicate plots each) within two distinctly different restoration projects associated with Alcoa's bauxite mining operation—one using largely intact topsoil, directly transferred to a restoration site (mining restoration system) and the other using novel soil created from bauxite residues (residue restoration system). Soil ecosystem function and ground invertebrates in each restoration area were compared to those found in neighbouring remnant vegetation. The research tested the species redundancy theory by relating ecosystem functions to macro-invertebrate species and functional group diversity. Temporal changes in physiochemical and microbiological properties of the upper soil profile were characterised across the chronosequence to assess how microbial activity affected the reconstruction of soil in the two restoration systems (Chapter 2). The degree of re-establishment of the ground-dwelling invertebrate community and the diversity of functional groups were investigated and compared between the two systems (Chapter 3). The differences between the restoration systems and restoration ages in the

degree of re-establishment of myrmecochory, seed dispersal rate, and seed dispersal distance were assessed by investigating the composition of the ant fauna (Chapter 4). The impact of ground-dwelling invertebrate communities on litter decomposition, and therefore nutrient cycling, was investigated using litter bags with and without ground-dwelling invertebrate access (Chapter 5). The impact of wood mulch application on accelerating the return of ground-dwelling invertebrates during restoration was assessed using experimental plots with 0 t/ha, 10 t/ha, and 300 t/ha snipped wood (Chapter 6).

Ground-dwelling invertebrate communities, functional group diversity, and ecosystem processes were observed to be gradually resembling those of the remnant vegetation as restoration age increased, with the recovery happening more rapidly in the mining restoration system than in the residue restoration system. Soil physiochemical and microbiological properties resembled those of the remnant systems closely in both restorations, but the rate of improvement was higher in mining restoration than in residue restoration. Soil invertebrate abundance and functional group diversity of both restoration systems increased with restoration age and increased more rapidly in mining restoration than in residue restoration. Invertebrate functional group diversity and ant abundance and functional group diversity were higher in the mining restoration system than in the residue restoration system. Seed dispersal rate at the mining restoration system resembled that of the remnant site as age since restoration increased. In contrast, no seed dispersal events were recorded at the residue restoration sites, although some dispersal events were observed in the adjacent remnant site. Ground-dwelling macro-invertebrates improved the rate of decomposition in both restoration systems; as restoration age increased the rate of decomposition returned to pre-disturbance levels. The decomposer invertebrate functional group diversity was higher but the abundance was lower in the mining system than in the residue system. The succession of the decomposer community (Collembola and Diplopoda) had a significant, positive effect on litter decomposition. Application of additional snipped wood accelerated the abundance or diversity of ground-dwelling invertebrates. Decomposer invertebrate community composition—Collembola, Diplopoda, mites, Dermaptera, and Blattodea—were

all higher in additional snipped wood treated sites than in the un-treated and remnant sites; the amount of snipped wood added (100 t/ha vs 300 t/ha) made little difference.

Although both restoration practices were improving habitat characteristics and were changing in the direction of the reference sites, change in soil ecosystem functions was more rapid in mining than in residue restoration and neither has yet fully reached pre-mined functionality. Encouraging the return of missing functional groups and diverse ground-dwelling invertebrate communities may help to accelerate succession and ecosystem functions such as decomposition and seed dispersal, and help to return the land to previous ecosystem values more quickly. The invertebrate functional group concept provides a framework for identifying the type of management requirements needed to achieve restoration goals. Further soil characterisation during restoration can also be used to compare the re-establishment of ecosystem function. Application of woody debris at the lowest rate (100 t/ha) and topsoil application are recommended to enhance the abundance and diversity of invertebrates in restored areas. This study highlights that efforts to help invertebrate recolonisation will speed-up the return of soil ecosystem function during restoration. The re-establishment of missing ecosystem processes will likely assist both mining and residue restoration systems in developing self-sustaining ecosystems.

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List of Abbreviations

ANOSIM	Analysis of Similarity
ANOVA	Analysis of Variance
AIC	Akaike Information Criterion
Alcoa	Alcoa of Australia Limited
ANOSIM	Analyses of similarities
AU$	Australian Dollar
C:N	Carbon to nitrogen ratio
$C_3Cl_2N_3NaO_3$	Sodium dichloroisocyanurate
$C_7H_6O_3$	Salicylic acid
CaCl2	Calcium chloride
CaSO4	Calcium sulphate
CCS	Cold climate specialists
CEC	Cation exchange capacity
CO_2	Carbon dioxide
$Cr_2O_2^{2-}$	Dichromate
Cr^{3+}	Chromium
CS	Cryptic species
D	Mean Dominance index
dbRDA	Distance-based Redundancy Analysis
DCR	Digital colour reader
DD	Dominant dolichoderinae
DistLM	Distance-based Linear Modelling
Dp	Particle density
e^H'/S	Shannon evenness
EC	Electrical conductivity
GM	Generalized myrmicinae
H'	Shannon diversity index
H_2O	Water
H_2SO_4	Sulphuric acid
HCS	Hot climate specialists
HDS	Tukey's honesty significance difference
M10	10 year old mining restoration
M2	2 year old mining restoration
M20	20 year old mining restoration

M5	5 year old mining restoration
MDS	Multidimensional scaling
MR	jarrah remnant
N	Total nitrogen
NaOH	Sodium hydroxide
NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
$(NH_4)_2HPO_4$;DAP	Di-ammonium phosphate
OPP	Opportunists
P	Extractable phosphorus
PAST	Paleontological statistics
PCO	Principle Coordinate Analysis
R10	10 year old residue restoration
R2	2 year old residue restoration
R5	5 year old residue restoration
RR	tuart remnants
SC	Subordinate camponotini
SD	Standard deviation
SE	Standard error
SMR	Soil microbial respiration
SOC	Soil organic carbon
SP	Specialist predators
TCS	Tropical climate specialists
HSD posthoc test	Honestly significant difference post hoc test
WA	Western Australia

Chapter 1   Introduction

1.1   Mining and restoration

Most ecosystems on earth are influenced by anthropogenic pressures, due in part to the increasing demand for natural resources as the human population increases (Kremen, 1992; Rosenfeld, 2002; Tok, 2015; Brejcha et al., 2016; Rawashdeh et al., 2016; Spiegel, 2016; Tripathi et al., 2016; Fernandes et al., 2018). Natural ecosystems are steadily being degraded or replaced by human-managed land-uses (Tscharntke et al., 2012), with land clearing as the foremost cause of biodiversity loss worldwide (Majer, 1989a; Walker, 1992; Dobson et al., 1997; Nichols and Nichols, 2003; Tscharntke et al., 2012). Resource extraction is one anthropogenic activity that can result in severe environmental impacts (Society for Ecological Restoration, 2004; Meyer Iii et al., 2011; Cristescu et al., 2012). Mining based environmental impacts include habitat and biodiversity loss, soil erosion, surface and ground water contamination, soil contamination, and sinkhole formation (Dontala et al., 2015; Tripathi et al., 2016), although impacts can vary with the nature of the ore deposit, method of extraction, toxicity of residues produced, and local environmental vulnerability (Castilla-Gómez and Herrera-Herbert, 2015; Lamb et al., 2015; Kim et al., 2018). Mining exposes bedrock, creates novel residue substrates and landforms, and results in areas of land compacted and devoid of vegetation that, if left unattended at the end of the mine's lifetime, increase the likelihood of environmental impacts (Aronson et al., 1993; Chauhan and Ganguly, 2011; Alcoa, 2013). The restoration or rehabilitation of this modified and degraded land is essential to remediate or minimize ongoing impacts and prepare the disturbed land for post-mining land use.

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (Society for Ecological Restoration, 2004). It comprises the re-establishment of composition, structure, and functioning of the degraded ecosystem as much as possible so as to resemble the original ecosystem prior to degradation (Bowman and Facelli, 2013; Lamb et al., 2015; Alexander et al., 2016). Ecosystem restoration is a multidimensional activity as it involves the physical,

biological, and chemical elements of the environment (Society for Ecological Restoration, 2004).

When ecosystem degradation has passed the line of "no return", rehabilitation may be the only solution, as much of the soil biota and seeds have been lost or removed (Alexander et al., 2016). The structure, composition, and process of select species in the restored degraded ecosystem may not fully complete (Stanturf et al., 2014), such that the resulting ecosystem may not include all the original ecosystem functions and services due to economic and ecological constraints (Lamb and Gilmour, 2003; Kollmann et al., 2016). However, some of these original functions may re-establish over time (Aronson et al., 1993; Lamb and Gilmour, 2003; Society for Ecological Restoration, 2004).

The ultimate goal of both restoration and rehabilitation is to establish a self-sustaining, functioning ecosystem that does not require further human support (Society for Ecological Restoration, 2004; Ruiz-Jaen and Aide, 2005). For restoration, this would mean that the restored site would resemble an "indigenous, historic system" (Society for Ecological Restoration, 2004). Therefore, in addition to species composition, ecosystem functions such as litter decomposition and nutrient cycling need to be considered as vital components in restoring a system; additionally, evidence of the resilience of the restored ecosystem to future disturbances is needed (Ruiz-Jaen and Aide, 2005; Courtney et al., 2009; Kollmann et al., 2016). However, ecosystems are complex, and restored ecosystems will never be identical to reference ecosystems (Society for Ecological Restoration, 2004); nevertheless, reference ecosystems provide a model tool to monitor and evaluate the success of the restoration effort.

1.2 Field of dreams and the role of invertebrates in ecosystem function

Mining restoration typically focuses on the re-establishment of physical structure (Hilderbrand et al., 2005) and the reinstatement of flora (Babin-Fenske and Anand, 2010), and often assumes that the presence of diverse plant life will encourage the recovery of fauna (Palmer et al., 1997). However, recovery of physical and vegetative structures may not always lead to a similar fauna structure as the measures of climatic conditions, topography, and herbivory differ (Palmer et al., 1997; Hilderbrand et al., 2005). Therefore, re-establishment of pre-disturbed physical structure and flora

structure may not be the best solution. Rather than focusing on restoring to the original state, it is more practical to achieve maximum ecosystem functions (Hilderbrand et al., 2005; Stanturf et al., 2014). Due to shared ecological function of many species, functional replacement might be more easily achieved than taxonomic composition replacement (Stanturf et al., 2001).

Invertebrates are a diverse and abundant component of the animal community; they perform a number of important roles, and their presence has been demonstrated to enhance ecosystem health (Babin-Fenske and Anand, 2010; Barton and Moir, 2015; Casimiro et al., 2019). Ground-dwelling invertebrates are considered ecosystem engineers as they can mediate several key ecosystem processes, including organic matter decomposition; for example, collembola and mites increase surface area for microbes by litter fragmentation (Blakely et al., 2002). Invertebrates also facilitate water infiltration and soil aeration by creating nests and "highways", aid pedogenesis by grazing and mixing the soil, promote plant productivity and community structure by herbivory, and facilitate soil greenhouse gas emission by producing biogenic structures (Lal, 1988a; Bruyn, 1999). Ants can mediate soil chemical property changes mainly by neutralizing soil pH and increasing soil nutrient content, especially nitrogen and phosphorus (Frouz and Jilková, 2008). Predatory invertebrates—for example, collembola, termites, and predatory ants—serve an important role in controlling pest outbreaks in newly revegetated areas (Nichols and Burrows, 1985). Termites are involved in litter decomposition, soil turnover, and the creation of deep burrows and galleries, which aerate the soil (Jouquet et al., 2006b). Understanding the link between the recovery of invertebrate communities and the recovery of ecosystem processes may enhance the success of restoration projects.

The pioneering work of Jonathon Majer (1980) led to the consideration of the importance of ant-seed dispersal (i.e. myrmecochory) during ecosystem restoration. Ants species not only disperse elaiosome-bearing seeds from the parent plant, but also remove the elaiosome from the seed in-situ (Andersen and Morrison, 1998), or transport seeds to their nest to feed on them (Harris and Standish, 2008). Seed predation by ants (Andersen and Morrison, 1998; Harris and Standish, 2008a), Coleopterans, Orthopterans (Honek et al., 2003; O'Rourke et al., 2006; Ichihara et al., 2011), and other invertebrates (Koprdová et al., 2010) is one of the main mortality

factors of seeds, resulting in the reduced availability of viable seeds for germination (O'Rourke et al., 2006; Koprdová et al., 2010; Ichihara et al., 2011). Seed predation influences the quantity of seeds entering the soil seed bank (Honek et al., 2003).

There is ample evidence to demonstrate that recolonizing animals play a significant role in the functioning of ecosystems (Frouz et al., 2006; Barton and Moir, 2015), for example, when they act as "ecosystem engineers" by changing the physical condition of the soil via digging and foraging activities (Hole, 1981; Folgarait, 1998; Andersen and Majer, 2004; Jouquet et al., 2006a). However, an area less studied is the reestablishment of invertebrate assemblages during restoration and the importance of species redundancy within the invertebrate community to promote ecosystem function during a restoration chronosequence (Cristescu et al., 2012).

1.3 Species redundancy and ecosystem function

The relationship between species loss and ecosystem function is explained by the concept of species redundancy (Rosenfeld, 2002). Some species perform similar functions and may therefore be substituted with little impact on ecosystem processes (Lawton and Brown, 1993; Rosenfeld, 2002; Doherty et al., 2011). Approximately two decades ago, Walker (1992) applied the species redundancy concept to conservation ecology—if there are several species performing similar functions, the absence or loss of some species might not affect ecological functions to the same extent as that of other species. Although the species redundancy concept is relatively new in conservation ecology, its origin dates back to the "ecological guild" concept where species were assigned to similar clusters based on their role in a particular community (Gitay et al., 1996; Hooper et al., 2005).

Over the past two decades, the species redundancy concept has been very popular among ecologists and environmental decision makers (Moir et al., 2010; Baiser and Lockwood, 2011; Alexander et al., 2016). In a restoration context, however, it is important to note that some ecological functions may not be performed in the absence of key species, when there is no functional redundancy among species (Walker, 1992).

The demand for the establishment of self-sustaining, resilient ecosystems is increasing in ecological restoration (Society for Ecological Restoration, 2004). Restoration brings

back ecosystems to their former or original condition, or to a state of health, soundness, and vigour (Environmental Protection Authority, 2006). The degree of functioning and the presence of redundancy, implying resilience, will depend on the collective roles of the biodiversity present (Bihn et al., 2010). Relative species abundance and diversity are not always good ecosystem interpreters as they can be impacted by ecosystem energy (or material) flow pathways (Hooper et al., 2005). Quantifying ecological processes, with a focus on measures of diversity and community structure, are rarely a component of ecosystem restoration projects as assessing ecosystem function is more demanding, and the process more time consuming (Ruiz-Jaen and Aide, 2005). Therefore, for most ecosystems, little is known about the relationship between species composition and functional diversity (Bihn et al., 2010), and the resultant functioning of the ecosystem.

The reconstruction of ecosystems in a restoration context offers the opportunity to consider the role of species and functional diversity in ecosystem function. Mining restoration provides an ideal environment for restoration research; generally, impacts are within a relatively small area, where a legislated requirement will be relatively well-documented and well-resourced and where there is progressive restoration within the same system. Some mining companies are also resourced with well-trained professional environmental staff (Bell and Hobbs, 2007). As a result, mining closures offer "mega-ecological laboratories" sourced with experts of various research aspects.

1.4 Mining operations in Australia

All states and territories of Australia conduct mining activities for a range of minerals and resources including Iron, Nickel, Aluminium, Copper, Gold, Silver, and Uranium (Hajkowicz et al., 2011). Mining (excluding petroleum products) is a significant contributor to Australia's economy, amounting to approximately 56 % of all export merchandise and contributing around 9 % to the gross domestic production of Australia (Geoscience Australia, 2016).

Mine site restoration needs to fulfil environmental values and public interest according to rules of each province, region, or country (Cristescu et al., 2012; Lamb et al., 2015; Spiegel, 2016). Initial attempts at mining rehabilitation in Australia only focused on the return of vegetation, often using non-local or non-native, fast-growing species (Gardner and Bell, 2007). In the 1980's, restoration efforts progressed to creating analogous

vegetation to that of the former or surrounding habitat (Gardner and Bell, 2007), with assumptions that flora restoration would promote the return of fauna (Cristescu et al., 2012). However, mining restoration projects are often challenging. Many mine sites are highly degraded and extremely expensive to repair at the time of closure.

Despite the high number of mining operations in Western Australia, there has been a lack of fauna recovery monitoring after mining restoration (Thompson and Thompson, 2004; Lythe, 2012). One exception is Alcoa of Australia Limited (hereafter termed Alcoa), the world's leading bauxite producer, and world's second largest alumina producer (Geoscience Australia, 2016). Alcoa operates bauxite mines in Western Australia and has contributed a large body of research into mining restoration techniques.

1.5   Alcoa bauxite operation

Alcoa has mined bauxite in the northern jarrah forest of south-western Australia since 1963 (Grant and Loneragan, 2001). Alcoa currently operates two open cut mines at Huntly and Willowdale. Before mining takes place, the entire vegetation cover is cleared, followed by topsoil and subsoil stripping for direct application to previously mined areas (Tacey, 1979; Nichols and Burrows, 1985). Bauxite is then excavated by heavy machineries from depths of up to 1–4 m (Nichols et al., 1985).

Alcoa restores approximately 550 ha of mined forest per year at a cost of about AU$ 34,000 per ha (Gardner and Bell, 2007; Koch, 2007a). Alcoa's restoration aim is to return the mined areas to a self-sustaining jarrah forest ecosystem that maintains timber, water, recreation, conservation, and other services (Tacey, 1979; Grant, 2006; Koch and Hobbs, 2007; Grant, 2008). In bauxite mine site rehabilitation, topsoil is removed from one site and spread to another as promptly as possible (Koch, 2007b; Koch and Samsa, 2007). This "direct return" top soil contains organic material, microbial activity, the majority of natural soil seed bank, and plant nutrients, which encourage plant growth, faunal return, and ecosystem functions (Koch, 2007a; Koch and Hobbs, 2007). Over the past 30 years, Alcoa has used a range of methods and gradually improved their bauxite mine site restoration. The present method involves adding broadcast seed (which represent a mixture of 78–113 plant species), hand planting some recalcitrant species (recover mainly by resprouting and produce little or no viable seeds— family Cyperaceae (sedges) and Juncaceae (rushes)), and applying 229 kg ha^{-1} of diammonium phosphate

sulphur extra as fertilizer (one-time application; 17.5 % N, 20.0 % P, 1.2 % S with micronutrients—Cu, Zn, Mo, and Mn—at 51 kg ha^{-1}) (Grant et al., 2007; Koch, 2007a). Waste rocks and timber are placed in the restoration area to provide shelter and habitat for both vertebrates and invertebrates (Koch, 2007a). This method of bauxite mining rehabilitation has proven successful in restoring a diverse jarrah forest, meeting completion criteria and establishing on-going recruitment of many species to and from the seed bank (Koch and Ward, 2005; Norman et al., 2006b)

Alcoa also operates three alumina refineries within Western Australia—at Kwinana, Pinjarra, and Wagerup. The excavated bauxite from mining is supplied to the alumina refineries where aluminium is refined from its oxide form (Courtney et al., 2009). In the alumina refineries, Alcoa uses the Bayer refining process, a common method worldwide, to refine aluminium from bauxite (Jones et al., 2012). The Bayer refining process involves a number of key steps including bauxite grinding, slurry storage, digestion by washing with sodium hydroxide (NaOH), clarification by filtering the solid impurities or red mud (residue), and precipitation by cooling and calcination (Alcoa, 2005; Chauhan and Ganguly, 2011).

The residue that is produced during the clarification stage consists of caustic-insoluble constituents (primarily oxides of iron and silicon and is highly alkaline) and a quantity of NaOH that is retained after the washing stage (Alcoa, 2005). Nearly two dry tonnes of slurry residue is generated for every one tonne of aluminium produced (Phillips and Chen, 2010). The residue is separated into different size fractions as part of the refining process. The coarse fraction is known as "sand" (approximately 60 %) and the fine fraction is known as "mud" (approximately 40 %). The "mud", which contains toxins, is stored so as to prevent the release of harmful elements into the environment (Alcoa, 2005, 2007). The embankment of the residue storage area is made up of the "sand" and the "mud" forms the "lake" within the embankment. The "mud" is subjected to a pre-thickening process that converts it into 50 % firm slurry, after which it is deposited in the "lake" in 0.4–0.7 m layers. After preliminary drying, bulldozers are used to rotate the wet mud onto the surface of the dry top layer, which accelerates the "mud" drying process and minimizes dust emissions. These mud lakes are developed progressively upwards to a height of 80 m, making the outer slopes highly visible. The highly modified residue storage soil is very different compared to the topsoil transferred in the mine

pits. Residue storage area soils are highly alkaline, have high electrical conductivity, a large amount of salt, and a high bulk density (Gräfe et al., 2011).

The rehalitation program at Alcoa focuses on developing vegetation cover on the outer residue sand embankments (Thiyagarajan et al., 2009; Jones et al., 2010; Thiyagarajan et al., 2011; Thiyagarajan et al., 2012), with the intention of improving the aesthetic value by covering and concealing the residue storage area with self-sustaining native vegetation (Dobrowolski et al., 2009) and minimizing dust emissions (Alcoa, 2007). The establishment of vegetation cover over the residue storage area can also contribute toward reducing deep drainage of alkaline water and lowering the potential risk of ground water contamination (Dobrowolski et al., 2009; Jones et al., 2010; Banning et al., 2011c). As residue sand is highly alkaline, it is amended with gypsum ($CaSO_4$, i.e. Calcium sulphate) (Dobrowolski et al., 2009), which alters the alkalinity from pH 12 to pH 9.5 (Chen et al., 2010b). Wood waste added on top of the soil layer promotes the return of ground and litter dwelling invertebrates and the development of predatory invertebrates, providing an early source of nutrients without affecting plant growth (Nichols and Burrows, 1985; Lythe, 2012). Native plant species assemblages of tuart woodland ecosystems are hand planted and broadcast seeded as they are tolerant of alkaline soils with relatively high salt content. This is accompanied by an application of di-ammonium phosphate ((NH_4)$2HPO_4$; DAP) fertilizer at 2.7 t ha^{-1} and some trace elements used in residue restoration (Dobrowolski et al., 2009). Alcoa is expected to restore many hundreds of hectares of residue storage areas by the end of their mining activities (Chen et al., 2010b) based on environmental requirements.

1.5.1   Completion criteria

Restoration completion criteria are the qualitative or quantitative measures of performance used to assess the success or failure of a restoration project, and are required for the closure of a mine site (Chamber of Minerals and Energy, 2004; Environmental Protection Authority, 2006). Objectives and benchmarks for success vary between restoration projects, depending on the degree of landform modification, the scale of the restoration project, the types of plant communities to be restored, and the local context (Society for Ecological Restoration, 2004; Environmental Protection Authority, 2006). Thus, while successful restoration is required in every restoration

project, full restoration of the original ecosystem is usually not possible due to permanent changes to soils, landforms, and hydrology; further, restoration projects are generally not monitor over adequate timeframe for full restoration (EPA, 2006).

Setting completion criteria provides a clear pathway for both the government and Alcoa to manage restoration on a regional basis. However, the completion criteria state that the minimum requirements for closed mine pits is to restore the pre-mining land uses and ecosystem functions (Koch and Hobbs, 2007) such that the Department of Parks and Wildlife (DPW) can manage restored mine pits within normal procedures of forest management (Gardner and Bell, 2007). It is required that restored mine pits meet regional land use practices, integrate into the local landform, establish a self-sustaining system, have the capability to integrate with standard forest management practices, and exhibit comparable resilience to native jarrah forests (Gardner and Bell, 2007). Alcoa has developed a long term residue management strategy for sustainable management of residue sand; further, this strategy helps Alcoa remain on track with their completion criteria and report the condition of their residue storage facilities to the state and local governments (Alcoa, 2013).

1.6   Justification of the study

Studies focused on ecosystem function restoration are often limited by inadequate budgets and capabilities (Society for Ecological Restoration, 2004; Ruiz-Jaen and Aide, 2005). Moreover, projects based on ecological processes need to be monitored long-term (Craft et al., 2002) and require multiple interventions (Herrick, 2000), which may incur further cost and time.

For mining restoration, the value of considering fauna and flora establishment (Bunn, 1983; Nichols and Bamford, 1985; Nichols and Burrows, 1985; Koch and Samsa, 2007; Courtney et al., 2009; Kim et al., 2018), soil microbial physiological profiles (Banning et al., 2008; Banning and Murphy, 2008; Banning et al., 2012), and soil physiochemical development (Gräfe et al., 2011; Courtney and Kirwan, 2012; Goloran et al., 2013) has been highlighted. To date, in terms of plant species similarity, Alcoa's bauxite mine restoration has mostly been successful in restoring pre-mining land uses and conservation (Koch and Hobbs, 2007). However, there is limited knowledge on

invertebrate establishment and their link to ecosystem function re-establishment in bauxite mining and residue restoration.

In terms of residue sand, generated during the processing of bauxite, previous studies of Alcoa bauxite mine restoration have provided information on changes to the chemical, physical, and microbial environment associated with this activity; poor recovery of chemical and physical properties (deficiency of macro and micronutrients, low water and nutrient retention capacity, high alkalinity) and poor microbial biomass of residue sand (Chen et al., 2009; Jones et al., 2010; Phillips, 2010a, b, c; Banning et al., 2011c). It is crucial to improve soil properties to establish a self-sustainable vegetation cover on residue embankments (Banning et al., 2014). Being a disturbed environment— i.e. with limited nutrients, a skewed soil pH, and an imbalanced ecological state—early stage plant recruitment is very limited (Banning et al., 2014). A number of studies have been carried out on microbes and plants, which mostly indicate that subsoil chemical composition of residue sand limits root growth and that the addition of organic amendments is required to enhance the microbial properties of residue sand (Gwenzi et al., 2011; Jones et al., 2011); however, few studies have monitored invertebrate community abundance and diversity. This information is necessary to estimate community richness of invertebrates and their ecological role in promoting the development of a functioning residue ecosystem.

Although organic and inorganic amendments to residue soils and the establishment of vegetation, soil biota, and fauna for mining restoration are well studied, the link between ground-dwelling invertebrates and ecosystem function development is little known in both mining and residue restoration systems (Grant et al., 2007; Majer et al., 2007a; Courtney et al., 2010); furthermore, research related to ground-dwelling invertebrate community development in residue sand embankments is lacking.

1.7   Research aims
1.7.1   General objective

This research aims to investigate the succession of soil invertebrates and the development of key ecosystem processes along a chronosequence of restored sites within two distinctly different restoration projects following bauxite mining. The restoration sites were managed by Alcoa in south-western Australia; the first site was a

previously mined restoration site with intact topsoil that was directly transferred from another bauxite mining operation (mining restoration system), and the second site comprised novel soil created from bauxite residues (residue restoration system). Each study site was compared to remnant vegetation in close proximity. The study relates ecosystem function to species and functional group diversity to test theories of species redundancy significance in relation to the development of self-sustaining ecosystems.

1.7.2   Specific objectives

The specific objectives of this research for each of the two distinct restored bauxite mine sites were to:

- Investigate the development of soil invertebrate diversity by:

  1. Quantifying changes in soil invertebrate fauna over time;
  2. Characterising functional group diversity of soil invertebrates and ant assemblages
  3. Comparing seed removal rate and seed dispersal distances to assess soil invertebrate role in the development of ecosystem functions;
  4. Quantifying litter decomposition rates as a measure of ecosystem functioning; and
  5. Comparing soil microbial activity as a measure of ecosystem functioning.

- Report composition and function changes to soil properties by:

  6. Quantifying soil pH and chemistry; and
  7. Quantifying soil structural changes (porosity measurements and bulk density) as a result of loss/ gain of invertebrate functional group diversity.

- Manipulate availability of ground litter cover to improve invertebrate diversity by:

  8. Assessing the effects of coarse woody debris addition on recovery of soil invertebrate communities within the mining restoration system.

1.8   Hypothesis

The hypotheses to be tested are:

- Recovery of invertebrate diversity and soil ecosystem function will be more rapid in the natural soil/ bauxite mine restoration than in the residue restoration as natural soil contains more biotic materials than modified residue soil;

- Soil invertebrate species and functional group diversity will increase with restoration age and increase more rapidly in bauxite mine restoration than in residue restoration;

- As the stage of rehabilitation progresses with time, soil invertebrate composition will resemble reference sites more closely;

- As rehabilitation progresses, ecosystem functions (rate of seed dispersal and litter decomposition) will resemble reference sites more closely;

- The development of soil invertebrate fauna abundance and diversity will enhance the quality of soil properties (soil physiochemical characters);

- An increase in soil organic content will increase soil invertebrate diversity and improve ecosystem function.

Chapter 2   Soil reconstruction using two contrasting restoration methods associated with bauxite mining and residue sand storage area revegetation

2.1   Abstract

Soil reconstruction is a crucial component of achieving successful restoration. The suitability of the upper topsoil layer of the soil profile as a growing medium is affected by key physical, chemical, and biological properties. Understanding the functionality of these properties can markedly improve ecosystem performance. Alcoa of Australia Limited (Alcoa) mines and refines bauxite for alumina, resulting in two systems that require restoration: the bauxite mine pits and the residue sand storage areas. Two contrasting restoration methods are used; in the former, soil is directly transferred to the restoration site (mining site), and in the latter, bauxite residue sand—a high pH, anthropogenic substrate—is amended with gypsum and fertilizer to create a suitable growing medium. This study investigated the temporal changes in soil and residue properties in the upper soil profile across a chronosequence of up to 20 years since restoration. It was hypothesized that, with time, the restored areas will progressively resemble the native (remnant) sites. Composite soil samples (n = 5) to the depth of 10 cm in 10-, five-, and two-year-old revegetated and adjacent coastal remnant sites in bauxite residue sand embankments and 20-, 10-, five-, two-year-old revegetated and adjacent jarrah forest remnant sites were tested for electrical conductivity (EC), pH, organic carbon, total N, NH_4-N, NO_3-N, extractable phosphorus, P (total), cation exchange capacity (CEC), bulk density, particle density, porosity, and soil microbial respiration. The results indicated that soil quality improved over time for both restoration methods. However, soil quality improvement was lower and on a different trajectory to remnant soil in residue restoration than in intact soil (mining site) restoration. The information collected highlights the long-term nature of abiotic changes in a restoration project and the need for further amendments to accelerate

changes. Characterisation of these soils can also be used to compare biodiversity and function.

2.2 Introduction

Soil ecosystem health is commonly defined as the ability of soil to function within an ecosystem by sustaining animal and plant productivity, promoting the health of fauna and flora, and maintaining the quality of air and water (Doran and Zeiss, 2000). Soil function and balance is divided into three main components: sustainable biological productivity, environmental quality, and flora and fauna health (Karlen et al., 1997). Approximately 80 % of earth's ecosystem functions are linked to soil functions (Lal, 2001); thus, the survival and extinction of most land based life forms are determined by the thin layer of soil that covers earth's surface (Doran and Jones, 1996). Soil is a slow-forming and non-renewable natural resource; unfortunately, similar to other ecosystems components on earth, soil is also facing numerous threats due to anthropogenic disturbances such as mining.

Mining alters the structure, chemistry, and function of soil by causing rapid reduction in soil fertility and biodiversity (Doran and Zeiss, 2000; Lewis et al., 2010; Muscolo et al., 2015; Jónsson and Davíðsdóttir, 2016; Muñoz-Rojas et al., 2016a; Tripathi et al., 2016). Open-cut mining operations remove the top soil layer before excavation operations take place, which disturbs soil ecosystem functions greatly (Rivera et al., 2014; Kim et al., 2018) but offers the possibility of reusing the topsoil in post mining operations.

2.2.1 Soil quality as an indicator of ecosystem health

Assessment of soil characteristics was first integrated into land management practices in the early 1990s (Karlen et al., 1997) with several indicators being used to assess soil quality. Soil physical indicators are mainly based on select soil structural features that have a large impact on plant growth (Hazelton and Murphy, 2007). Soil bulk density, i.e. the dry weight of soil per unit of volume soil (Hazelton and Murphy, 2007), is an indicator of soil compaction influencing soil density, pore space for water and solute movement, soil aeration, plant root penetration and growth, and seedling germination (Peverill et al., 1999; USDA, 2008; Barros et al., 2013; Bowman and Facelli, 2013). Soil chemical properties such as soil organic carbon, electrical conductivity, pH, and soil nitrogen and other nutrients are normally associated with soil fertility (USDA, 2008). In particular, soil

organic carbon content is important as it increases soil fertility, aeration, and water retention (Thomsen et al., 2012; Prober et al., 2014).

Soil pH controls the availability of nutrients in soil and directly impacts soil biota (Bardgett, 2005). For example, insoluble aluminium (Al^+) is released into soil solution at low soil pH (i.e. pH 4–5), which can be toxic to microorganisms and plants (Kennedy, 1992). The availability of P, essential for plant growth, is typically low in acidic soils as it is precipitated onto Fe and Al colloids under low pH conditions (Kennedy, 1992). In extremely alkaline soils the diversity of soil fauna is low—with fewer fungi, bacteria, and earthworms (Bardgett, 2005). Soil biological properties are more sensitive to anthropogenic disturbances as they respond to environmental changes quicker than soil physical and chemical properties (Doran and Zeiss, 2000; Bastida et al., 2008; Muscolo et al., 2015). Soil microbial communities, alongside soil invertebrate populations, play a significant role in soil ecosystem function (Frouz and Nováková, 2005). In general, soil biological properties such as soil microbial respiration and biomass are indicators of soil health (Muñoz-Rojas et al., 2016a). However, soil quality assessments are challenging as soil quality is linked to human activities and different soil types undergo different changes depending on their original composition (Barros et al., 2013).

The relationship between soil quality indicators and ecosystem functions are reasonably well documented in agriculture (Peverill et al., 1999; Hazelton and Murphy, 2007; Bowman and Facelli, 2013; Muscolo et al., 2015; Creamer et al., 2016); however, there are limited studies in mining and contaminated land rehabilitation. Several studies have focused on soil microbial parameters and chemical properties in post-mining chronosequences (Frouz and Nováková, 2005; Frouz et al., 2007). A few studies have focused specifically on bauxite mining in Western Australia. In one such study, Ward (2000) concluded that the soil chemical parameters—i.e. nitrogen, extractable phosphorus, extractable potassium, and pH—of 12-year-old restored bauxite mine pits were approaching those of an unmined forest. In another study, Thiyagarajan et al. (2009) identified that the distribution of micronutrients such as boron (B), zinc (Zn), and

manganese (Mn) in bauxite residue soils was influenced by soil organic carbon, pH, and exchangeable sodium (Na).

A number of authors suggest that soil management practices should consider ecosystem functions, i.e. how they impact soil biota and their interaction with soil physical and chemical properties (Frouz and Nováková, 2005; Banning et al., 2011b; Banning et al., 2011c; Thomsen et al., 2012). There are relatively few studies that consider ecosystem health following mining. This study aims to evaluate ecosystem recovery by characterising changes to select physical, chemical, and microbiological soil properties across a chronosequence following bauxite mining restoration and bauxite residue restoration. It is predicted that changes in soil properties will be relatively minor over the chronosequence, and soil properties will remain distinct to those of the reference sites, particularly for residue restoration.

2.3   Methods

2.3.1   Study area

This study was located in Western Australia, between 40 and 120 km south-west of Perth, and was conducted within two different restoration systems associated with bauxite mining. The two restoration systems investigated were 1) bauxite mining restoration on the Darling Scarp, and 2) bauxite residue restoration on the Swan Coastal Plain. The bauxite mining restoration sites were located at Alcoa's Huntly mining site in the northern jarrah forest (32° 36' S, 116° 06' E). The extent of the jarrah forest is approximately 3.3 million ha, of which 712,900 ha fall within Alcoa's mineral lease area (Figure 2.1). The bauxite residue restoration sites were located at Alcoa's Kwinana aluminium refinery, a 1,000-ha site within the Kwinana industrial area (32° 12' S, 115° 49' E).
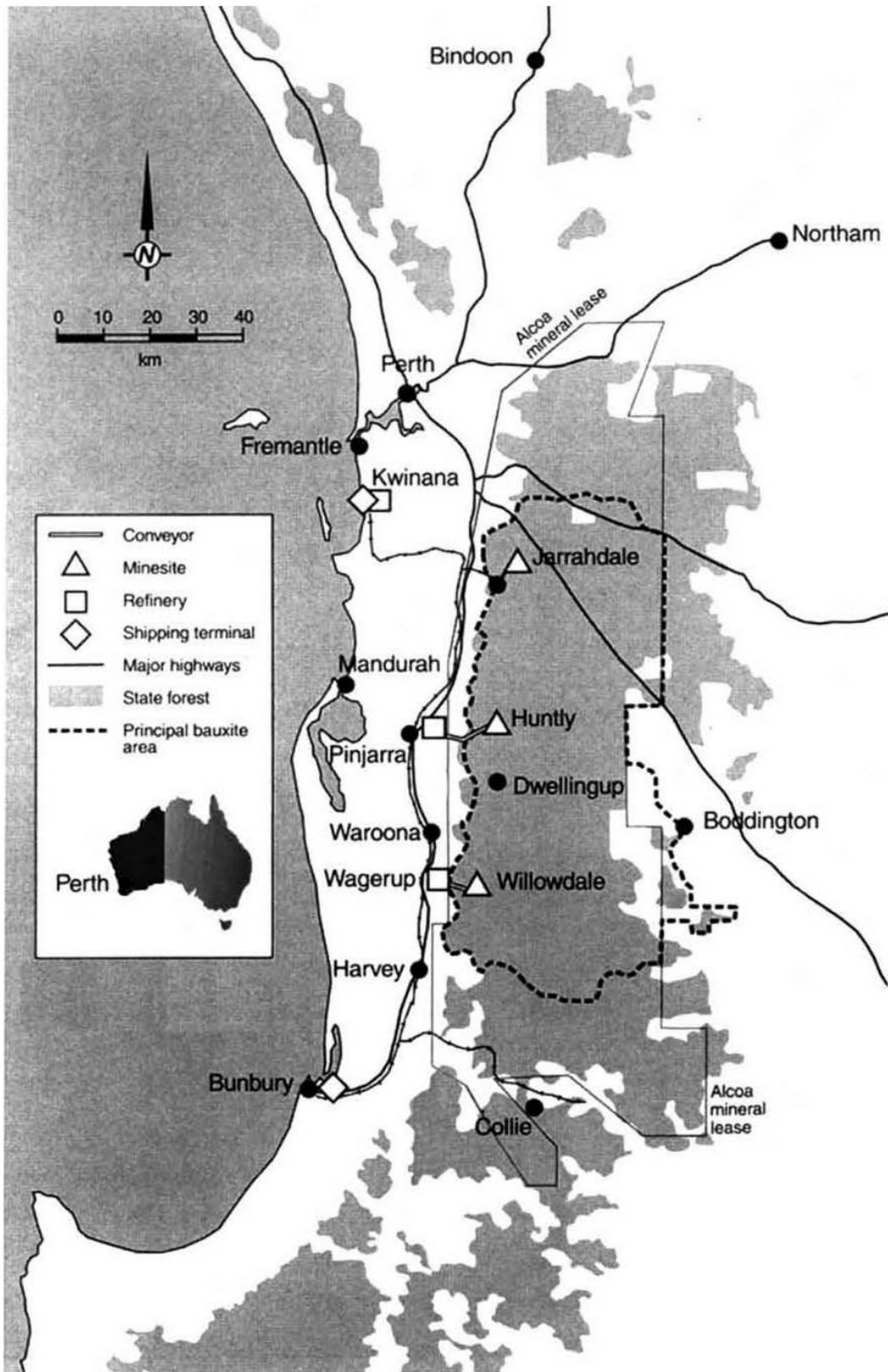
Figure 2.1 Map illustrating Alcoa's mineral lease area, past and current mining, and restoration areas within the jarrah forest of south-west Western Australia (Smith et al., 2004)

2.3.2 Restoration process in each site

Alcoa's bauxite mining commenced in the jarrah forest in 1963; rehabilitation was first initiated in 1966, and involved the planting of 6.7 ha of exotic *Pine* spp. and *Eucalyptus* spp. (Koch, 2007a). As the operation expanded, the methods changed and improved from rehabilitation to a restoration-focused approach (Norman et al., 2006a; Koch, 2007b). Alcoa restores approximately 550 ha of land each year using methods such as double soil stripping, landscaping and pre-ripping, overburden and topsoil return, logs and rock return for fauna habitat, contour ripping, seeding, planting, fertilizing, and monitoring to check that the number of established plants are in accord with agreed targets (Koch and Ward, 2005; Koch, 2007a). The current restoration practice has been in use since 1988, and involves seeding with *Eucalyptus marginata*, *Corymbia calophylla*, and 76–111 local understory species, alongside hand planting of recalcitrant species (Koch, 2007a; Koch and Samsa, 2007; Craig et al., 2015)

Kwinana's bauxite refinery commenced in 1963 and was the first Western Australian alumina refinery (Alcoa, 2015). Revegetation of residue sand embankments with native species is a relatively recent addition and is undertaken to enhance the physical stability of the site, control erosion and pollution, and provide water balance and aesthetics. The bauxite residue sand embankments share soil chemical and physical characteristics as the Quindalup Dune coastal system (dunes are made up of creamcoloured calcareous sands) in the south-west of Western Australia (Banning et al., 2011c; Cresswell and Bridgewater, 1985; Jasper et al., 2000); hence plant species were selected from that environment as most likely to have the best chance of survival (Goloran et al., 2013). The standard residue restoration involves incorporating 225 t ha^{-1} of gypsum to a depth of 1.5 m and 2,751 kg ha^{-1} of fertilizer (di-ammonium phosphate) to the depth of ~20 cm; this is followed by broadcasting a mixture of seeds containing about 55 native coastal plant species of Western Australia (Banning et al., 2011c; Goloran et al., 2013). Wood mulch is then added to the surface at a depth of 3 cm to suppress dust. Finally, seedlings that cannot establish from seeds are hand planted.

2.3.3   Climate

The climate of the two study areas is Mediterranean, with long hot and dry summers (which may last up to 7 months) alternating with cool, wet winters. The mean annual rainfall of the mining restoration site ranges from 900 to 1,300 mm (Gardner and Bell, 2007; Craig et al., 2015) whereas the mean annual rainfall of the residue restoration site is 760 mm (Silberstein et al., 2001; Banning et al., 2011c); approximately 75 % of rainfall occurs from late autumn (May) to early spring (September) in both sites (Figure 2.2). At both sites, summer drought can last up to four to six months, though unseasonal rain can occur as a result of tropical cyclones during the hottest periods of the year (Gardner and Bell, 2007). The mean daily temperature of both sites is similar, with that of the mining restoration site varying from 15 °C to 30 °C and that of the residue restoration site ranging from 18 °C to 31 °C (Figure 2.2).
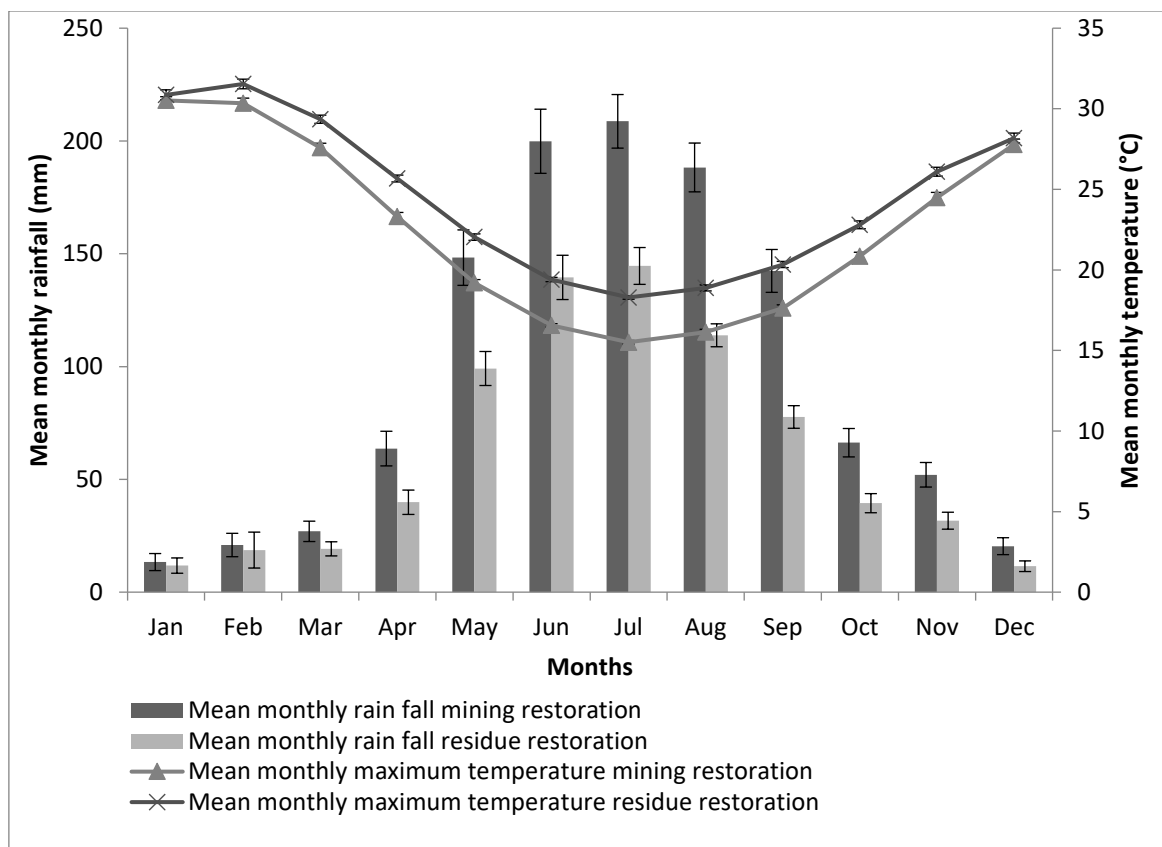
Figure 2.2 Mean monthly rainfall and maximum temperature patterns (means ± SE) of Dwellingup (12 km south to mining restoration) and Kwinana town centre (10 km south to residue restoration) for an average year based on daily rainfall and temperature data (1983 to 2016) obtained from Australian Government Bureau of Meteorology (2017)

2.3.4   Geomorphology

The Darling Plateau and Swan Coastal Plain are the two main geographic regions of south-western Australia. The Swan Coastal soils are of aeolian origin with some incursions of riverine material (Nichols et al., 1985). The Swan Coastal Plain contains a series of distinct sand dunes that run approximately parallel to the coast (McArthur and Bettenay, 1960). The residue restoration study sites are located in the coastal dunes of the Quindalup Dune geological system within the Swan Coastal Plain. The soils of the Quindalup Dune geological system are derived from underline aeolianite of the Tamala Limestone, and comprise variable depths of silicious, yellow, and brown leached sands (Ruthrof et al., 2010). Further inland, at the west edge of the Darling Plateau, rising approximately 300 m above sea level, is the Darling Scarp. The soils of the Darling Scarp are ancient, formed by in situ weathering of Darling range granite that formed during the tertiary period (Mulchay, 1967). A number of theories have been formulated to explain the weathering process, i.e. laterization, but the one most widely believed is that the lateritic gravel in WA formed from in situ weathering when the climate was warmer and wetter (Mulchay, 1967; Ruthrof et al., 2010). The topsoil layer consists of coarse ferruginous gravel and beneath it is bauxite (aluminium oxide), which is rich in aluminium. The bauxite occurs in isolated pods on the flanks and gentle hill slopes (Mulchay, 1967; Nichols et al., 1985; Armstrong and Nichols, 2000).
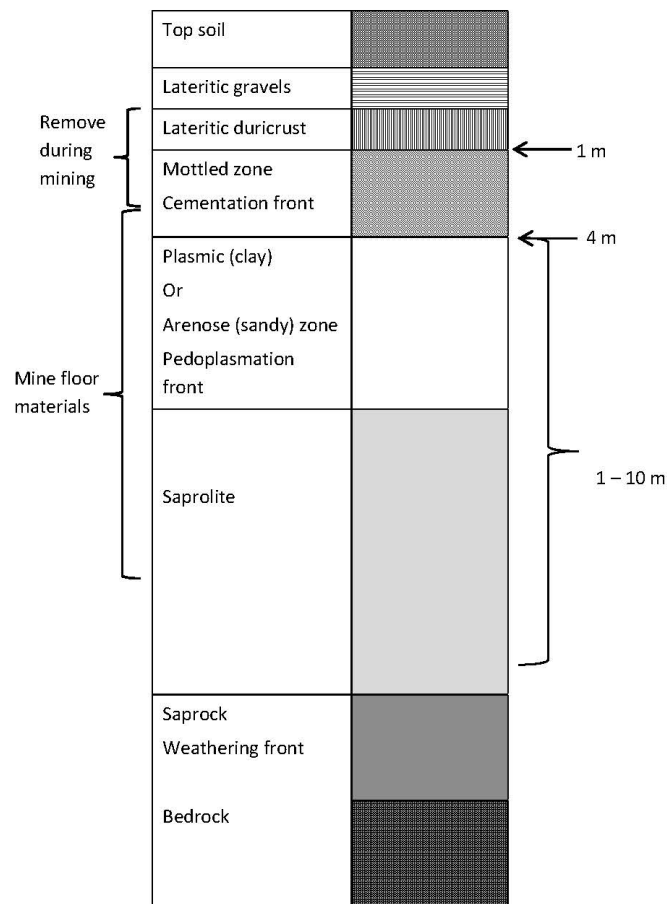
Figure 2.3. An idealised Darling Scarp laterite soil profile (adapted from Koch (2007a))

2.3.5 Vegetation characteristics

The south-west corner of the Darling Scarp in Western Australia is covered by dry, sclerophyll jarrah forest (*Eucalyptus marginata*), the state's most extensive forest reserve, providing timber production, water catchment, agriculture, recreation, and national parks for conservation (Hingston et al., 1980; Nichols et al., 1985). The Jarrah forest overstorey is dominated by jarrah and marri (*Corymbia calophylla*) trees; *Banksia grandis* and *Persoonia longifolia* are prevalent in the mid-story, and *Acacia pulchella, Allocasuarina fraseriana, Hibbertia hypericoides, Kennedia coccinea, Lasiopetalum floribundum, Trymalium ledifolium, Xanthorrhoea preisii*, and numerous other species are present in the understory (Nichols and Bamford, 1985; Banning et al., 2011a; Craig et al., 2015).

The Quindalup Dune system vegetation is composed predominantly of *Acacia rostellifera, A. cochlearis,* and *A. cyclops*; in the residue restoration system, plant communities characterised by Open Heath (height ≤ 1.7 m) include *A. rostellifera* or *A.*

cyclops coastal heath on dry dunes with *Melaleuca systena*, *Hibbertia subvaginata*, *Rhagodia baccata, Spyridium globulosum,* and *Olearia axillaris* (Cresswell and Bridgewater, 1985; Banning et al., 2011c; Dixon, 2011). Compared to the Darling Scarp ecosystem, the Swan Coastal Plain Quindalup Dune ecosystem has less vegetation richness as the coastal alkaline soils are geologically recent, providing insufficient time for species to evolve and adapt to the high pH and novel soil structure (Dixon, 2011). The remnant vegetation surrounding the Kwinana site differs from that used in the revegetation; in addition to *A. rostellifera, A. cyclops, Melaleuca systena, Hibbertia subvaginata, Olearia axillaris*, and *Spyridium globulosum*, the remnant vegetation also consists of mixed herbs and sedges as understory species*.*

2.3.6   Experimental design and site selection

This study was conducted across two restoration systems: the Huntly bauxite mining restoration (hereafter mining restoration) and Kwinana residue restoration (hereafter residue restoration). A total of nine study sites were selected, comprising four and three restoration ages in mining restoration and residue restoration, respectively, with two adjacent forest remnant sites (jarrah forest at Huntly and tuart forest at Kwinana). Study sites had been revegetated two, five, 10, and 20 years ago (20-year-old sites were only found at mining restoration). Three replicates were established for each treatment, resulting in 15 experimental plots (hereafter plots) for mining restoration (i.e. 5 study sites × 3 reps) and 12 plots for residue restoration (i.e. 4 study sites × 3 reps) (

Figure 2.4). Each plot was 20 m × 20 m in size. Mining restoration plots were established at least 100 m from the edge, while residue plots were placed at least 50 m from the edge, reflecting the smaller areas revegetated in each year.
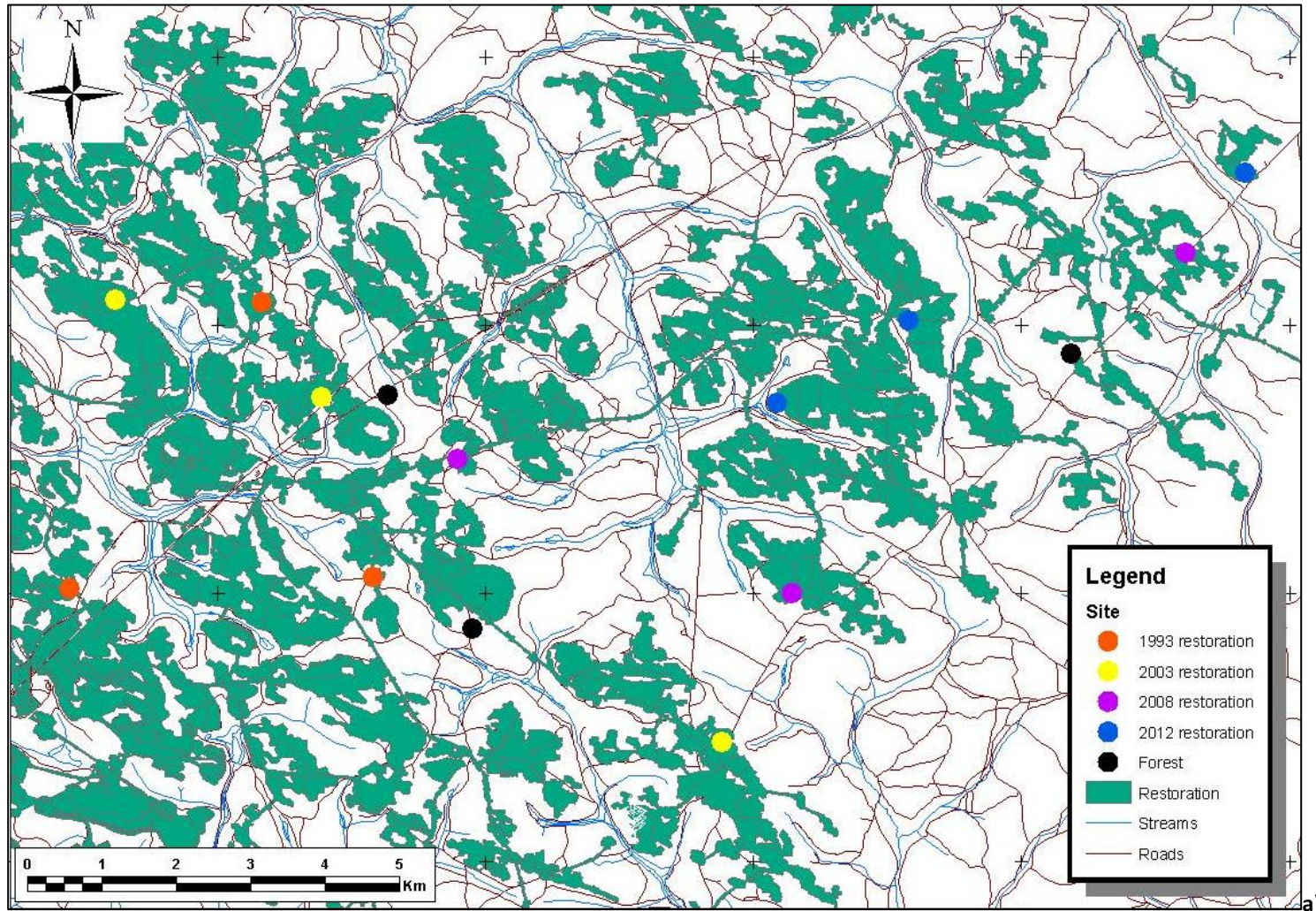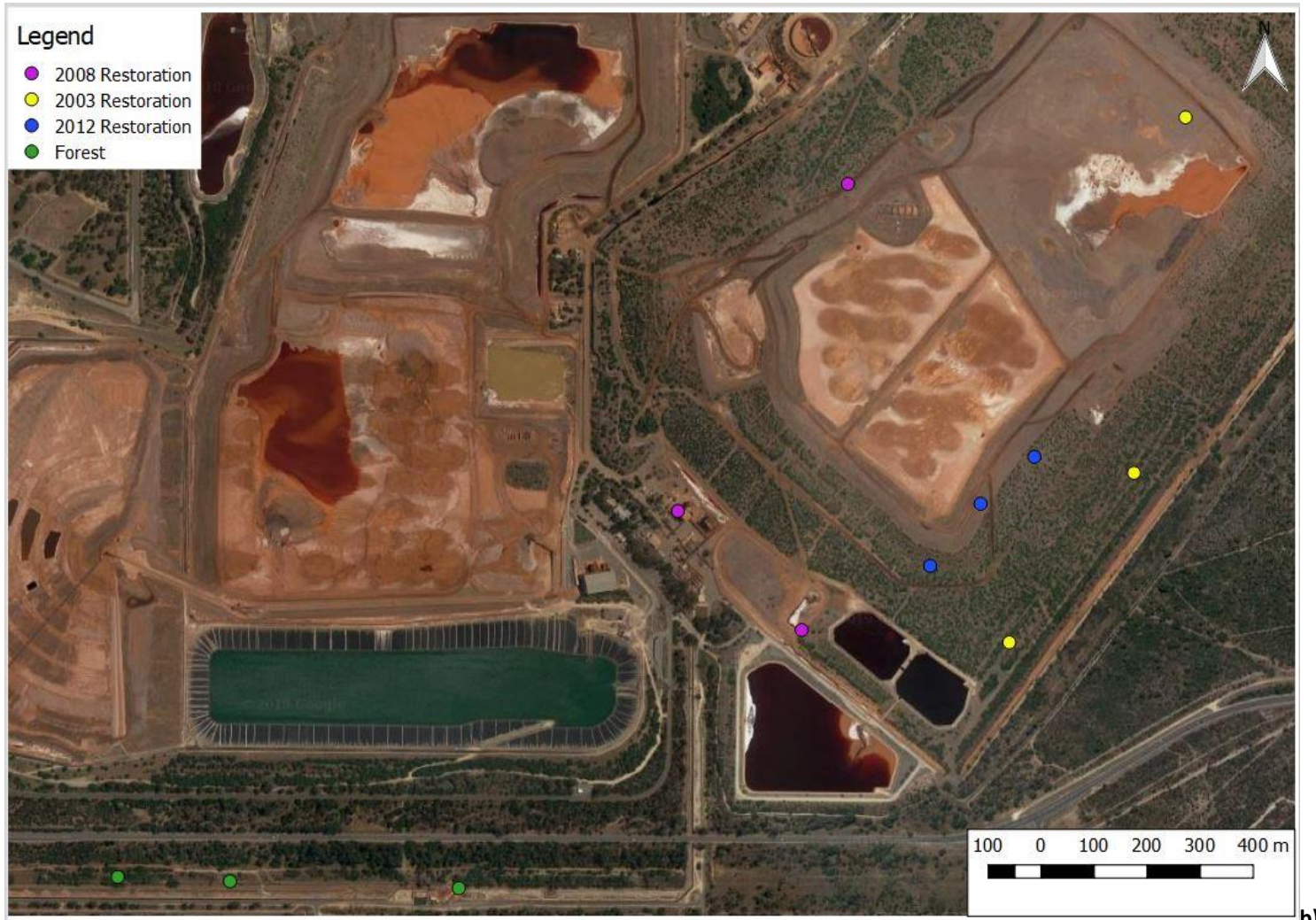
Figure 2.4. Map showing the location of a) the experimental plots within mining restoration (two, five, 10, and 20 years since restoration), and b) the experimental plots within residue restoration (two, five, and 10 years since restoration), including the reference ecosystems (forest); there were three replicate plots for each treatment.

2.3.7   Soil Sampling

The field sampling was conducted in August 2015. A composite soil sample comprising five locations in each plot (centre and north, south, east, and west quarters) was collected from the top 5 cm of the soil profile using a soil core sampler 10 cm in diameter. Leaf litter was cleared prior to sampling. Two soil samples were collected from each five locations of the plot, comprising 10 bulked soil samples per plot. Soil samples were air-dried and sieved (2 mm); then, 100 g sub-samples were stored at 4 °C for soil microbial analysis (see Section 2.3.7.2 for more details), and the remaining 200 g of samples were used for soil physiochemical analysis.

2.3.7.1   Soil Chemical analysis

Soil organic carbon (OC), total nitrogen (N), nitrate nitrogen (NO_3-N) ammonium nitrogen (NH_4-N), extractable phosphorus (P), and cation exchange capacity (CEC) analyses were conducted by the Chem Centre (Resources and Chemistry Precinct, Bentley, Western Australia; hereafter, Chem Centre). Soil organic carbon (OC) was measured by using the Walkley-Black chromic acid wet oxidation method (Rayment and Lyons, 2011). Oxidisable matter in soil samples was oxidized by $Cr_2O_2^{2-}$ in the presence of sulphuric acid (H_2SO_4) using the heat of dilution to accelerate the reaction. The residual Cr^{3+} ions were measured using a spectrophotometer at 600 nm. Calibrations were carried out using the known weight of sucrose to soil organic matter (Rayment and Lyons, 2011). Total soil nitrogen was determined using the Kjeldahl digestion method (Rayment and Lyons, 2011). Ground soil samples (< 0.2 mm) were digested with a mixture comprising sulphuric acid, potassium sulfate, and copper in a test tube digestion block. The digest was diluted and citrate and tartrate were added to form complexes with the heavy metals. Ammonia content was determined by using the Berthelot reaction with chlorine and salicylic acid to form a blue compound. Then, the ratio of organic C to total N (% org C/ % total N) was calculated. Ammonium and nitrate ions were displaced from soil using 1 M KCl solution. Ammonium ions in the extract were determined colorimetrically following oxidative coupling with salicylic acid ($C_7H_6O_3$) using sodium dichloroisocyanurate ($C_3Cl_2N_3NaO_3$) as a source of chlorine. Citrate and tartrate were added to sequester interfering metal ions (Rayment and Lyons, 2011). Nitrate ions were determined colorimetrically following reduction to the nitrite ion with

hydrazine, followed by diazotisation of 1-naphthylethylenediamine and subsequent coupling with sulphanilamide (Rayment and Lyons, 2011). Extractable soil P was measured by mixing soil and 0.5 M sodium bicarbonate solution (pH 8.5) for 16 hours at 23 °C using an end-over-end shaking technique. Extractable P was measured using automated colourimetry with 882 nm spectral filters. CEC was determined by desorbing the ammonium ions from the exchange sites by extraction with calcium and potassium nitrate and measuring the displaced ammonium ions using formol titration. Chloride was measured to correct for entrained ammonium chloride (Rayment and Lyons, 2011).

Electrical conductivity (EC), and soil pH were measured at Curtin University laboratories. Soluble salts were extracted from soil with water (1:5) after shaking for 1 h. The electrical conductivity of the extract was measured with a calibrated conductivity meter and the result expressed as mS/cm at 25°C. The pH of the extract was measured with a calibrated pH meter and combination glass electrode. Soil samples were equilibrated by shaking for 1 h with 0.01 M $CaCl_2$ solution (1:5).

Soil physiochemical parameters, organic C, total nitrogen, and cation exchange capacity were categorized according to typical soil composition, as reported by Hazelton and Murphy (2007); Rayment and Lyons (2011) (Table 2.1).

Table 2.1. Soil organic carbon, total nitrogen, and cation exchange capacity rating (adapted from Hazelton and Murphy (2007))

Rating	extremely low	very low	low	moderate	high	very high	extremely high
Org C (%)	< 0.4	0.4-0.59	0.6–0.99	1-1.59	1.6–1.99	2-2.99	>3
total N (%)	-	<0.05	0.05-0.15	0.15-0.25	0.25-0.5	>0.5	-
C:N ratio	-	<10	10-15	15-25	25-70	70-100	>100
Extractable P (mg/kg)	-	<5	5-10	10-17	17-25	>25	-
CEC (cmol/ kg)	-	<6	6-12	12-25	25-40	>40	-

Org C: total organic carbon; total N: total Nitrogen; C:N: ratio of organic C to total N; Extractable P: extractable phosphorus; CEC: cation exchange capacity.

2.3.7.2 Soil Microbiological analysis

Soil microbial activity was measured with the rapid, 1-day Solvita soil CO_2 respiration test, which measures release of CO_2 as a function of substrate availability (Muñoz-Rojas et al., 2016a). Soil samples (100 g) from each experimental plot were dried at 40 °C in a convection oven for 24 h. Sub-samples (40 g) of soil were then weighed into a 50 ml plastic beaker containing four < 2 mm punctures on the bottom to wick up moisture into the soil by capillary action. The plastic beakers were then placed inside a 240 mm glass jar and 20 ml of deionised water was added into the glass jar. As soon as the water was added, a CO_2 probe was inserted, and the glass jar was sealed closed. The jars were incubated at 25 °C for 24 h, and measurements were taken with a mini spectrometer, digital colour reader (DCR), specified for Solvita tests.

2.3.7.3 Soil Physical Analysis

Separate soil samples were collected in August 2015 to conduct soil physical analysis. Ten random soil samples were taken from the top 10 cm of the soil surface of each plot using a core sampler 7.5 cm in diameter. The soil samples were subsequently weighed and then oven dried at 105 °C to a constant weight and reweighed to the nearest 0.0001 g. The bulk density (BD) of each sample was calculated according to methods of Rayment and Lyons (2011). Particle density (Dp) was calculated (g/cm^3) by obtaining the mass of

dry soil and volume of soil solids, and porosity was calculated by using the bulk density and particle density values, according to Rayment and Lyons (2011).

2.3.8   Data analysis

Data were normally distributed and no transformations were necessary. Shapiro-Wilk and Levene's test were performed with soil characteristic data to test for normality and homogeneity, respectively. Two-way analysis of variance (ANOVA) was performed to test the differences in soil characteristics between the two restoration systems and restoration sites, and to test the interaction effect between restoration system and site. Tukey's honesty significance difference (HDS) posthoc test was performed to compare means when a significant difference was observed in the ANOVA. Analysis was performed using SPSS statistical software version 21. Principal coordinate analysis (PCO) analysis was performed to produce ordinations of similarity of soil characteristics between sites on the Bray–Curtis matrix. Further relationships between measured soil characteristics were analysed with Pearson's correlations. The R-statistic was considered significant at $P < 0.05$.

2.4   Results

2.4.1   Soil structure and chemistry

Four of the measured parameters—i.e. ammonium nitrogen, nitrate nitrogen, phosphorus Colwell, and cation exchange capacity—showed no differences across the two restoration systems or across the chronosequence. Nine of the 15 measured soil characteristics were significantly different between the two restoration systems (Table 2.2). In particular, porosity, conductivity, pH, and microbial respiration had lower values whereas organic carbon had a higher value in the mining restoration system as compared to the residue system. Changes across the chronosequence were not always consistent between systems; there were, however, significant interaction effects between pH, total nitrogen, and microbial respiration (Table 2.2).

Generally, mining restoration soil was similar to jarrah remnant site soil; however, in the remnant soil, bulk density tended to be lower and organic carbon and microbial respiration tended to be higher as compared to the restoration soil (Table 2.2). In contrast, characteristics of the residue soil were generally distinct to that of the tuart

forest remnant soil. Some parameters tended to have a lower value (particle density, bulk density, conductivity, pH, phosphorus, potassium, and microbial respiration) while others tended to have a higher value (organic carbon, nitrate and ammonium nitrogen, and cation exchange capacity) in the tuart remnant compared to the residue site (Table 2.2). For some characteristics (bulk density, pH, organic carbon, nitrate, cation exchange capacity), the values in the residue soil tended to approach the values of the remnant site as time since restoration increased; for microbial respiration, however, the values observed in the residue soil tended to move away, with time, from the values recorded in the remnant site. Bulk density was significantly correlated with particle density (r = 548, $P < 0.01$) and C:N ($r = 0.442$, $P < 0.05$) (Table 2.2). Soil organic carbon showed a positive correlation with total nitrogen (r = 935, $P < 0.01$), ammonium nitrogen (r = 0.752, $P < 0.01$), and CEC (r = 876, $P < 0.01$) and a negative correlation with porosity (r = -724, $P < 0.01$), EC (r = -681, $P < 0.01$), and pH (r = -863, $P < 0.01$). There was no significant relationship between soil organic carbon and microbial respiration.

Table 2.2. Comparison of soil physical, chemical, and biological characteristics (mean ± SE) across all nine study sites. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant. Different lower-case letters in the same row indicate significance differences within soils across sites; upper case letter A indicates significant differences between the two restoration systems, and * indicates a significance interaction effect between sites and restoration system at $P < 0.05$

Soil parameter (Mean ± SE)	Sites								
	M2	M5	M10	M20	MR	R2	R5	R10	RR
Physical characteristics									
Particle Density (g/cm^3)	3.52 ± 0.13^b	2.88 ± 0.15^{ab}	3.11 ± 0.23^{ab}	2.63 ± 0.19^{ab}	2.75 ± 0.26^{ab}	2.97 ± 0.09^{ab}	3.03 ± 0.12^{ab}	2.94 ± 0.09^{ab}	2.45 ± 0.12^a
Porosity (%)^A	10.00 ± 0.33^a	8.00 ± 1.00^a	9.00 ± 0.88^a	8.00 ± 1.33^a	12.00 ± 1.20^a	50.00±1.45^b	52.00 ± 1.16^b	52.00 ± 2.33^b	49.00± 1.67^b
Bulk Density ^{A}	2.07 ± 0.11^c	1.94 ± 0.10^{bc}	2.07 ± 0.12^c	1.71 ± 0.04^{bc}	1.44 ± 0.21^{ab}	1.48 ± 0.05^{ab}	1.44 ± 0.03^b	1.40 ± 0.04^b	1.24 ± 0.02^a
Chemical characteristics									
Electrical Conductivity (mS/cm^3)	2.67 ± 0.33^a	3.00 ± 0.58^a	2.67 ± 0.33^a	3.00 ± 0.00 ^{a}	4.00 ± 0.00^b	8.33 ± 0.88^c	14.67 ± 2.40^d	7.67 ± 0.88^c	4.67 ± 0.33^b
pH (H$_2$O) ^{A*}	6.10 ± 0.06^{ab}	5.70 ± 0.06^a	5.67 ± 0.03^a	5.77 ± 0.09^{ab}	5.90 ± 0.06^{ab}	8.73 ± 0.07^{bc}	8.30 ± 0.35$^{b\,c}$	8.33 ± 0.15$^{b\,c}$	6.47 ± 0.07^b
pH (CaCl$_2$) ^{A*}	5.10 ± 0.10^{ab}	4.60 ± 0.10^a	4.60 ± 0.12^a	4.73 ± 0.15^{ab}	4.93 ± 0.09^{ab}	7.93 ± 0.03^c	7.83 ± 0.22 ^{c}	7.77 ± 0.09^c	5.23 ± 0.03 ^{b}
Total Organic Carbon (%)^A	1.98 ± 0.39^c	2.08 ± 0.21^c	1.64 ± 0.02^c	1.96 ± 0.36^c	2.40 ± 0.48^c	0.16 ± 0.03^a	0.27 ± 0.10^{ab}	0.33 ± 0.05 ^{ab}	1.86 ± 0.39 ^{c}
Total Nitrogen (%)^{A*}	0.054 ± 0.013^{bc}	0.066 ± 0.006^c	0.058 ± 0.003^{bc}	0.058 ± 0.013^{bc}	0.067± .011^c	0.05± 0.000^a	0.054 ± 0.007^{ab}	0.066 ± 0.005^{ab}	0.058 ± 0.014^c
C:N (%)^A	38.3 ± 3.43	31.4 ± 2.09	28.5 ± 1.40	35.6 ± 4.56	36.0 ± 4.23	31.3 ± 6.36	22.2 ± 3.11	18.4 ± 2.29	24.6 ± 0.99
Ammonium Nitrogen (mg/kg)^A	5.00 ± 1.53	4.67 ± 0.88	4.33 ± 0.67	5.00 ± 1.53	7.33 ± 2.33	0.50 ± 0.00	0.67 ± 0.17	0.83 ± 0.17	2.00 ± 0.58

Soil parameter (Mean ± SE)	Sites								
	M2	M5	M10	M20	MR	R2	R5	R10	RR
Nitrate Nitrogen (mg/kg)	0.67 ± 0.17	1.33 ± 0.83	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	1.17 ± 0.44	0.67 ± 0.17	1.67 ± 0.44
Phosphorus Colwell (mg/kg)	19.00 ± 7.81	12.33 ± 4.41	7.00 ± 3.79	2.00 ± 0.58	1.67 ± 0.33	8.33 ± 2.73	17.00 ±6.25	7.00 ± 2.00	1.33 ± 0.33
Potassium Colwell (mg/kg)	180.0 ± 60.28[ab]	187.0 ± 38.44[ab]	126.0 ± 22.06[ab]	118.0 ± 45.94[ab]	61.0 ± 15.54[a]	140.0 ± 20.00[ab]	260.0 ± 0.65[b]	183.0 ± 0.28[ab]	49.0 ± 8.66[a]
Cation Exchange Capacity (cmol/kg) [A]	6.33 ± 0.88	6.33 ± 1.20	4.33 ± 0.33	4.33 ± 0.88	5.67 ± 1.45	2.00 ± 0.00	2.67 ± 0.33	3.33 ± 0.33	5.33 ± 1.45
Microbial Characteristics									
Microbial Respiration* (mg/kg of CO_2)	2.7 ± 0.15 [a]	7.3 ± 2.05 [a]	12.9 ± 6.1 [a]	33.8 ± 1.82 [b]	157.9 ± 6.09 [d]	68.6 ± 55.41[bc]	80.6 ± 21.91[bc]	116.2 ± 51.56 [d]	15.9 ± 11.97 [a]

Table 2.3. Pearson's correlation with all tested soil variables (n = 3). Significant correlation coefficients (r) relationships are in boldface. ** Correlation is significant at the 0.01 level and * correlation is significant at the 0.05 level. Dp: particle density; BD; bulk density; EC: electrical conductivity; OC: total organic carbon; N: total nitrogen; C:N: ratio of organic C to total N; NH₄-N: ammonium nitrogen; NO₃-N: nitrate nitrogen; P: phosphorus Colwell; K: potassium Colwell; CEC: cation exchange capacity; SMR: soil microbial respiration

Soil parameter	Dp	Porosity	BD	EC	pH (H₂O)	OC	N	C:N	NH₄-N	NO₃-N	P	K	CEC	SMR
Dp	1													
Porosity	-0.106	1												
BD	**0.548****	**-0.693****	1											
EC	0.083	**0.741****	**-0.448***	1										
pH (H₂O)	0.084	**0.872****	**-0.511****	**0.767****	1									
OC	-0.107	**-0.724****	0.366	**-0.681****	**-0.863****	1								
N	-0.249	**-0.581****	0.23	**-0.629****	**-0.826****	**0.935****	1							
C:N	0.18	**-0.616****	**0.442***	**-0.484***	**-0.444***	**0.473***	0.197	1						
NH₄-N	-0.128	**-0.743****	0.278	**-0.552****	**-0.705****	**0.752****	**0.705****	0.312	1					
NO₃-N	-0.191	0.152	-0.098	0.132	-0.044	0.203	0.314	-0.16	-0.137	1				
P	**0.409***	0.02	0.301	0.316	0.1	-0.132	-0.123	-0.133	-0.044	0.034	1			
K	**0.384***	0.164	0.102	**0.471***	0.244	-0.251	-0.208	-0.315	-0.149	0.135	**0.735****	1		
CEC	0.026	**-0.514****	0.332	**-0.480***	**-0.633****	**0.876****	**0.838****	0.307	**0.597****	**0.390***	0.06	-0.021	1	
SMR	-0.025	0.251	-0.374	**0.432***	0.33	-0.2	-0.277	-0.07	-0.047	-0.116	-0.144	-0.061	-0.202	1

.

A Principle Coordinate Analysis (PCO) of soil physical properties clearly separated the two restoration systems along axis 1, which captured 64.7 % of the total variation, and correlated with bulk density (BD), soil porosity, and particle density (Dp) (Figure 2.5.a). Soil porosity (P) was high in residue restoration, with corresponding data points clustered away from those of all other sites. Furthermore, the tuart remnant plots were clearly separated from the residue restoration sites; mining restoration sites were also separated from the jarrah remnant, but to a lesser extent. PCO analysis based on soil chemical properties clearly separated residue restoration sites from all other sites along axis 1, which captured 49.3 % of the total variation (Figure 2.5.b). The tuart remnant plots were clustered with the mining restoration sites and the jarrah remnant plots, without a clear separation between sites. Variations in the distribution of OC, total nitrogen, C:N ratio, ammonia nitrogen, and CEC differentiated mining restoration, jarrah remnant, and tuart remnant sites from residue restoration sites.
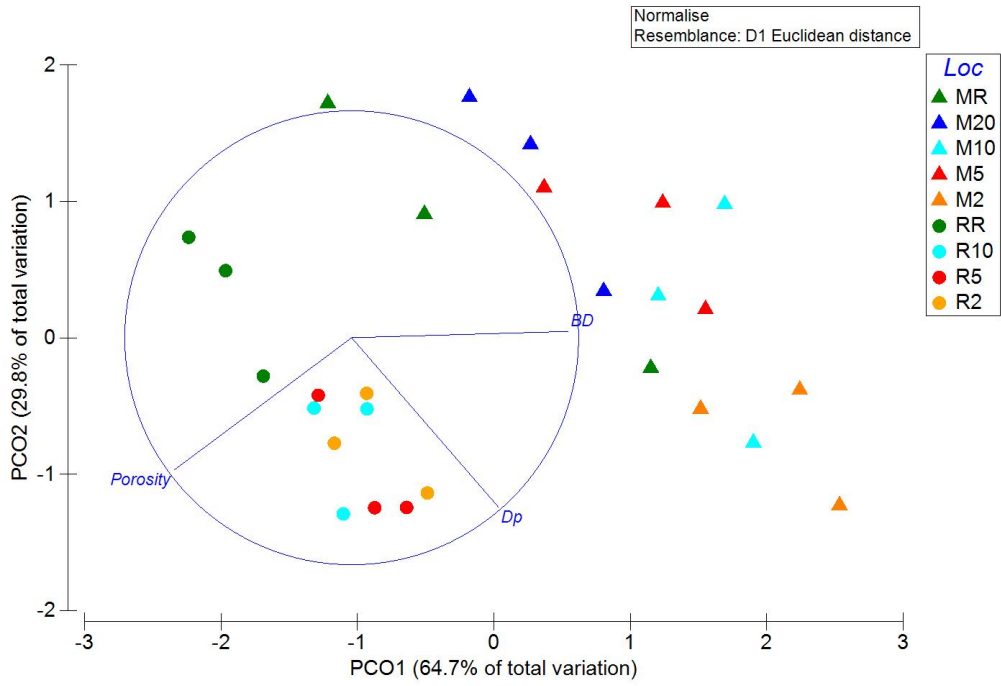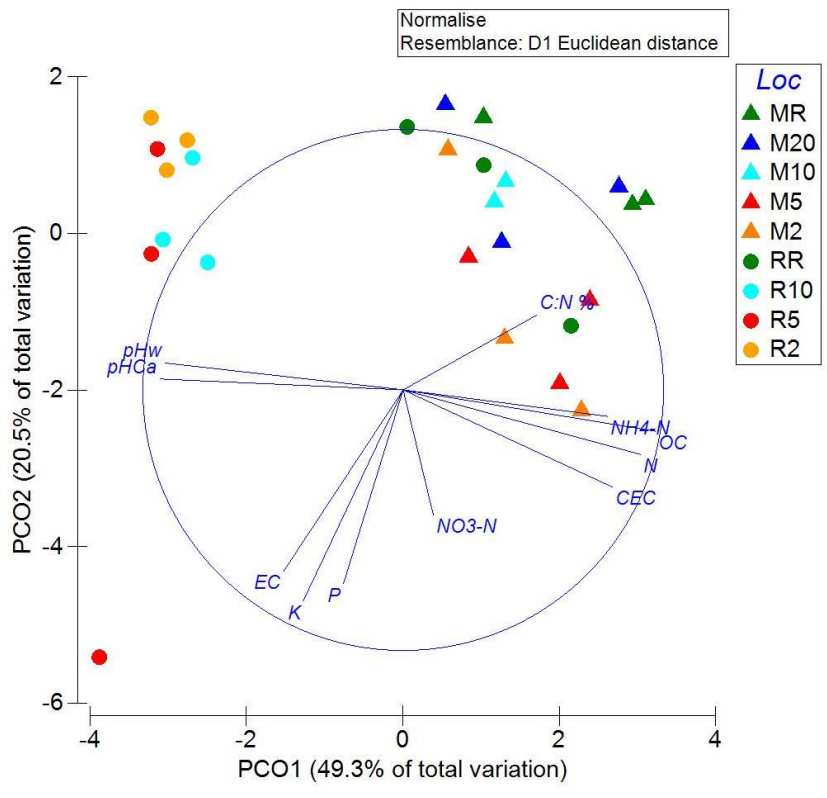
Figure 2.5. Principal coordinate analysis of soil a) physical, and b) chemical components of each site showing dependencies between soil indicators. Points represent sites (n=27). Sites: M2 = 2 year old mining restoration, M5 = 5 year old mining restoration, M10 = 10 year old mining restoration, M20 = 20 year old mining restoration, MR = jarrah remnant, R2 = 2 year old residue restoration, R5 = 5 year old residue restoration, R10 = 10 year old residue restoration, and RR = tuart remnant. Dp: particle density; BD: soil bulk density; EC: electrical conductivity; OC: total organic carbon; N: total nitrogen; C:N: ratio of organic C to total N; NH4-N: ammonium nitrogen; NO3-N: nitrate nitrogen; P: phosphorus Colwell; K: potassium Colwell; CEC: cation exchange capacity.

2.5   Discussion

This study assessed a number of soil characteristics across a chronosequence within two different restoration systems, mining restoration and residue restoration, and compared them to adjacent remnant vegetation sites. The results support the hypothesis that the physical, chemical, and biological characteristics of the soils would be distinctly different between the two restoration systems, and that there would only be limited changes in these parameters across the chronosequence. The three residue sand treatments are fundamentally different to the tuart remnant forest, which is not surprising given that residue sand has high alkalinity, salinity, sodium content, and poor nutritional status and remains as such across the chronosequence. There is considerably less distinction between the jarrah forest remnant and the four restored mining restoration sites, with the indications of similarity increasing across the chronosequence.

Soil characteristics can be used as direct indicators of soil properties or as indirect indicators of soil ecosystem functions (Thomsen et al., 2012; Muñoz-Rojas et al., 2016a). Therefore, soil characteristics are used to assess, monitor, and evaluate the direction of change of restoration success after anthropogenic disturbances (Barros et al., 2013; Muñoz-Rojas et al., 2016b). Defining "ideal" soil characteristics is challenging as the target varies between location and soil type (Karlen et al., 1997; Bastida et al., 2008). The soil physiochemical characteristics defined in this study clearly show that the residue substrate remains highly distinct with minimal change occurring across the chronosequence.

Soil organic carbon is one of the key differences. In a given ecosystem, many soil characteristics—including cation and anion exchange capacity, nutrient turnover, soil colour, water holding capacity, and soil aeration—are influenced by organic C within the soil (Banning et al., 2008; Muñoz-Rojas et al., 2012; Muñoz-Rojas et al., 2016a). Therefore, soil organic C has been widely used as a key soil indicator. The extremely low levels of soil organic C observed in the residue restoration sites of this study are an indication of highly degraded surface soils and are similar to the

results of previous studies on this system (Banning et al., 2011c; Goloran et al., 2013), with soil carbon values distinctly lower than that of reference coastal sands.

Total C:N ratio is also associated with soil productivity. Banning et al. (2011b) found a lower C:N ratio in older rehabilitation sites than in reference forest plots. Further, Ganjegunte et al. (2009) reported significantly lower C:N ratios in mined soils than in unmined (undisturbed) soils. However, in this study, the soil C:N ratio was observed to be higher in two-year-old rehabilitation sites as compare to reference sites in both rehabilitation systems. This might be because of the very low amounts of N or N fixing microorganisms in the two-year-old rehabilitation sites as compared to the reference sites. Moreover, the high alkalinity of residue sand may cause denitrification, and N leaching and volatilization (Sharifi, 2007; Chen et al., 2010a; Goloran et al., 2013), which may result in low N levels in residue sand.

The two-year-old mining restoration sites have little or no organic matter turnover as there is a lack or absence of organic matter input to the soil. The high level of organic carbon observed in the two-year-old mining restoration sites might be because of the topsoil application practice at mining restorations. In the mining process, the above ground vegetation is logged and cleared prior to topsoil stripping (Koch, 2007a). This process could result in a lot of organic matter being left on the topsoil that is then incorporated when it is stripped and transferred. Initially, added topsoils might improve with the input of organic matter from both flora and fauna as the rehabilitation progresses, resulting in very-high levels of soil organic C at five-year-old mining restoration sites. However, mining restoration sites might reach a transition stage and become independent from initial topsoil applications when they mature further, which might be the reason that a decrease in organic C levels was observed in 10-year-old as compared to five-year-old mining restoration sites. High and very high soil organic C levels in mining restoration sites indicate high soil structural stability, sound structural conditions, good water holding and pH buffering capacities, and sufficient soil nutrient levels. On the other hand, low soil organic C content is generally associated with low water holding capacity, low soil biodiversity, high soil bulk density, unstable soil

structure, and poor soil fertility (Lal, 2004; Shrestha and Lal, 2006; Ganjegunte et al., 2009).

Soil biological indicators can be sensitive parameters to detect differences between soil types as soil microbial indicators provide information on the pedogenesis trajectory following restoration. Measuring soil microbial respiration provides important information on soil carbon and nutrient cycling (Muñoz-Rojas et al., 2016a). Surprisingly, residue restoration sites were observed to have higher microbial respiration compared to mining restoration sites, despite having low soil carbon. This might be because of the surface wood mulch addition in residue restoration, which might bring in associated microbial communities (Koch, 2007a). However, the gradually increasing microbial respiration in mining restoration sites indicates that soil function development is moving towards the trajectory of the undisturbed jarrah remnants. Soil organic carbon accumulation is the principal factor that affects soil microbial properties (Frouz and Nováková, 2005). Although soil respiration was high and variable in residue restoration sites compared to mining restoration sites, the results of this study showed no significant correlation between soil microbial respiration and soil organic carbon. This might be because, instead of establishing as a result of soil organic matter, the existing microbial communities are travelling with the wood mulch and topsoil. Moreover, elevated soil microbial respiration levels can be an indication of an unhealthy system that loses soil organic matter because of intense tillage or other soil health degrading factors (NRCS, 2012). It is essential to establish self-sustaining microbial communities on restored sites.

Ground-dwelling invertebrates distribute organic matter in the soil profile (Wolters, 1991; Frouz et al., 2003; Kilpelainen et al., 2007). Understanding their distribution and abundance across the study system will help understand the functioning and trajectory of these sites and is the focus of the rest of this thesis. It is predicted that community function and invertebrate species composition will differ across the two study systems and older restoration sites will resemble remnant sites more closely. Further, it is predicted that the development of ground-dwelling invertebrate fauna will be correlated with the development of soil ecosystem functions like seed dispersal and litter decomposition.

Chapter 3　Invertebrate taxa and functional group diversity in two different bauxite mining restoration systems

3.1　Abstract

Invertebrates are described as ecosystem drivers as they are involved in numerous ecological functions. This study compared the recovery of ground-dwelling invertebrate communities and functional group diversity in a chronosequence of restored forest sites within two distinctly different restoration projects associated with bauxite mining: transfer of topsoil stockpiled for less than a year into the mine pit (mining restoration) and revegetation on bauxite residue generated during the refining process (residue restoration). Ground-dwelling invertebrate assemblages and functional group diversity in each restoration area were compared to those found in undisturbed remnant forests. Sampling was undertaken over a two-year period, covering both wet and dry seasons. Surface active invertebrates were sampled using pitfall traps and litter invertebrates were extracted using Tullgren funnels. Invertebrates were sorted to the ordinal level and were subsequently classified into functional groups. Ants (Family Formicidae) were sorted to the level of species and classified into functional groups in relation to stress and habitat disturbance. Soil invertebrate and ant abundance was correlated with soil physiochemical and microbiological properties (Chapter 2). Soil invertebrate abundance and functional group diversity increased with restoration age, more rapidly so in mining restoration than in residue restoration. Invertebrate functional group diversity was higher, but abundance was lower in mining restoration as compared to residue restoration. Further, ant abundance and functional group diversity were greater in mining restoration than in residue restoration. Soil porosity, bulk density, total organic carbon, ratio of organic C to total N, and soil microbial respiration were significantly correlated to ant community assemblages. Coleopteran species richness was significantly different between the two restoration systems and between study sites but did not show any clear pattern in relation to restoration age. The return of missing invertebrate

community assemblages and ant functional groups will assist both restoration systems in achieving a fully functioning ecosystem.

3.2 Introduction

Monitoring of biological diversity is essential for determining the success of restoration (Ruiz-Jaen and Aide, 2005). The comprehensive assessment of every element of an ecosystem is unrealistic (Nally and Fleishman, 2002), so select indicators are used to assess community structure and functioning (Majer, 1983; Bisevac and Majer, 1999; Agosti et al., 2000; Andrés and Mateos, 2006; Fattorini et al., 2011). Typically, higher plants and vertebrates are used as they are easy to collect, and many are easily identifiable by sight (Greenslade and Greenslade, 1984; Cristescu et al., 2012).

Invertebrates are infrequently used in assessment but should not be overlooked as they comprise the majority of animal biomass, are highly diverse (Lavelle et al., 2006), and have an important role in many ecosystem functions. For example, soil invertebrates represent 23 % of the total described diversity of living organisms (Decaëns et al., 2003). Soil invertebrates perform very important ecosystem functions including litter decomposition, pedogenesis, facilitating soil aeration, seed dispersal, pollination, and providing a food source for vertebrates (Nichols and Bamford, 1985; Langmaack et al., 2001; Majer et al., 2006; Jouquet et al., 2011). They also act as ecosystem engineers, maintaining and improving the stability of the biological, physical, and chemical properties of soil (Nichols and Burrows, 1985; Casimiro et al., 2019).

3.2.1 Ground-dwelling invertebrates as a soil quality and environment assessment tool

Although under-utilised in restoration monitoring, ground-dwelling organisms such as protozoa, nematodes (Park et al., 2011), earthworms (Jouquet et al., 2014), and insects (Langmaack et al., 2001; Andersen and Majer, 2004; Cole et al., 2016) have all been used as biological indicators of soil health. The indicator qualities of terrestrial invertebrates are widely recognized in the context of detecting ecological change associated with human land-use (Rosenberg et al., 1986; Kremen, 1992; Lindenmayer et al., 2002; Fattorini et al., 2011; Barton and

Moir, 2015) and are useful for quantifying the ecological impact of chemically contaminated soils (Blakely et al., 2002).

Many invertebrates are substrate specific, demanding particular habitat requirements for their occurrence and survival (Griffin, 1998). Therefore, it is recommended that key arthropods are monitored in restoration assessment (Lindenmayer et al., 2002; Courtney et al., 2010; Barton and Moir, 2015; Cole et al., 2016). Invertebrates groups such as ants, beetles, termites, collembolans, hemipterans, and spiders have been used to track the state of a recovering ecosystem (Majer, 1989a; Greenslade and Majer, 1993; de Bruyn, 1997; Pik et al., 2002; Majer et al., 2006; Orabi et al., 2010), and ecosystem functions have been correlated to changes in specific functional groups (Salminen and Haimi, 1997). For example, earthworms have been promoted as indicators of soil health to monitor the sustainability of Australian farms (de Bruyn, 1997). In a similar study, soil fauna has been used to assess decomposition processes under conditions of chemical contamination (Salminen and Haimi, 1997).

Collembolans are the primary colonizers of disturbed environments, and temporal changes in their composition are considered useful for monitoring restoration progress (Greenslade and Majer, 1993). The species richness, composition, and diversity of collembolan communities differ according to environmental conditions such as soil type, soil water content, and peat composition (Sławska, 2000).

Soil invertebrate fauna influences soil texture and consistency, turnover rate, total and macro porosity, organic matter mineralization, infiltration rate, and water retention characteristics (Lal, 1988b). Typically, ants mediate soil chemical property changes by shifting soil pH to neutral and increasing soil nutrient content, especially nitrogen and phosphorus (Frous and Jilkova, 2008). Collembolans and mites are responsible for surface litter decomposition, which releases carbon and nutrients to the soil (Blakely et al., 2002; Prober et al., 2014). Predatory invertebrates—i.e. collembolans, termites, and predatory ants—serve an important role in controlling pest outbreaks in newly re-vegetated areas (Nichols and Burrows, 1985). Termites are involved in litter decomposition, soil turnover,

and the creation of deep burrows and galleries, which aerate the soil (Jouquet et al., 2006b).

3.2.2 Ants as ecological indications

Ants are one of the most diverse and abundant animal taxa in Australia and are found in almost all terrestrial environments of the continent (Grimbacher and Hughes, 2002), playing vital roles as soil aerators, predators and prey, herbivores, granivores, scavengers, and plant mutualists (Lach and Thomas, 2008; Casimiro et al., 2019). There are 13 subfamilies, 61 genera, and 497 described ants species in Australia, with many more species still to be described (Heterick, 2009). Little is known about the ecology and biology of many of the described and undescribed species (Lach and Thomas, 2008).

Ants have commonly been used as ecological indicators in Australia due to their high abundance occupancy of high trophic levels, sensitivity to changes in the environment, ease of sampling, relative ease of identification (compared to other terrestrial invertebrates), and due to the presence of many specialist predators in the taxa (Majer, 1983; Greenslade and Greenslade, 1984; Pik et al., 2002; Andersen and Majer, 2004; Casimiro et al., 2019). The number and composition of ant species in an area can indicate the health of an ecosystem and provide insight into the presence of other organisms, since many ant species have obligate interactions with plants and other animals (Agosti et al., 2000).

Many ants build nests that are entirely or partially submerged in the soil, which they inhabit for several months or for many years (de Bruyn and Conacher, 1990). These nests increase soil microporosity and reduce bulk density, with soil particle separation based on the body size of ant species (Holec and Frouz, 2005; Frouz and Jilková, 2008). When ant communities are severely disturbed, they are unable to effect soil bulk density (Kilpelainen et al., 2007), which could lead to a decrease in water permeability and soil aeration (Hazelton and Murphy, 2007). Ant nests also increase water repellence during times of low soil moisture by increasing soil organic matter content within the nest (Frouz and Jilková, 2008). The amount of ant soil excavation varies depending on habitat and ant species. Estimates vary from 350–420 kg of soil per year per ha in Australian prairie habitats (Briese, 1982)

to 600 kg per year per ha in the USA (Lyford, 1963). In Australian prairie habitats, *Pheidole* sp. and *Iridomyrmex* sp. are known to excavate 75–90 kg and 150–180 kg of soil per year per ha, respectively (Briese, 1982). Ants are also capable of regulating the temperature inside their nests—by trapping solar radiation (Frouz, 2000) and metabolic heat production (Horstmann and Schmid, 1986), and maintaining in-situ microorganism cultures (Frouz, 2000)—which helps regulate soil temperature. Many ant species use surrounding plant remnants as insulation material for anthills in order to maintain constant temperature inside ant nests (Frouz and Jilková, 2008).

Ants can alter soil chemistry (Chen, 2005). Ants shift soil pH towards neutral values and increase soil nutrient content (Frouz et al., 2003; Frouz and Jilková, 2008). However, the influencing factor behind the pH shifting mechanism is unclear. It is assumed that accumulation of basic cations may increase the pH of acidic soils and organic matter accumulation may decrease the pH of basic soils (Frouz et al., 2003). Ants also play an important role in changing aboveground C, N, and P content (Frouz et al., 2003; Frouz et al., 2005; Kilpelainen et al., 2007). Micronutrient accumulation in and close to ant nests varies between ant species (Briese, 1982; Wagner and Jones, 2006); furthermore, within a species, nutrient accumulation varies based on surrounding soil properties and available building material (Frouz et al., 2003). Ants are also responsible for accumulating easily degradable materials and increasing available P content (Frouz and Jilková, 2008).

3.2.3   Ant responses to disturbance

Ant community responses to disturbance are well studied compared to other invertebrate groups, especially in Australia. Pioneering work in Australia by Majer (1976) and colleagues identified patterns of ant recolonization relating to restoration age (Majer, 1976). Subsequent surveys identified the importance of plant species richness and diversity, presence of litter cover and large logs, and age since restoration in influencing the reestablishment of a diverse ant community in post-mining restorations (Majer, 1983, 1985; Majer, 1989b; Majer et al., 2007b). Surveys in 37-year-old Western Australia bauxite mining restorations, which used a range of restoration methods, found that although ant

diversity was achieved to the same level as that of the remnant sites, species composition was not the same (Majer et al. (2013), indicating the challenge of achieving full species recovery.

Ants have been used as bio-indicators as they respond to a wide range of disturbances such as fire (Andersen, 1991a; Vanderwoude et al., 1997; Gunawardene and Majer, 2005; Andersen et al., 2009; Majer et al., 2011), mining (Majer et al., 1982; Majer, 1983, 1984b, 1985, 1992; Andersen, 1993; Majer, 1996; Holec and Frouz, 2005), agricultural practices (Weir, 1978; Burbidge et al., 1992; Bruyn, 1993; Perfecto and Snelling, 1995), road construction, and urbanisation (Majer and Brown, 1986; Samways, 1997). Ants often abandon their nest after forest clearcutting as it changes microhabitats and removes food resources (Rosengren et al., 1979; Punttila et al., 1996; Majer et al., 1997). Ants species do not always show consistence responses to disturbances; responses vary based on the intensity of disturbance, exposure period, and habitat type (Hoffmann and Andersen, 2003; Casimiro et al., 2019). Hoffmann and Andersen (2003) describe three confounding factors to explain variable ant responses to disturbances: 1) habitat variability, as disturbance affects habitat suitability; 2) non-liner species response, as responses might vary according to the severity of the disturbance; and, 3) time since initial disturbance, as instantaneous responses might vary from long term responses.

Australian ants have been categorized into functional groups, predominantly at the generic level, according to their environmental preference and responses to habitat disturbances (Table 3.1). Greenslade (1978) originally proposed a functional group classification for Australian ants based on their competitive interactions, evolutionary history, and habitat requirement. The original classification was later modified by Andersen (1990) so as to be based more on community dynamics than on evolutionary history. This ant functional group scheme has been a useful tool to assess disturbance in mesic Australia (Hoffmann and Andersen, 2003; Gove et al., 2007). For example, these functional groups have subsequently been used to monitor the revegetation success in Uranium mining restorations (Andersen, 1993) and to assess the ant community response to logging and experimental fire in a eucalyptus forest in south-eastern Australia

(Andersen et al., 2009). In terms of their response to stress, disturbances, and competition, Dominant Dolichoderinae (DD) are highly abundant in hot, open habitats, and are highly active and aggressive species with large foraging ranges (Andersen, 1995). Unlike DD, Generalized Myrmicinae (GM) have small foraging ranges, and are not highly active or aggressive. Opportunists (OPP) are considered as unspecialized, less competitive species that are usually more prominent in stressed and disturbed habitats (Andersen, 1995).

Table 3.1. Classification of Australian ants into functional groups, depending on their continental scale response to environmental disturbances and stress (Adopted from Andersen (1995, 1997); Hoffmann and Andersen (2003); Majer et al. (2013))

Functional group	Characteristics	Taxa
Dominate Dolichoderinae (DD)	Highly abaundant in hot and open habitats, employ highly competitive and aggressive behavior on other ants, highly active foragers	*Iridomyrmex* *Anonychomyrma*
Subordinate Camponotini (SC)	Co-occur with DD but subordinate competitors, dominate habitat in the absence of DD, relative abundance is normally low in a given community, large body size, nocturnal foragers	*Camponotus,* *Polyrhachis,*
Hot-climate Specialists (HCS)	Occur where abundance of DD is highest, have a range of different behavioural, morphological, and physiological foraging ecology, little interaction with other ants	*Melophorus,* *Meranoplus*
Cold-climate specialists (CCS)	Occur where abundance of DD is low, in cool environments	*Prolasius,* *Notoncus*
Tropical-climate specialists (TCS)	Occur where abundance of DD is low, in tropical environments	*Oecophylla,* *Tetraponera*
Cryptic Species (CS)	Generally minute to small species, occur within litter and soil, little interaction with other ants	*Hypoponera,* *Solenopsis*
Generalized Myrmicinae (GM)	Highly abundant and unspecialized species, rapid foragers, defend food resources, highly competitive for resources	*Crematogaster,* *Monomorium,* *Pheidole*
Opportunists (OPP)	Poorly competitive and unspecialized ants, occur in high densities in disturbed habitats and low densities in undisturbed habitats, distribution is strongly limited by the competition of other ant species	*Rhytidoponera,* *Tetramorium,* *Paretrechina*
Specialist Predators (SP)	Large body size, solitary predators, specialized diet, occur in low population densities, little competitive interaction with other ant species for food	*Leptogenys,* *Brachyponera*

Bauxite mining in the jarrah forest of Western Australia highly alters the soil profile; as such, it is vital to re-establish the soil ecosystem and associated

processes once mining operations cease. In this type of restoration system, the presence or absence of ants is relatively well-studied (Majer and Nichols, 1998; Majer et al., 2007b; Majer et al., 2013). However, no surveys have looked at ant communities within a residue restoration environment.

This study aims to quantify the degree of re-establishment of the ground-dwelling invertebrate community and invertebrate functional group diversity in relation to time since restoration and physiochemical changes occurring across a chronosequence in jarrah forest bauxite mining restoration and residue sand revegetation in Western Australia. Here, Formicidae and Coleoptera are used to assess restoration success. The following research questions were addressed:

- Do ground-dwelling invertebrates differ in composition and diversity between mining and residue restoration systems?
- Does age since restoration affect ground-dwelling invertebrate assemblages and do older sites resemble remnant sites more closely?
- Do ant assemblages and functional group diversity differ between mining and residue restoration systems and, further, do they gradually resemble the assemblages and diversity of adjacent remnant forests as time since restoration increases?
- Are there invertebrate species that could be considered indicators of successful restoration?
- Does the development of soil invertebrate fauna correlate with changes in soil physiochemical and microbiological characteristics?

3.3   Methods

3.3.1   Study sites

Invertebrate sampling was conducted in the same plots used for soil sampling (see Chapter 2.3.1 for details of study sites).

3.3.2   Invertebrate collection methods

Ground-dwelling invertebrate fauna were sampled over a two-year period to include two wet months (early September 2013 and 2014) and two dry months

(early March 2014 and 2015). Litter samples were collected from five locations within each plot (obtained by placing a 19 cm × 19 cm quadrat randomly in five locations, and gathering the litter within), and combined into a calico bag (Majer and Greenslade, 1988; Postle et al., 1991; Majer et al., 2002). Upon collection, litter samples were kept under cool conditions (approximately 20 °C) until transportation to the laboratory (3 to 12 h post-collection). Litter-dwelling invertebrates were extracted within 24 h of collection by using Tullgren funnels (Figure 3.1a), as described in Postle et al. (1991), and raising the temperature from ambient to 40 °C over a period of 7 d. The litter was suspended in funnels that had two types of meshes: a smaller polyester mesh and a larger metal mesh. The smaller polyester mesh sat on top and prevented litter fragments from falling into the preservative while the larger metal mesh sat on the bottom and permitted the passage of larger invertebrates.

In each plot, six pitfall traps (40 mm internal diameter, 120 ml volume, and ¼ filled with ethylene glycol) were established at least 2 m apart. Traps were left open for seven consecutive days (Greenslade and Majer, 1993). When collecting the traps for the first time, new tubes with lids were inserted to maintain the sampling location but not to trap invertebrates.

Invertebrate samples were transferred from ethylene glycol to 85 % ethanol within 7 d of collection for preservation. All invertebrates were sorted and counted to at least the ordinal level. For the most abundant subclass, Collembola, a gridded line insect counting tray with a total of 25 squares was used to estimate the abundance. Collembolans were separated and evenly distributed across the gridded line insect counting tray, and individuals in the four corner cells and the middle cell (five cell counts) were counted and multiplied by five to estimate the total abundance.
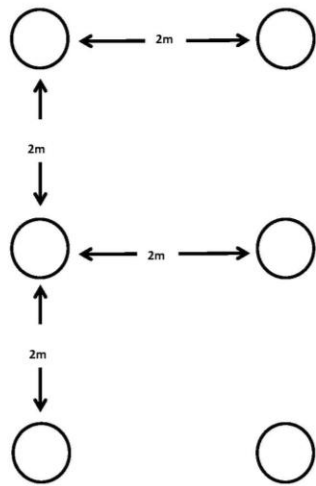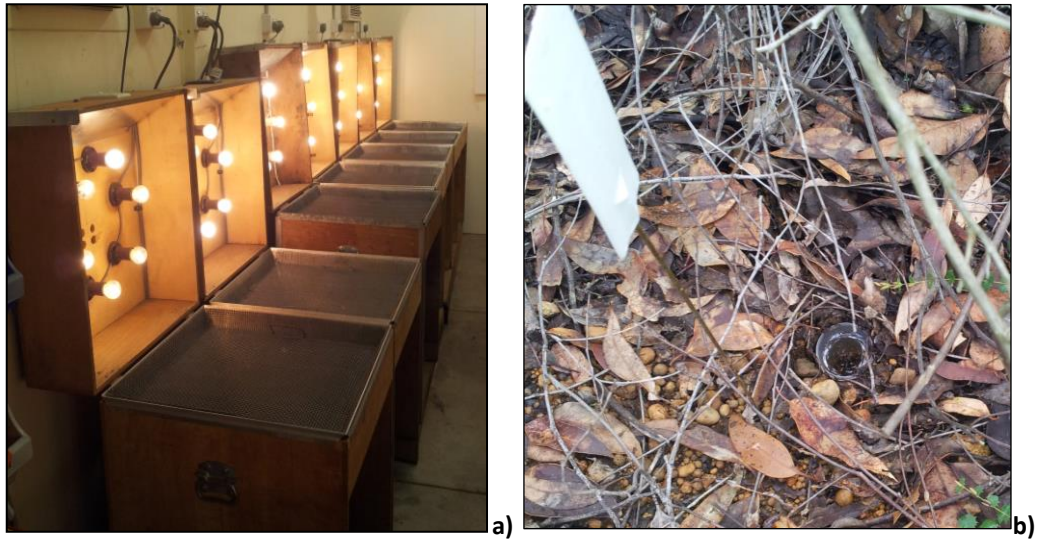
Figure 3.1. Invertebrate sampling methods: a) Tullgren funnels for extracting litter invertebrates b) pitfall trap to collect ground-dwelling invertebrates and c) diagram of pitfall trap layout in the field

Within the Hymenoptera, the ants (Family: Formicidae) were removed, counted separately and further sorted to the species level. Ant species collected from pitfalls were assigned to functional groups at the generic level (Table 3.1) based on their response to ecological interaction, niches, and habitat disturbance

(Andersen, 1995, 1997; Andersen et al., 2002; Andersen and Majer, 2004; Heterick, 2009).

3.3.3 Data analysis

All abundance data were log (x+1) transformed to minimize the effect of large variations in abundance of certain orders/species.

Shannon diversity index (H'), Shannon evenness index (e^H'/S), and Mean Dominance index (D) were used; the analysis of the most conspicuous and abundant species (Michener, 1997) and taxonomic richness were obtained for each rehabilitated site using Paleontological statistics (PAST) version 3.14. Shapiro-Wilk tests were performed using SPSS (version 21) to check the normality of diversity indices.

Differences in invertebrate species abundance and diversity between restoration ages within the two restoration systems were compared using two-way analysis of variance (ANOVA) in SPSS. For two-way ANOVA, restoration age and study sites were used as independent variables and the log-transformed abundance of a) all invertebrate orders, b) ants only, and c) ant functional group abundance were used as dependant variables. A Tukey's HSD posthoc test was used to compare treatments where a significant ANOVA was obtained.

Multivariate analyses were performed using PRIMER (version 6). A similarity matrix was constructed using Bray-Curtis measures from the log-transformed abundance of a) all invertebrate orders, b) ants, and c) ant functional groups.

Principal coordinate analysis (PCO) analysis and cluster analysis were performed to produce ordinations of similarity on the Bray–Curtis matrix. Sites positioned closer on the ordination plots were more similar to each other. Analyses of similarities (ANOSIM) and pairwise ANOSIM were used to test for differences in invertebrate composition between time since restoration (two, five, 10, 20 years

since restoration, and remnant) and between systems. The R- statistic was considered significant at $P < 0.05$.

Soil physiochemical and microbial characteristics (see Chapter 2 for more details) were correlated with a) abundance of all invertebrate orders, b) abundance of ants, and c) abundance of coleopterans.

To investigate if soil physiochemical and microbial characteristics (see Chapter 2 for more details) explain the variation in a) abundance of all invertebrate orders, and b) abundance of ants, a distance-based redundancy analysis (dbRDA) was applied via the distance-based linear modelling (DistLM) procedure using PRIMER 6 (version 6.1.16) with the add-on PERMANOVA (version 1.0.6). Euclidean distance is inappropriate for some type of data; therefore, dbRDA was applied for environmental data as it gets around this limitation (Anderson et al., 2008). Prior to the analysis, abundance data were log $(x+1)$ transformed, and environmental variables and soil physiochemical and microbial characteristics were normalised and Euclidean distance was used to generate a resemblance matrix. The amount of variation in the abundance data, which was explained by the environmental variables, was determined using DistLM. BEST selection and Akaike Information Criterion (AIC) selection criterion were used with +9999 random permutations (Anderson et al., 2008).

3.4   Results

3.4.1   Invertebrate species richness and abundance

A total of 89,420 individual invertebrate specimens were recorded over the four sampling periods. Generally, invertebrates were more abundant in the residue restoration system than the mining restoration system (Figure 3.2). Collembola was the most abundant group (77 % of the total sample), followed by Formicidae (8 %), Acari (6 %), Diplopoda (3 % - but with only four individuals in the mining plots), and Coleoptera (2 %). Overall, 76 Formicidae species (Table 3.2) and 28 coleopteran morphospecies were identified.

Higher level invertebrate group abundance differed between restoration ages and between the two restoration systems (Figure 3.2). Some groups showed clear

patterns; for example, Isopoda was only sampled when the restoration site matured to five years of age and was present in remnant sites of both restoration systems (Figure 3.2). In mining restoration, Blattodea, Chilopoda, and Neuroptera were not recorded at the two-year-old restoration site, and only started to appear from sites five years and older, including the jarrah remnant site. In the mining restoration system, molluscs were recorded only at the 20-year-old mining site and the jarrah remnant. However, in the residue restoration system, molluscs were recorded in all of the sites, including the tuart remnant site.
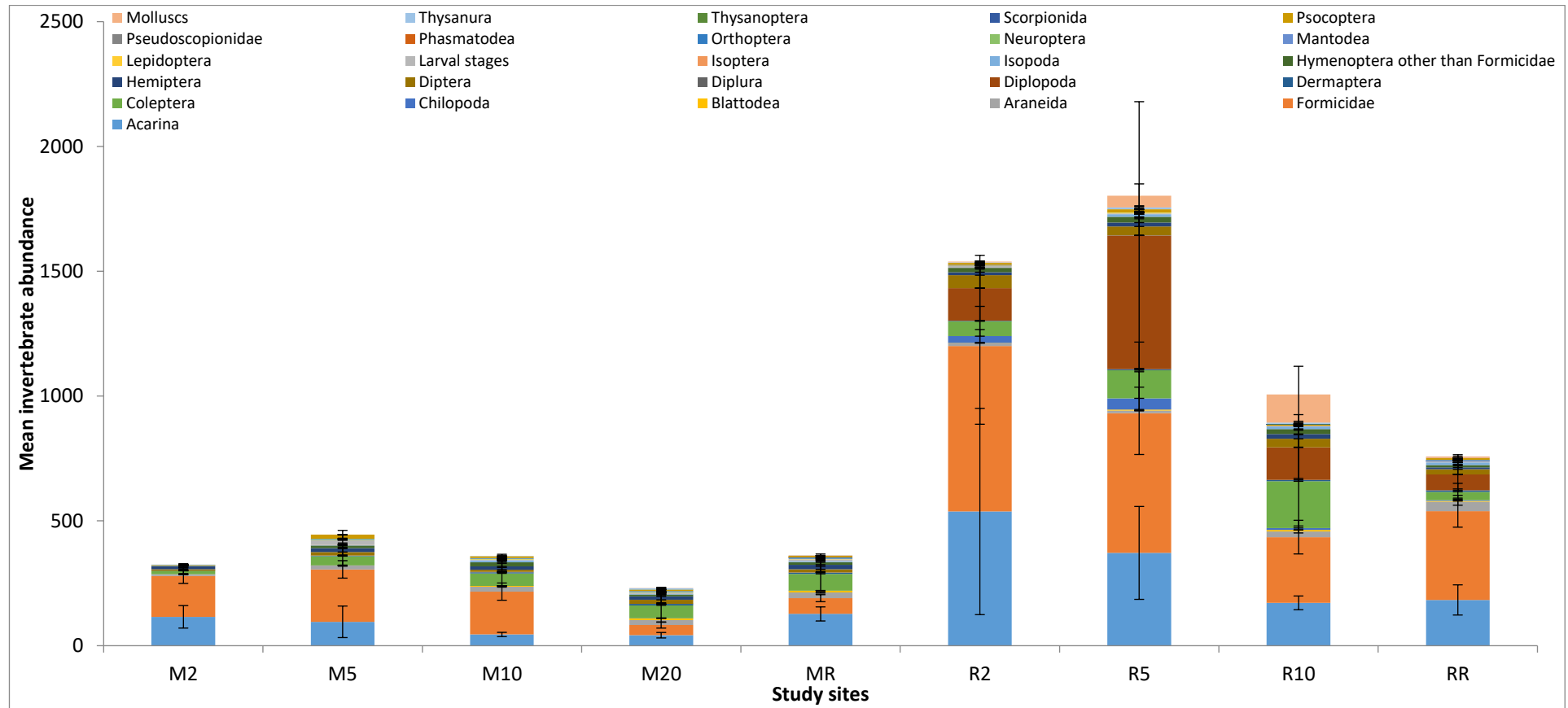
Figure 3.2. The mean invertebrate group abundance without Collembola (± SE) of three replicate plots in each site. Study sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

Mining restoration sites had significantly higher diversity (P < 0.05) than residue restoration sites, with the jarrah remnant site having the highest diversity (mean $H' = 1.537 \pm 0.104$) and the two-year-old residue restoration site the lowest (mean $H' = 0.619 \pm 0.137$). Invertebrate group diversity was affected by restoration system ($F_{1,89} = 54.907$, P < 0.001), age since rehabilitation ($F_{8,89} = 10.915$, P < 0.001), and sampling season ($F_{1,89} = 5.031$, P < 0.05). There was a significance interaction effect between age since restoration and sampling season ($F_{8,89} = 2.342$, P = 0.025) on the diversity of invertebrate groups. Invertebrate diversity was higher in the dry season (mean = 1.131 ± 0.065) compared to the wet season (mean = 0.968 ± 0.072). Restoration age showed a significant negative correlation to invertebrate ordinal diversity ($r_{107} = -0.426$, P < 0.001), evenness ($r_{107} = -0.606$, P < 0.001), and dominance ($r_{107} = -0.469$, P < 0.001).

The main objective of this study was to quantify the degree of ground-dwelling invertebrate community re-establishment in relation to time since restoration, and not to evaluate seasonal variation in invertebrate communities. Therefore, for each site, seasonal samples (i.e. samples collected during wet and dry months) were combined, providing the following results.

Ordinal diversity differed significantly between restoration systems ($F_{1,8} = 9.469$, P < 0.001). Mean invertebrate Shannon diversity (H') increased in both restoration systems as the age since restoration increased (

Figure 3.3a). All mining restoration sites had significantly higher mean Shannon diversity indexes (P < 0.05) as compared to the residue restoration sites, ranging from 1.29 in the five-year-old mining site to 1.55 in the jarrah remnant site; the exception was the two-year-old mining restoration site, which—with a diversity of 1.05—was not significantly different to the residue restoration sites (

Figure 3.3a). All sites in the residue restoration system had similar diversity, ranging from 0.62 in the two-year-old site to 0.74 in the 10-year-old site (

Figure 3.3a). The mean Shannon evenness of recorded invertebrate orders differed significantly between the two study systems ($F_{1,8} = 11.299$, P < 0.001); the

mining restoration system had greater evenness, with the two-year-old site having the highest evenness among all sites (

Figure 3.3b).

The mean Dominance index (D) of invertebrate groupings (see Figure 3.2) differed significantly between the restoration systems ($F_8 = 8.982$, $P < 0.001$). The highest D (0.726 ± 0.066)—significantly higher than the corresponding values of the mining restoration sites—was recorded in the two-year-old residue restoration site (

Figure 3.3c). All mining restoration sites, with the exception of the two-year-old site, had a significantly lower ($P < 0.05$) D value than the residue restoration sites (

Figure 3.3c).
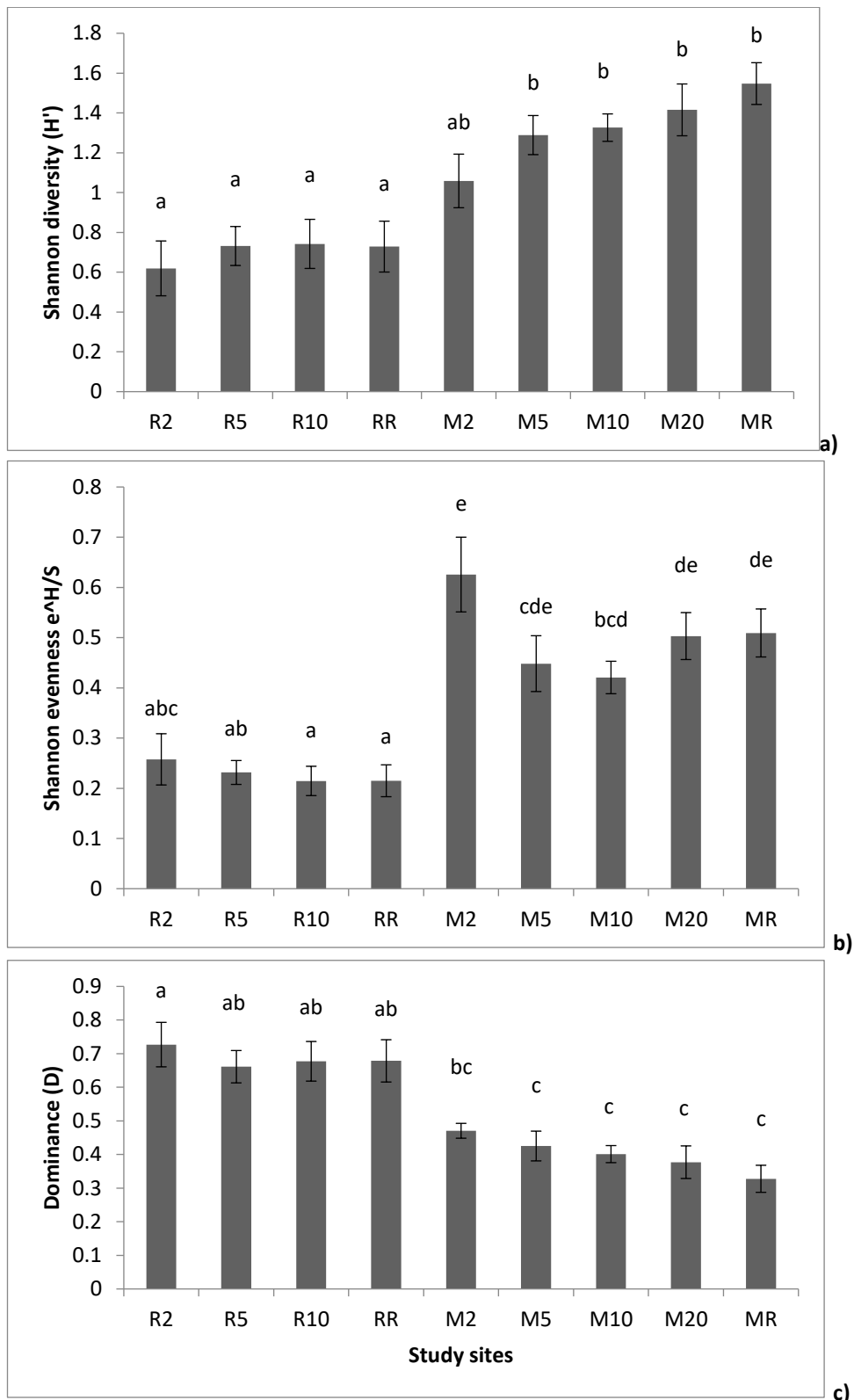
Figure 3.3. Comparisons of a) Shannon diversity (H'), b) Shannon evenness (e^H'/S), and c) Dominance (D) (mean ± SE) of higher level invertebrate groupings (see Figure 3.2) between different restoration ages (site means are calculated across all three replicates). Plots with different letters indicate significantly difference means (Tukey HSD, p < 0.05). Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

The results of a non-metric multidimensional scaling (NMDS) analysis showed that higher-level ground-dwelling invertebrate groupings were clearly separated into two large clusters based on the two restoration systems. Within the residue system, the restoration sites were clearly separated by age; further, all three restoration sites were distinct from the tuart remnant (

Figure 3.4). In the mining restoration system, the two-year-old site was distinct from the other sites.
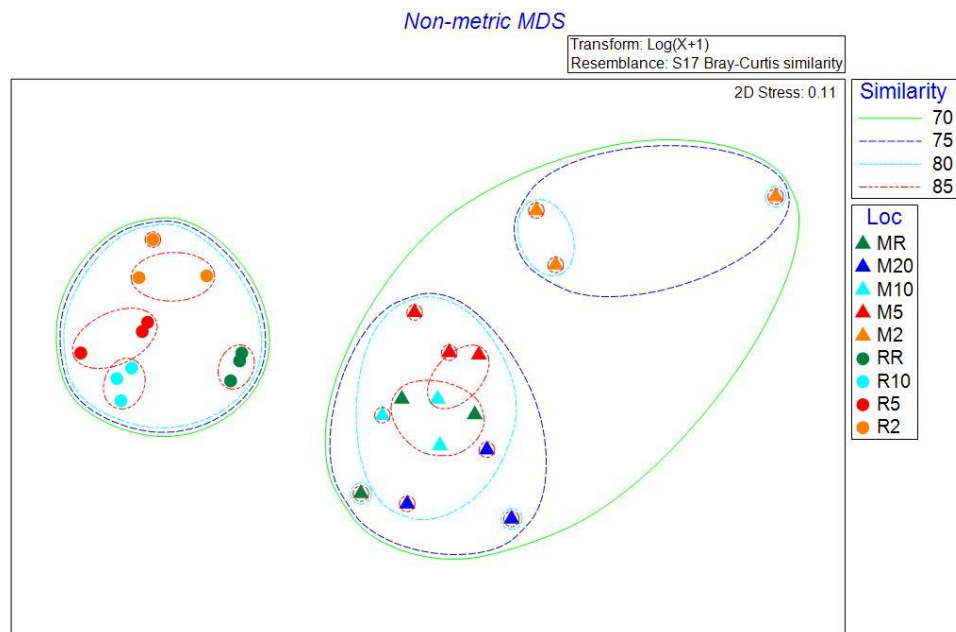
Figure 3.4. Non-metric multidimensional scaling (NMDS) plot of pitfall invertebrate abundance (log x+1) of each site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

3.4.2   Ant species richness, abundance, and diversity

A total of 7,865 ants representing eight families, 30 genera, and 74 species were recorded. Collectively, 44 and 50 species were recorded in the mining and the residue restoration system, respectively, with 24 species common to both systems (Appendix A). Ant species richness varied significantly between sites ($F_{8,35}$ = 3.309, P < 0.01), ranging from three to 25 species, with the younger restoration sites having significantly fewer species than the remnants; however, numbers

increased in both restoration systems with an increase in age since restoration (Figure 3.5a).

Ant diversity varied significantly between sites ($F_{8,35}$ = 3.309, P < 0.01), and increased with an increase in time since restoration (Figure 3.5c). The lowest diversity was recorded at the two-year-old mining restoration site.
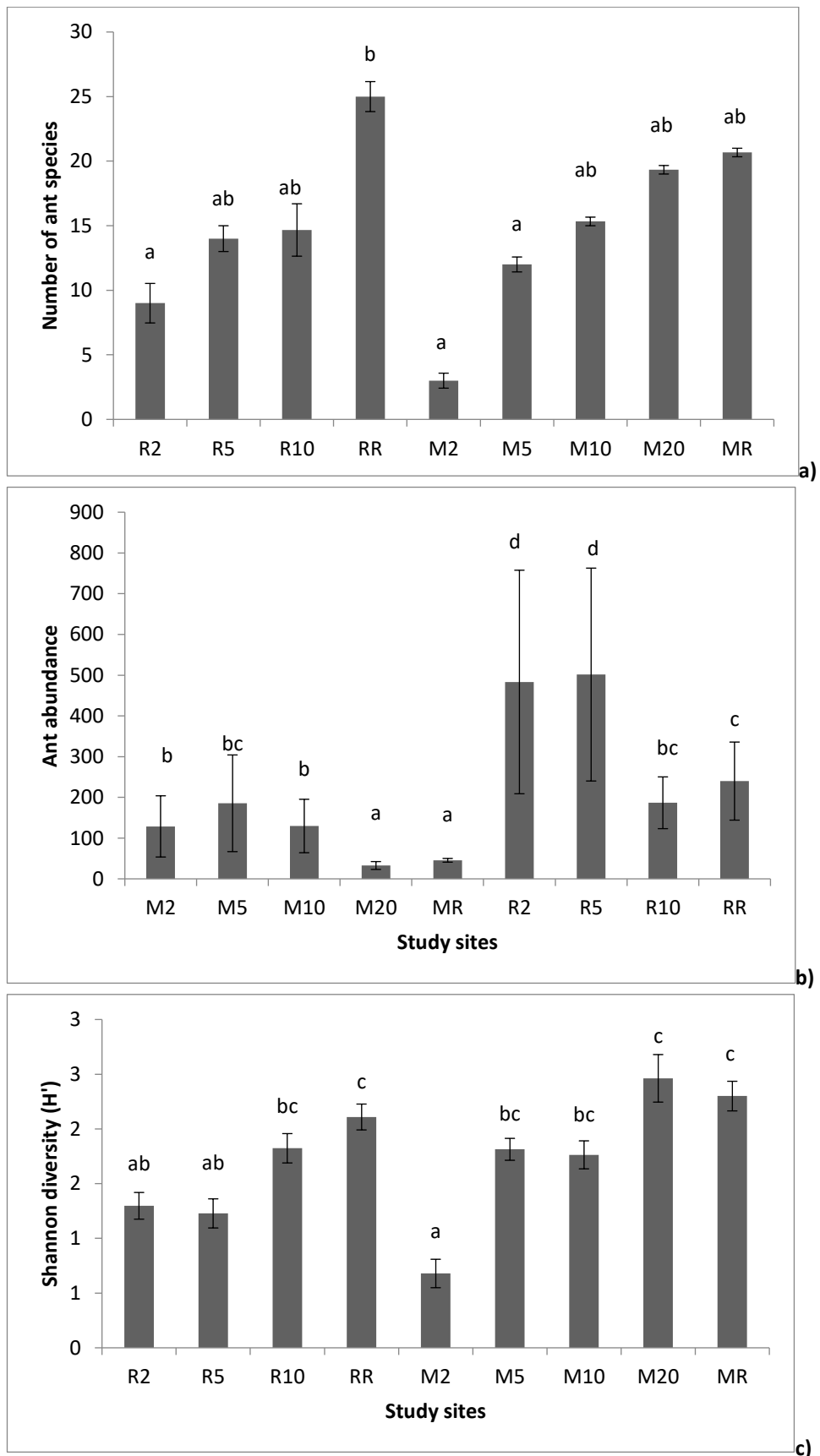
Figure 3.5. The mean (± SE) a) ant species richness, b) ant abundance, and c) Shannon diversity recorded at each site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

Ants were more abundant in the residue system than in the mining restoration system ($P < 0.05$), and differed significantly between sites ($F_{8,35} = 7.737$, $P < 0.01$). The highest mean abundance was recorded at the five-year-old residue restoration site (501.5 ± 261.2), followed by the two-year-old residue restoration site (483.25 ± 274.3), whereas the lowest mean ant abundance was recorded at the 20-year-old mining restoration site (32.75 ± 9.6) and the jarrah remnant site (45.5 ± 4.6). *Iridomyrmex* species *(I. chasei* Forel, *I. discors* Forel, *I. bicknelli*) were the most abundant taxa in most sites.

Several abundant species (present in many plots) were absent in young but plentiful in older rehabilitation and remnant sites (Table 3.2). *Arnoldius scissor* (Crawley) and *Prolasius antennatus* McAreavey were only sampled in jarrah remnant plots. *Myrmecia mandibulari* F.Smith, *Myrmecia vindex* F.Smith, *Stigmatomma panctulatum* (Clark), *Strumigenys perplexa* (F.Smith), *Crematogaster disper* Forel, *Leptogenys neutralis* Forel, and *Stigmacros bosii* (Forel) were recorded only in the jarrah remnant and the 20-year-old mining restoration site (Table 3.2). *Anonychomyrma itinerans perthensis* (Forel) was recorded in mining restoration sites older than 10 years and both jarrah and tuart remnant sites, but absent in younger mining restoration sites and all residue restoration sites. *Notoncus* spp. were only recorded during the wet season in the tuart remnant site, with the exception of one *Notoncus giberti* individual that was observed at the 10-year-old residue restoration site. Further, *Stigmacros glauerti* McAreavey, *Myrmecia urens* complex sp. JDM 1, *Hypoponera congrua* (Forel), *Rhytidoponera violacea* (Forel), and *Discothyrea turtoni* Clark were only recorded in the tuart remnant site. *Iridomyrmex gracilis spurcus* Wheeler, *Iridomyrmex rufoniger suchieri* Forel, *Nebothriomyrmex majeri* Dubovikov, *Camponotus* JDM 63, *Melophorus aeneovirens* (Lowne), *Melophorus ludius sulla* Forel, and *Solenopsis clarki* Crawley were only occurred in residue sites (Table 3.2).

Other ant species were predominatly sampled in the newly restored, i.e. disturbed, sites. For example, *Iridomyrmex bicknelli* Emery was the most abundant ant in the two-year-old mining restoration site, followed by *Rhytidoponera metallica*; however, none of the recorded species were only sampled in the

youngest sites, apart from *I. suchieri* Forel, which was only recorded in the two-year-old residue site.

Several abundant species were specific to mining or residue locations. Six species of *Melophorus* were recorded in all residue sites but were missing from the mining restoration system, with the exception of *Melophorus turneri perthensis* Wheeler, which was also recorded at the five- and 10-year-old mining restoration plots. The large meat ant, *Iridomyrmex purpureus* (F.Smith), was only recorded in the two-, five- and 10-year-old mining sites.

Other species were present in older restoration sites but not in the remnant sites. *Camponotus terebrans* (Lowne) was observed in sites aged five and above in both restoration systems, but not found in either of the remnant or the two-year-old sites.

Table 3.2. Mean ant species abundant (mean ± SE) with most abundant species (red font), and key indicator species (highlighted in blue) at each study site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant

Ant species	Study sites								
	M2	M5	M10	M20	MR	R2	R5	R10	RR
Arnoldius scissor (Crawley)	0±0	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Anonychomyrma itinerans perthensis (Forel)	0±0	0±0	1.33±0.33	1.33±0.33	9.33±6.83	0±0	0±0	0±0	36.66±29.48
Doleromyrma darwiniana fida (Forel)	0±0	0±0	0.66±0.66	2±0.57	20.33±8.37	0±0	0±0	0±0	0±0
Dolichoderus semiorbis Shattuck & Marsden	0±0	16.66±15.67	0.33±0.33	1.66±0.66	0±0	0±0	0±0	0±0	0.66±0.66
Iridomyrmex bicknelli Emery	109±19.13	44.66±8.19	54.66±39.5	6.33±4.48	0.66±0.33	51±21.51	11±6.55	25±23.5	5.66±3.84
Iridomyrmex chasei Forel	0±0	0±0	0±0	0±0	0±0	359.33±276.85	217.33±54.57	91.33±30.94	114±67.11
Iridomyrmex discors Forel	0±0	0±0	0±0	0±0	0±0	133.33±28.95	252.66±215	21.33±7.21	9.66±9.17
Iridomyrmex dromus Clark	0±0	8.33±7.35	0±0	0±0	0±0	1.66±0.33	6±1.52	2.33±1.85	0±0
Iridomyrmex gracilis spurcus Wheeler	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.66±1.66
Iridomyrmex notialis Shattuck	0±0	0±0	0±0	0±0	0±0	0±0	1.33±1.33	0±0	0±0
Iridomyrmex mjobergi Forel	0±0	19±7.21	5±3	0±0	0±0	0±0	1.33±0.88	1.33±0.33	0.33±0.33
Iridomyrmex purpureus (F.Smith)	0.33±0.33	35±20.64	22.66±13.01	0±0	0±0	0±0	0±0	0±0	0±0
Iridomyrmex rufoniger suchieri Forel	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Iridomyrmex splendens Forel	0±0	7.33±4.37	0±0	1±0.57	0±0	0±0	0±0	0±0	0±0
Iridomyrmex suchieri Forel	0±0	0±0	0±0	0±0	0±0	15.66±15.66	0±0	0±0	0±0
Iridomyrmex suchieroides Heterick & Shattuck	0±0	0±0	0±0	0.33±0.33	0±0	0±0	0±0	19.33±18.34	12±6.55

Nebothriomyrmex majeri Dubovikov	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Ochetellus glaber (Mayr)	0±0	0.33±0.33	3.33±2.02	4.33±1.45	2±0.57	0±0	0±0	0±0	0±0
Tapinoma minutum broomense Forel	0±0	0.66±0.66	2.33±0.88	2.66±1.66	4.66±2.72	0±0	0.66±0.66	0±0	1±1
Camponotus JDM 63	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.33
Camponotus claripes minimus Crawley	0±0	0±0	0±0	0±0	1±0.57	0±0	0±0	1±0	0.33±0.33
Camponotus claripes JDM 25	0±0	0±0	0.33±0.33	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Camponotus scratius Forel	0±0	1±0.57	0±0	0±0	0±0	0±0	1±1	0±0	0±0
Camponotus terebrans (Lowne)	0±0	10.66±5.36	36.66±8.66	9.33±7.33	0±0	0±0	9±8.5	2±2	0±0
Camponotus walkeri Forel	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.66	0±0	0±0
Melophorus aeneovirens (Lowne)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Melophorus JDM 230	0±0	0±0	0±0	0±0	0±0	0.66±0.66	0±0	0±0	5.66±1.76
Melophorus JDM 520	0±0	0±0	0±0	0±0	0±0	2±2	0.33±0.33	1.66±1.2	0±0
Melophorus JDM 783	0±0	0±0	0±0	0±0	0±0	0±0	3.66±2.66	16.33±6.64	3±2.51
Melophorus ludius sulla Forel	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	10.66±4.33
Melophorus turneri perthensis Wheeler	0±0	11±10	7.33±1.2	0±0	0±0	1±0.57	2.66±1.2	3±2.08	2.66±1.76
Notoncus enormis Szabo`	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Notoncus gilberti Forel	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33	3.33±1.85
Notoncus hickmani Clark	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.66±0.88
Prolasius JDM109	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Prolasius antennatus McAreavey	0±0	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0

Stigmacros aemula Forel	0±0	0.33±0.33	0.66±0.66	1±0.57	2.66±1.2	0.33±0.33	1.33±0.33	1±1	0±0
Stigmacros bosii (Forel)	0±0	0±0	0±0	2.33±0.33	1.66±1.2	0±0	0±0	0±0	0±0
Stigmacros brachytera McAreavey	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Stigmacros epinotalis McAreavey	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Stigmacros glauerti McAreavey	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1±0.57
Stigmacros JDM 115	0±0	0±0	0.66±0.66	0±0	0.33±0.33	0±0	0±0	0±0	0±0
Brachyponera lutea (Mayr)	0±0	0±0	0.33±0.33	0.66±0.33	1±0.57	0±0	0.33±0.33	1±1	0.66±0.66
Hypoponera congrua (Forel)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Hypoponera eduardi (Forel)	0±0	0±0	0.33±0.33	0±0	0±0	0±0	0.66±0.66	0±0	0±0
Leptogenys neutralis Forel	0±0	0±0	0±0	1±0.57	1.66±1.66	0±0	0±0	0±0	0±0
Austroponera rufonigra (Clark)	0±0	0±0	0.66±0.66	0.33±0.33	0.33±0.33	0±0	0±0	0±0	0±0
Rhytidoponera inornata Crawley	0±0	14.33±14.33	0±0	1.33±1.33	1.66±1.2	0±0	0±0	0±0	1±0.57
Rhytidoponera metallica (F.Smith)	57.66±19.93	76±13.52	35.33±3.17	11±9.53	10±10	0±0	0.33±0.33	7.33±0.88	12±4
Rhytidoponera violacea (Forel)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Discothyrea turtoni Clark	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Austromorium flavigaster (Clark)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Cardiocondyla atalanta Forel	4±4	1±0.57	0.33±0.33	2.66±0.88	3±2.51	36±16.52	0±0	0.66±0.33	0.33±0.33
Crematogaster disper Forel	0±0	0±0	0±0	0.66±0.66	2.33±0.88	0±0	0±0	0±0	0±0
Crematogaster laeviceps chasei Forel	0±0	0±0	1.33±0.33	2±0.57	1.33±0.33	0±0	0±0	0±0	6±2.64
Meranoplus ferrugineus Crawley	0±0	0±0	0.66±0.66	1.33±0.88	0±0	0±0	0±0	0.33±0.33	17.66±5.2

Meranoplus rugosus Crawley	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0	0.33±0.33
Meranoplus JDM 74	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Meranoplus JDM 968	0±0	0±0	1±0.57	0±0	0±0	0±0	0±0	0±0	0±0
Monomorium JDM 101	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Monomorium lacunosum Heterick	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.66	0.33±0.33	0.66±0.66
Monomorium leae Forel	0±0	0.66±0.66	0.66±0.66	1.33±0.66	2.66±1.2	0±0	0±0	0±0	5.33±5.33
Monomorium sordidum Forel	0±0	1±1	1.33±0.33	2±1	1.66±0.33	3±3	127.66±53.07	45.33±16.73	29.66±12.41
Pheidole JDM 164	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.33±1.33	2.66±0.88
Pheidole ampla perthensis Crawley	0.33±0.33	0±0	0±0	0.33±0.33	1±0.57	0±0	0.33±0.33	0±0	7.33±3.52
Solenopsis clarki Crawley	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	21.66±16.67
Strumigenys perplexa (F.Smith)	0±0	0±0	0±0	1±0.57	3.33±2.33	0±0	0±0	0±0	0±0
Tetramorium impressum (Viehmeyer)	0±0	0±0	0±0	0±0	0±0	34.33±9.59	26.33±6.35	6.66±1.85	0.33±0.33
Tetramorium simillimum (F. Smith)	0±0	0±0	0±0	0±0	0.66±0.66	6±3.78	3.33±3.33	0±0	0.66±0.66
Myrmecia clarki Crawley	0±0	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Myrmecia tepperi Emery	0±0	0±0	0.33±0.33	0±0	0±0	0±0	0±0	0±0	0±0
Myrmecia mandibulari F.Smith	0±0	0±0	0±0	0.33±0.33	0.66±0.33	0±0	0±0	0±0	0±0
Myrmecia urens complex sp. JDM 1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.33
Myrmecia vindex F.Smith	0±0	0±0	0±0	0.33±0.33	0.33±0.33	0±0	0±0	0±0	0±0
Stigmatomma panctulatum (Clark)	0±0	0±0	0±0	0.66±0.33	1±0.57	0±0	0±0	0±0	0±0

The Principle Coordinate Analysis (PCO) based on the abundance (log x+1) of ant taxa clearly separated sites into four groups, by restoration system (mining and residue) along axis 1 and by restoration age along axis 2 (Figure 3.6a). Residue sites were grouped together irrespective of age, although the remnant plots were somewhat distinct and the older sites tended to be closer to the remnant plots. The mining sites, however, showed a clear progression with time since restoration; the two-year-old plots were highly distinct from the remnant plots, the five- and 10-year-old sites were grouped closer to the remnant, and the oldest restoration site (20-years) was grouped with the remnant site. ANOSIM results indicated that ant community composition differed significantly between the two restoration systems (Global R = 0.765; P = 0.001).

Cluster analysis based on ant species richness supported these groupings. The two-year-old mining restoration sites were separate from all other sites (Figure 3.6b). The remaining mining and residue plots were divided into two distinct groups. Overall, there were four separate groups: Jarrah remnant plots and 20-year-old mining restoration plots, five- and 10-year-old mining restoration plots, tuart remnant plots, and all residue restoration sites, although the two-year old residue site was somewhat distinct from the five- and 10-year-old residue sites.
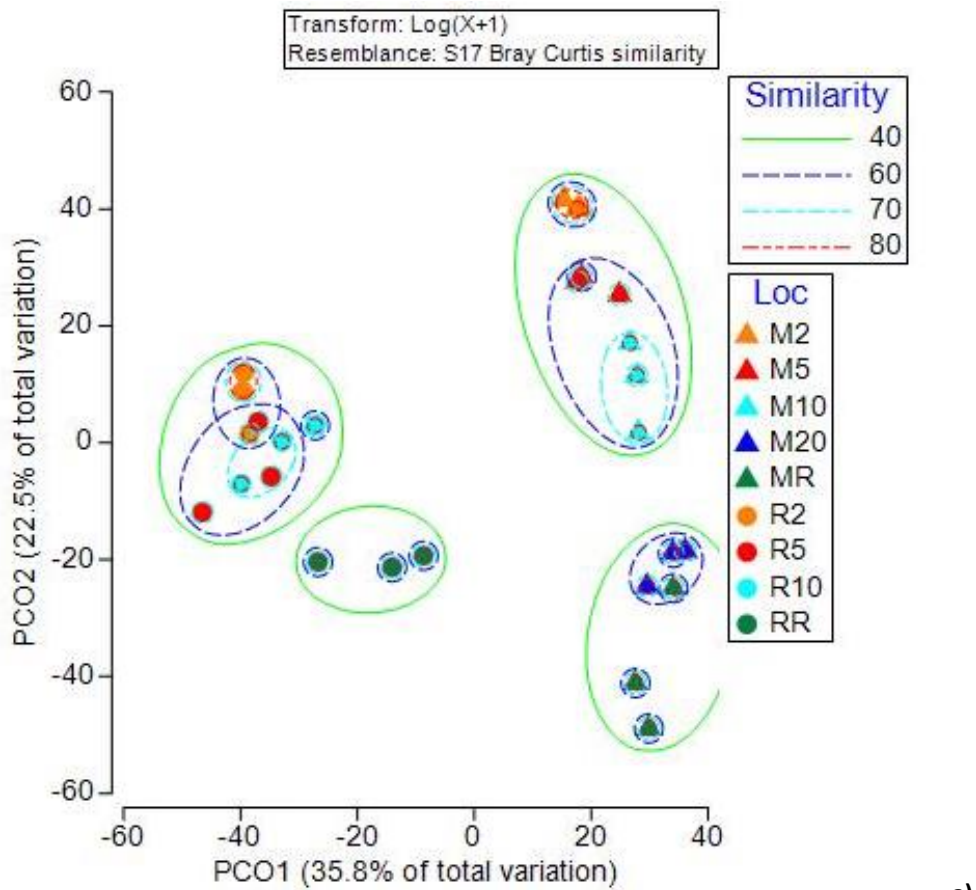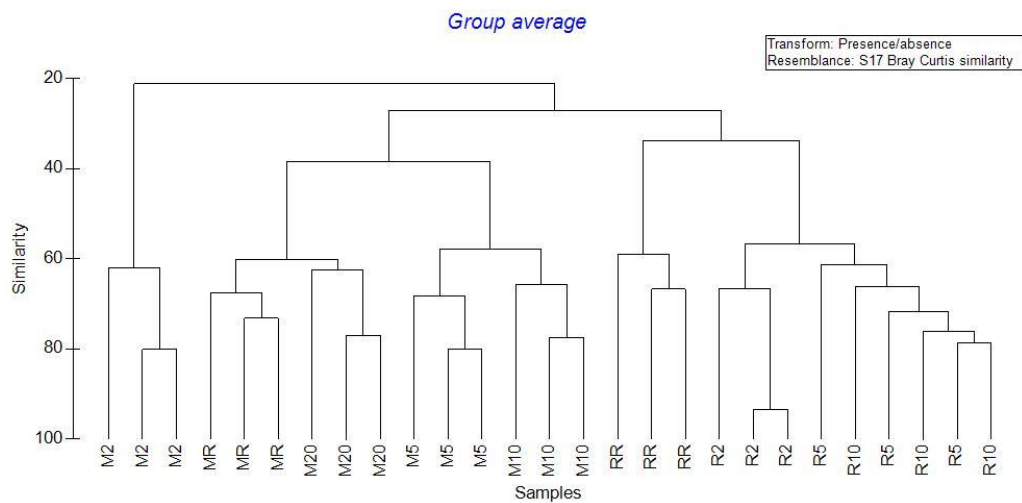
Figure 3.6. a) Principle Coordinate Analysis based on ant abundance (log x+1), and b) Bray-Curtis cluster similarity at 40 %, 60 %, 70 %, and 80 % of each site overlayed. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

3.4.3   Ant functional groups

A total of eight ant functional groups were recorded (Figure 3.7). In the residue restoration system, the number of species in each functional group increased as the age since restoration increased, except in the Subordinate Camponotini group. Specialist Predators and Subordinate Camponotini became evident at the restoration age of five and persisted in tuart remnant plots (Figure 3.7a). Restoration system had a significant effect on the abundance of Dominant Dolichoderinae ($F_{1,27}$ = 10.983, P < 0.01), Generalized Myrmicinae ($F_{1,27}$ = 16.409, P < 0.05), Hot-climate Specialists ($F_{1,27}$ = 14.098, P < 0.01), Opportunists ($F_{1,27}$ = 6.807, P < 0.05), Specialist Predators ($F_{1,27}$ = 4.778, P < 0.05), and Subordinate Camponotini ($F_{1,27}$ = 6.019, P < 0.05). Study site had a significant effect on the abundance of Generalized Myrmicinae ($F_{7,27}$ = 3.308, P < 0.05), Hot-climate Specialists ($F_{7,27}$ = 5.678, P < 0.01), Opportunists ($F_{7,27}$ = 4.748, P < 0.01), Specialist Predators ($F_{7,27}$ = 7.847, P < 0.001), and Subordinate Camponotini ($F_{7,27}$ = 5.153, P < 0.01). There was a significant interaction effect of restoration system (mining and residue) and age since restoration on the abundance of Generalized Myrmicinae ($F_{3,27}$ = 3.845, P < 0.05), Hot-climate Specialists ($F_{3,27}$ = 7.891, P < 0.01), Specialist Predators ($F_{3,27}$ = 6.225, P < 0.01), and Subordinate Camponotini ($F_{3,27}$ = 5.295, P < 0.01). In the mining restoration system, as the age since restoration increased, the number of species in Cold-climate Specialists, Generalized Myrmicinae, Opportunists, and Specialist Predators tended to increase, while Hot-climate Specialists were present in restoration but not sampled in remnant plots (Figure 3.7a). Cold-climate Specialists, Cryptic Species, Subordinate Camponotini and Specialist Predators appeared as the restoration reached five years of age and were also present in jarrah remnant plots. Specialist Predators appeared as the restoration matured further (10 and 20 years old) and were also present in the remnant site.

In both systems, ants were more abundant in the five-year-old restoration site as compared to the other sites (Figure 3.7b). Dominant Dolichoderinae were the most numerically abundant functional group in all sites, except in the 20-year-old mining restoration site and the reference mining site where Opportunists (OPP) were the most abundant. The proportional of Dominant Dolichoderinae ants to

others decreased and Specialist Predator ants increased in both restoration systems as the age since restoration increased (Figure 3.7b).
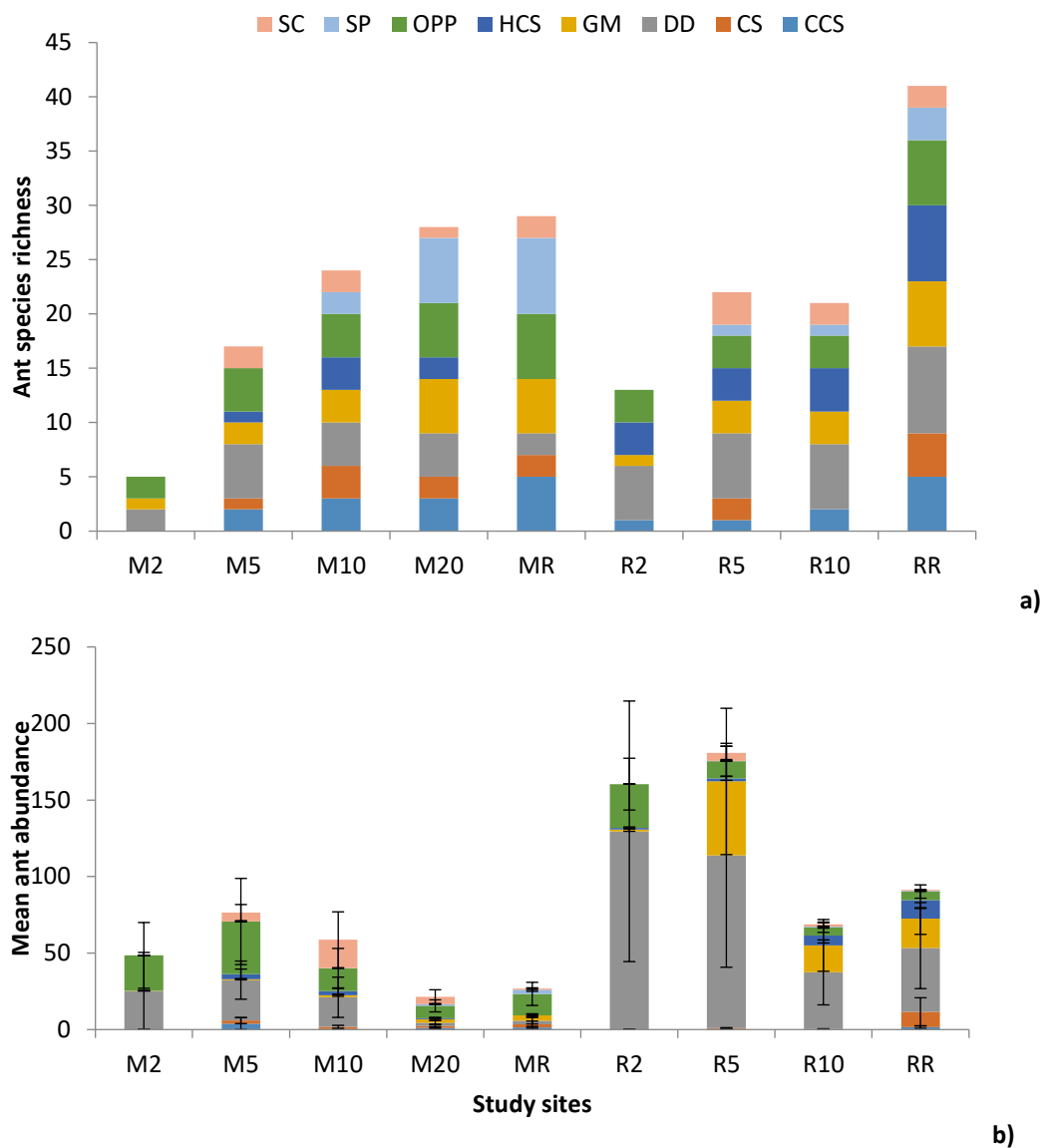
Figure 3.7. a) Ant species richness, and b) mean (± SE) ant abundance in each site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant. SC, Subordinate Camponotini; SP, Specialist Predators; OPP, Opportunists; HCS, Hot-climate Specialists ; GM, Generalized Myrmicinae; DD, Dominant Dolichoderinae ; CS, Cryptic Species; CCS, Cold-climate Specialists.

Principal coordination (PCO) analysis of ant functional group abundance (log + 1) separated sites into four clusters, by age since restoration along axis 1 and restoration system along axis 2; a clear progression from young to old sites was evident in the mining restoration system, but less so in the residue restoration system (Figure 3.8). The youngest restoration sites (two-year-old) of both

restoration systems clustered together (apart from one two-year-old residue plot) (Figure 3.8). ANOSIM results indicated that ant functional group abundance was significantly different between the two restoration systems (Global R = 0.459; P = 0.001).
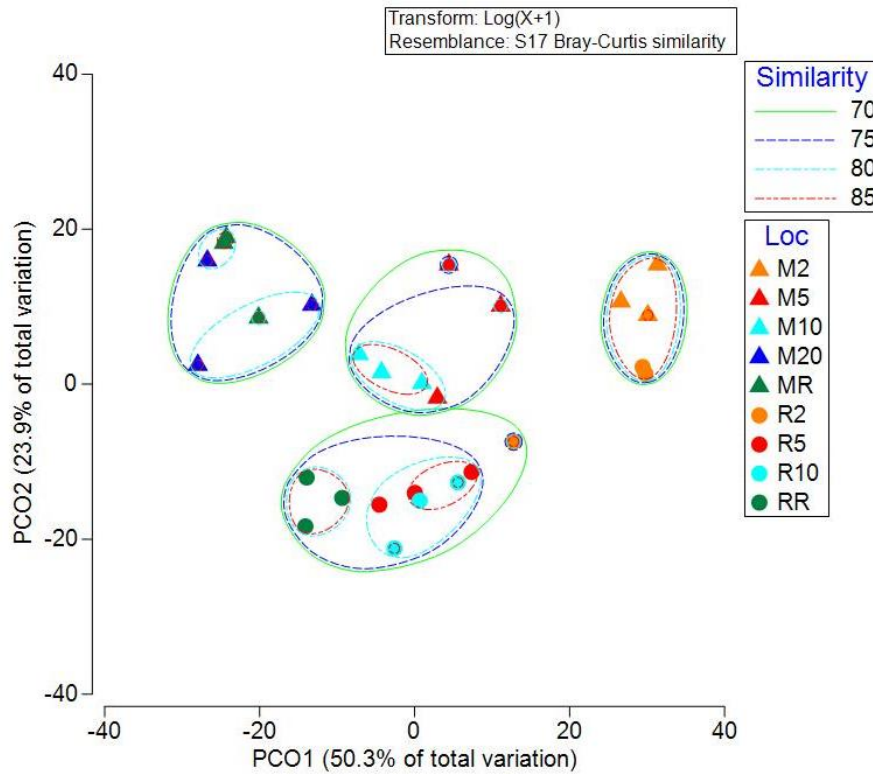
Figure 3.8. Principle Coordinate Analysis based on ant functional group abundance of each site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

3.4.4   Relationships between soil invertebrates and upper soil characteristics

Soil physiochemical characteristics were significantly correlated to ant abundance (r = 0.877, P< 0.01), but not correlated to ordinal abundance and coleopteran abundance. Soil bulk density (r = -0.511, P< 0.01) and particle density (r = -0.669, P< 0.05) showed significant negative correlation to ant species richness, indicating a decrease in soil bulk density and particle density as the number of ant species increased. A significant positive correlation was observed between ant abundance

and porosity (r = 0.604, P< 0.01), and a significant negative correlation was observed between ant abundance and organic C (r = -0.563, P< 0.01), total nitrogen (r = -0.519, P< 0.01), ammonium nitrogen (r = -0.522, P< 0.01), ratio of organic C to total N (r = -0.419, P< 0.05), and cation exchange capacity (r = -0.405, P< 0.05). No significant relationships were observed between ant abundance and soil bulk density and particle density. A negative correlation was found between ant diversity and particle density (r = -0.725, P< 0.01), but no significant correlation was observed between ant diversity and other soil parameters. Distance-based linear modelling on ant abundance separated the residue restoration system from the mining restoration system (Figure 3.9). Soil porosity, bulk density, total organic carbon, ratio of organic C to total N, and soil microbial respiration accounted for a large amount of variation between study sites.
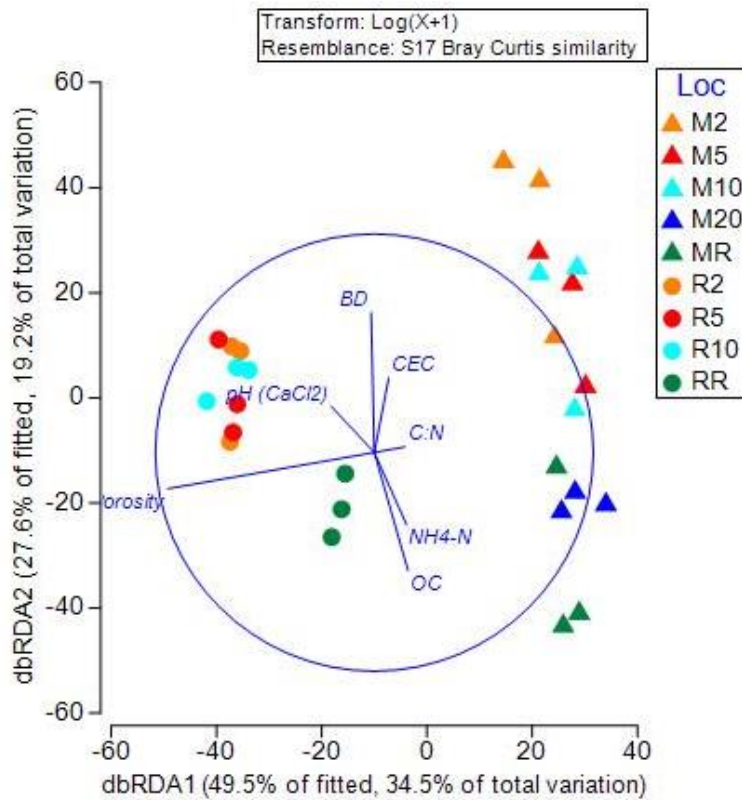
Figure 3.9. Distance-based redundancy analysis (dbRDA) based on ant abundance (log x+1) of each site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant. Dp: particle density; BD: bulk density; EC: electrical conductivity; OC: total organic carbon; N: total nitrogen; C:N: ratio of organic C to total N; NH4-N: ammonium nitrogen; NO3-N: nitrate nitrogen; P: phosphorus Colwell; K: potassium Colwell; CEC: cation exchange capacity; SMR: soil microbial respiration.

3.5   Discussion

The ultimate goal of restoration is the development of flora and fauna communities similar to those that were present pre-disturbance (Bisevac and Majer, 1999). Here, the composition of invertebrates was used as a bioindicator to assess the success of two restoration systems—one with topsoil and one with bauxite residue as restoration substrate—within Alcoa's bauxite mining operation. Unsurprisingly, the soil properties of both systems were very different, and the invertebrate communities could primarily be separated by system. However, within both systems, clearly changes in communities were observed with respect to age since restoration. Soil invertebrate abundance, richness, and functional group diversity were found to correlate with age since restoration. Similar to the findings of Majer (1996), and Hoffmann and Andersen, (2003), restored sites were

characterised by more generalised ant species, with fewer specialists than found in remnant forests. Similar to other studies, *Rhytidoponera metallica* was identified as an indicator species of anthropogenic disturbance (Hoffmann and Andersen, 2003; Andersen and Majer, 2004) and the presence or the absence of this species was associated with disturbance. The pattern of invertebrate community distribution within mining restoration was analogous to that observed in previous studies (Postle et al., 1991; Greenslade and Majer, 1993; Majer and Nichols, 1998; Majer et al., 2013).

The two restoration systems were not strictly comparable because of the differences in geographic parameters, vegetation characteristics, and level of disturbance. Additionally, although the tuart remnant plots—used as a reference system for the residue restoration sites—were close in proximity to the residue restoration sites, they were not comparable in terms of soil or vegetation community. Consequently, the invertebrate community composition of the residue restoration sites may never closely resemble that of the tuart remnant, whereas the mining restoration sites did appear to be resembling the jarrah remnant site with time; in fact, 20 years after restoration, there was considerable overlap between the restoration site and the remnant site. However, despite the unique soil structure, the residue restoration sites supported a large variety of invertebrate taxa and by 10 years after restoration, contained nearly 59 % of the ant taxa present at the nearby remnant site.

One of the issues with monitoring restoration and using invertebrates is the site-specific nature of communities, which results in scientists having to select indicators for each restoration project. For example, Carabidae beetles have been used as indicators of grazing pressure on Mediterranean Crete shrublands (Kaltsas et al., 2013), and meat ants (*Iridomyrmex purpureus*) have been used as indicators of pesticide contamination in cotton growing area in New South Wales, Australia (Weir, 1978; Majer, 1983). Further, ants were used as an indicator taxa to assess rehabilitated subtropical Queensland shrublands (Majer, 1985), savanna woodland of the tropical north (Majer, 1984b), success of mineral sand mines and heathland rehabilitations in Western Australia (Majer et al., 1982; Bisevac and Majer, 1999), and success of bauxite mining rehabilitations in Western Australia

(Postle et al., 1991; Majer et al., 2007b; Majer et al., 2013). The comparison of the two restoration systems also reveals the specific nature of invertebrate communities. Even though invertebrate orders overlapped, there was little overlap in genera and species across both restoration systems. Ant functional group profiles, ant species, and ant genera showed consistent response to restoration system and age since restoration, suggesting ants are useful indicator species to assess the success of mining and residue restoration. The younger mining restoration sites (two-, five-, and 10-year-old) were mostly dominated by Dominant Dolechoderinae, which was in agreement with the results obtained by Majer (1983). Five- and 10-year-old mining restoration sites were dominated by *Iridomyrmex bicknelli* and *I. purpureus* (meat ant). *Iridomyrmex purpureus* usually occur in disturbed or open habitats (Heterick, 2009; Majer et al., 2013) and its absence from the 20-year-old restoration site indicates that it has developed features that are unfavourable for meat ants.

Results suggest that both restoration systems are gradually developing assemblages resembling those of their respective model ecosystems. Increased ant species richness over time in both restoration systems might be an indication of the development of suitable habitats for ant recolonization. However, the number of ant species was observed to increase more rapidly in mining restoration than in residue restoration. This might be because mining restoration comprises topsoil, which may develop suitable habitats more rapidly than residue restoration with wood mulch. The ant species richness pattern was in agreement with other studies carried out in disturbed vs. native forests, where the ant species richness was higher in older restoration sites and remnant sites than in younger restoration sites (Majer, 1984b; Bisevac and Majer, 1999; Frouz et al., 2001; Holec and Frouz, 2005).

As age since restoration increased, the abundance of taxa decreased and the richness increased. The observed increase in diversity as the abundance of particular taxa reduced over time was in agreement with the results of other studies carried out in disturbed and undisturbed sites, where the ant abundance was higher and diversity was lower in even-aged forests and woodland pastures compared to old growth forests (Bisevac and Majer, 1999; Xavier et al., 2009). The

number of ant species and the number of ant functional groups were higher in the two-year-old residue restoration site than the two-year-old mining restoration site, which may reflect available resources (Topp et al., 2010). Alternatively, fewer ants may be introduced to mining restorations with stockpiled or direct-transferred topsoils as mechanical handling and anaerobic conditions within stockpiled topsoil are known to decrease the soil invertebrate biomass (Abdul-Kareem and McRae, 1984); in contrast, many ant species might get "free rides" to residue restoration sites with fresh wood mulch.

Ant functional group profiles, ant species, and ant genera showed variable response to restoration systems and age since restoration. The younger mining restoration sites (two-, five-, and 10-year-old) were dominated by Dominant Dolechoderinae, but as age since restoration increased, their abundance decreased; this was consistence with the findings of Burbidge et al. (1992) where, following disturbance, DD abundance decreased with the gradual formation of a thick litter layer that provided dense shaded areas. For some species disturbance can result in improved habitat suitability (Hoffmann and Andersen, 2003). *Iridomyrmex suchieri* is typically present in highly disturbed environments (Majer et al., 2013). The presence of this species in only the two-year-old residue restoration site in this study supports the notion that, during the early stages of restoration, residue sites are highly modified environments. In contrast, *Leptogenys neutralis* typically occurs in relatively undisturbed jarrah forests (Majer et al., 2013) and its presence in the 20-year-old mining restoration site indicates its improved state. *Anonychomyrma itinerans perthensis* is a sand dwelling ant species that inhabits open woodlands (Majer et al., 2013, and prefers wetter and cooler microhabitats. At the mining restoration sites, *A. itinerans perthensis* was observed in sites that were 10 years of age and older, but in the residue restoration system, it was only observed in the tuart remnant site. *Strumigenys quinquedentata* and *Tapinoma minutum broomense* typically occur in shaded forest conditions (Heterick, 2009) and were only sampled in the 20-year-old mining restoration site and the jarrah remnant. Thermophilic *Melophorus* spp*.* were mostly observed at the residue restoration sites, including the reference tuart remnant, except for *Melophorus turneri perthensis*, which was also sampled

at the five- and 10-year-old mining restoration sites. *Melophorus* spp. prefer open habitats and can generally be found in hot sandy soils and urban disturbed habitats (Heterick, 2009), indicating the presence of more sandy soil open habitats in residue restoration sites. They are generally absent in well-forest habitats unless there is a moderate disturbance, like fire (Majer, 1978; Andersen, 1991a). However, the occurrence of *M. turneri perthensis* only at young restoration sites, and it's disappeared from older (20-year-old) restoration and reference plots might be because of the reduction in open habitats, creating unfavourable habitats for thermophilic ant species. Apart from the general opportunist *Cardiocondyla atalanta* (Heterick, 2009), ants in the Myrmicinae and Ponerinae subfamilies were recorded in sites older than five years. Members of these subfamilies build their nests and forage within litter (Heterick, 2009), which is typically absent from the early stages of restoration. *Notoncus* spp. are winter active ants and Cold-climate Specialists; they were recorded only during the wet season in tuart remnant plots and were not observed in any of the mining restoration sites.

Monomorium lacunosum is a widespread but rarely sampled ant and has only been recorded in three occasions in Western Australia (once in Eneabba, once in Cape Arid National Park, and once in Queen Victoria Spring Nature Reserve) and once in South Australia (Heterick, 2009). Surprisingly, it was recorded multiple times in one of the five-year-old residue restoration plots. It was unclear if the individuals were from a single nest or multiple nests. It is possible that this species may have arrived with the wood mulch and found preferable habitat qualities to colonize in the five-year old residue restoration site.

A focus on the response of ant functional groups to disturbance potentially overcomes the limitations of focusing on particular species that vary from site to site. Functional groups are based on a variety of ecological characteristics of taxa and do not focus on a particular disturbance type, such that predictions can be made about the response of groups to restoration actions; however, some taxa are difficult to fit with a group (Hoffmann and Andersen, 2003). Ant functional group composition showed a noticeable difference between two-year-old and older restoration sites in both systems. Initial restoration stages likely provide a

hostile environment for ant species with specialized feeding characteristics and foraging habits. Generally, two-year-old restorations are devoid of understorey, with limited food resources for ants. Those ants that are slow to colonise new sites may be disadvantaged. Therefore, the approximation of function to that of the remnant site (using functional groups as the indicator) occurs much earlier than the approximation of individual taxa.

There was a clear succession of ant functional groups between restoration systems and across restored sites, in accordance with the findings of Andersen (1993). In the mining restoration system, as the age of restoration increased, Cryptic Species increased. Cryptic Species likely have a positive relationship with the development of litter as it results in decreased habitat suitability for these species (Majer et al., 1982). With the increase in age since restoration, the species richness of Generalized Myrmicinae and Opportunists was observed to increase. Both functional groups comprise widely adapted taxa with different competitive abilities and a variety of habitat preferences (Andersen, 1995; Hoffmann and Andersen, 2003); hence, with increasing restoration age, a variety of habitats and resources may become available for these functional groups. In the mining restoration system, as the age since restoration increased, the number of Cold-climate Specialist species increased while the number of Hot-climate Specialist species decreased. It is expected that habitat quality increase with time since restoration to ultimately resemble that of the remnant site; in the process, functional groups like Hot-climate Specialists, which favour open habitats, are eliminated and groups like Cold-climate Specialists, which prefer well-forested and moist habitats, are accommodated (Andersen, 1995). Cold-climate Specialists were not observed in the two-year-old mining restoration plots, which may have been due to the high abundance of Dominant Dolichoderines (Andersen, 1995) or the lack of understorey and moist habitats, creating an unfavourable environment for recolonization. Species abundance and richness of Hot-climate Specialists increased in the residue restoration system as the restoration age increased, indicating recolonization by arid environment-adapted taxa (Andersen, 1997) in residue restoration sites. Cold-climate species abundance increased with age since restoration in residue restoration sites, indicating a reduction in the disturbance

level and an improvement in habitat quality, consistent with the findings of Hoffmann and Andersen (2003). The thick exotic grass cover in residue restoration may help prevent soil moisture evaporation and as the restoration matures, the developing understorey may achieve favourable cool, moist habitats for Cold-climate Specialists to inhabit.

Specialized Predators started to appear in five- and 10-year-old restoration sites; their species richness increased as time since restoration increased, with highest numbers in jarrah remnant and 20-year-old mining restoration plots. Hoffmann and Andersen (2003) consider Specialized Predators as one of the last functional groups to recolonise restored mine sites. Their presence in the 10-year-old sites is encouraging, as it provides evidence that the restoration sites are on the trajectory towards achieving certain functions of the remnant, undisturbed plots. This contradicts the results of the Bisevac and Majer (1999) study where Specialist Predators were absent in control plots around mineral and sand mining restoration sites at Eneabba, Western Australia.

Given the importance of invertebrates on soil properties, this study further analysed the relationship between these parameters across the chronosequence. It is not clear if ground dwelling invertebrates modify the degraded soils or if the modified soil properties result in the recolonization of ground dwelling invertebrates to the restored areas. Frouz et al. (2006) found that the presence of macrofauna did not significantly increase mineralization, but did accelerate soil mixing. Hole (1981) highlighted many direct and indirect effects of soil invertebrates on soil structure: soil structure formation is thought to be a direct effect of nest building, feeding, and transporting materials while soil structure stabilization an indirect effect of soil invertebrates (Hole, 1981; Wolters, 1991; Folgarait, 1998). Soil particle density and bulk density were negatively correlated with soil taxa richness, which reveals that an augmented ant community composition increases soil structure formation. Shrestha and Lal (2006) and

Ganjegunte et al. (2009) also found that soil bulk density and stability improved as soil biodiversity increased.

Restoration of bauxite pits and highly modified residue sands clearly facilitate the return of a diverse invertebrate community, with species richness increasing more rapidly in topsoil restoration (mining) than in residue restoration. Invertebrate taxa and functional groups within each restoration system were shown to be gradually resembling those of the remnant reference sites; however, even after 20 years, original community structure was yet to be achieved in mining restoration.

Chapter 4   Ant-plant interactions: re-establishment of myrmecochory within bauxite mining and refinery restoration

4.1   Abstract

Re-establishment of ecosystem processes is one of the ultimate goals of ecosystem restoration, and plant-animal interactions are recognized as a vital component. Myrmecochory, i.e. seed dispersal by ants, is an ecologically significant mutualism in dry sclerophyll Australian ecosystems. This study investigated the composition of ant fauna, seed dispersal rate, and seed dispersal distance in bauxite mining and bauxite refinery residue restoration chronosequences. The mining restoration system consisted of four restoration sites (two-, five-, 10-, and 20-year-old) and an adjacent jarrah remnant (reference) site, while the residue restoration system contained three restoration sites (two-, five-, and 10- year-old), and an adjacent tuart remnant (reference) site; there were three replicate plots in each site. Elaiosome-bearing *Acacia extensa* seeds (mean weight 13.4 ± 0.02 mg) were offered to ants for 3 h and the fate of the seed (dispersed or elaiosome theft without dispersal) were recorded. When dispersal was observed, distance to nest was measured and the ant dispersers and thieves identified. During 2275 seed removal events, 12 species from four sub-families were observed dispersing seeds, and a further eight species were observed feeding on the elaiosome. Ant species richness associated with seeds was lower in the residue system than in the mining system, and no feeding stations were visited in the residue restoration sites. As restoration progressed, the richness of myrmecochorous ant species increased in the mining system but remained similar in the residue system. In the mining system, *Rhytidoponera metallica* F.Smith was the most common disperser (81% of observations), while *Iridomyrmex purpureus* (F.Smith) had the highest mean dispersal distance (18.2 m) and the highest recorded dispersal distance (40.5 m). Seed removal rate in the mining system declined as time since planting increased and was similar between the 20-year-old site (11%) and the remnant site (13%), indicating that this ecosystem process may

be returning to pre-disturbance levels. The reason for a lack of dispersal at the residue system, despite dispersal events in the adjacent remnant site and an abundant ant population, remains unclear.

4.2   Introduction

4.2.1   Seed Dispersal

Re-establishment of ecosystem processes is one of the ultimate goals of ecosystem restoration (Society for Ecological Restoration, 2004; Kollmann et al., 2016), and plant-animal interactions are recognized as a vital component of ecosystems (Bonte et al., 2012; Traveset et al., 2014; Leal et al., 2015). Pollination, seed dispersal, and plant protection are positive plant-animal mutualisms, and the composition of mutualist communities that perform these functions may differ temporally and spatially. One of the key interactions in Australian Mediterranean ecosystems is seed dispersal by ants (myrmecochory); hence, it is an essential plant-animal mutualism to re-establish to pre-disturbance levels in restored habitats (Gove et al., 2007; Hilley, 2014). Myrmecochory is not necessarily absent in restored habitats, but it may be in elevated levels (Gove et al., 2007).

The Australian continent is dominated by myrmecochorous vegetation (Orians and Milewski, 2007), with approximately 1500 plant species for which ants are the primary seed dispersal agents (Berg, 1975). These myrmecochorous plants are associated with fire-prone, dry sclerophyllous areas and woodland habitats (Berg, 1975). Intense bush fires can decrease the abundance of viable seeds, which in turn alters future plant diversity and abundance (Luna et al., 2007). In Australian myrmecochorous communities, ants not only disperse the seeds away from competitive siblings, resulting in better germination sites for some seeds, but also increase the chance of seed germination by aiding the seed to escape from the lethal effects of forest fires (Beaumont et al., 2011). Dispersal is always passive, as seeds have no control over where they will end up, but seed dispersal is influenced by a variety of parent plant traits (Traveset and Rodríguez-Pérez, 2008). For example, some plants produce seeds with rewarding structures, such as elaiosomes, to attract ants, birds, and rodents.

Not all interactions between seed and potential dispersal agents are positive. Seed predation is a core mortality factor that reduces the availability of seeds for germination (O'Rourke et al., 2006; Koprdová et al., 2010; Ichihara et al., 2011). However, in some cases, seeds collected by seed predators (dung beetles,

granivorous ant, rodents) are stored in nests, in soil, or tree holes, and "forgotten". In some cases, these seeds are abandoned in fertile soils which can promote germination (Traveset and Rodríguez-Pérez, 2008).

4.2.2 Ant–seed interactions

Ants interact with seeds through both predation and seed dispersal. Different ant taxa treat seeds in various ways depending on their body size and the size of the seed. For example, species of Ponerine ants and the genus *Rhytidoponera* actively move seeds into their nests (Hughes and Westoby, 1992a; Pizo and Oliveira, 1998); *Crematogaster* spp., *Ectatomma* spp., and *Monomorium* spp. manipulate seeds before carrying them into their nests (Munguía-Rosas et al., 2009); *Pheidole* spp. recruit worker ants to remove the elaiosome on the spot and carry it to their nests; smaller ant species, like *Solenopsis* spp., may recruit nest-mates to cover the seed with soil on the spot before removing the elaiosome (Pizo and Oliveira, 1998).

In some habitats, seeds predation by granivorous ants is one of the leading seed mortality factors (Andersen, 1991b). During seed predation, ants destroy a large portion of the diaspore as that represents a nutrient-rich, high-quality food resource that ants can store (Schöning et al., 2004), and the viability and condition of some small plant populations can be negatively affected by this seed predation (Albert et al., 2005). Some seeds have physical and/or chemical defensive mechanisms—such as containing thick or woody covers or low levels of toxins (e.g. non-protein amino acids, protease and amylase inhibitors, cyanogenic glycosides)—to diminish the impact of predators (Hulme and Benkman, 2009).

Myrmecochory is a beneficial dispersal syndrome in many parts of the world. Worker ants take elaiosome-bearing seeds to their nests, use the high nutrient elaiosome to feed the larval stages, and discard the leftover seed in or on their trash piles or areas outside their nest (Hughes and Westoby, 1992a; Kim et al., 2011). Consequently, seed harvesting ants influence the abundance and distribution of seeds locally. Seed dispersal without the aid of animals (i.e. wind or gravity dispersal) is generally only short distance; the mean seed dispersal distance can vary between 1–2 m, with the published global average dispersal distance being 2.24 m (± 7.19 SD) (Pascov et al., 2015). Ant seed dispersal distances

rarely exceed 4 m (Hughes and Westoby, 1992a), but some seeds produced the long distance dispersal, with distance over 400 m recorded (Pascov et al. (2015) and regular occurrences of dispersal beyond 20 m (Harris and Standish, 2008; Pascov et al., 2015). However, the high occurrence of ants and myrmecochorous plants in southern Australian and South African habitats leads to rapid seed removal, which substantially reduces the competition between parent and offspring plants (Andersen, 1988a; Westoby et al., 1991; Hughes and Westoby, 1992b).

4.2.3 Consequences of ant–seed interactions in restoration

Animal–seed interactions influence the trajectory of vegetation recovery in disturbed landscapes. During habitat restoration, ants affect the seed availability of a particular area by either: (1) removing the elaiosome from the seed without dispersing the seed (Andersen and Morrison, 1998); (2) dispersing the elaiosome-bearing seeds from adjacent remnants; or (3) predating on seeds, and consequently reducing the availability of seeds for dispersal (Harris and Standish, 2008) and germination in young restoration sites (Majer, 1984a, 1985). The motivation of ants to harvest and disperse the seed, either as an alternative food source or as a high nutrient source to re-establish the colony, might increase with habitat disturbance (Prinzing et al., 2008). Restoration success may be limited by the lack of re-colonization of seed dispersal species in restored sites (Baur, 2014).

Although there is a lot of research related to seed dispersal, it has only seldom been in the context of degraded forest ecosystem restoration (McConkey et al., 2012; Gallegos et al., 2014). Many gaps exist in our understanding of how seed predation and dispersal change as restored habitats mature. Therefore, it is vital

to shift research focus on seed dispersal from an organism-orientated perspective to one of functional groups.

4.2.4 Role of ants as indicators of monitoring the success of Australian ecosystem restoration

Ants have frequently been considered to be bio-indicators of ecosystem change in Australia (Majer, 1983; Bruyn, 1999; Andersen et al., 2009), and are appropriate for inventory and monitoring programs as they have stationary, perennial nests with relatively restricted foraging ranges (Agosti et al., 2000). For example, changes in ant seed removal rates were demonstrated between fertile and infertile soils (Mossop, 1989); further, the lack of seed-dispersing ant communities was proven to be a barrier to vegetation development at the Ranger uranium mine rehabilitation in Australia's Northern Territory (Andersen and Morrison, 1998).

Though myrmecochory is well documented worldwide, only a few studies focus on the response of myrmecochory to restoration (Majer, 1980; Majer, 1984a, 1985; Andersen and Morrison, 1998; Standish et al., 2007; Harris and Standish, 2008). There is a lack of information on how myrmecochorous functional groups change as restoration works proceed, and if they can be used as an indicator of the success of ecosystem restoration. Further, there is a lack of understanding of how seed dispersal and dispersal distance change as restored habitats mature and whether restored systems presents a shift towards their respective remnant systems (in terms of the abovementioned parameters) over time. This study aims to understand the patterns of myrmecochory in Alcoa's rehabilitation sites and to investigate whether there is a difference between ant community composition, seed dispersal rate, and seed dispersal distance in two distinctly different

rehabilitation practices (mining and residue restoration systems). Specifically, the study addresses:

- Whether seed removal rates and dispersal distances differ between mining and residue restoration systems and whether they resemble adjacent remnant forest sites more closely with increasing restoration age.
- Whether and how the myrmecochory differs between the two restoration systems and if ant taxa interaction with seeds is on the trajectory towards that of adjacent remnant forest sites as restoration age increases?

4.3   Methods

4.3.1   Site selection and experimental design

Seed removal rate and dispersal distance were assessed in four habitat categories: mining restoration sites of different ages, native jarrah forest remnant site (as a reference for mining restoration sites), residue restoration sites of different ages, and coastal tuart woodland remnant (reference) adjacent to residue restoration sites. Four mining restoration sites (two, five, 10, and 20 years since restoration) and three residue restoration sites (two, five, and 10 years since restoration) were selected to provide comparisons between restoration systems at different ages, with three replicates plots in each site (see Chapter 2 for more details of study sites). Sampling was conducted during April and November 2014 with two sampling efforts, morning and afternoon, at each plot at each period.

Seed removal rate and dispersal distance were measured by monitoring the removal of *Acacia extensa* seeds (approximately 13 mg) from feeding boards. *Acacia extensa* seeds (hereafter referred to as *Acacia* seeds) were selected because this species is common in both the mining and residue restoration systems. This species is considered a myrmecochorous plant as its seeds possess a white elaiosome, which is smaller than the seed. No *Acacia* seed rain (of any species) was observed at any of the sites during the sampling period, so the seed at the feeding stations were likely to be an attractive resource. Seeds were

obtained from Alcoa's nursery, having been collected within the local area, and not been subjected to any germination treatments.

Feeding boards were prepared by gluing a filter paper disc (70 mm diameter) to a 10 cm x 10 cm plywood board. For each replicate plot, 16 feeding boards were placed on the ground in a 6 m x 6 m grid with 2 m spacing in between, similar to Andersen (1988a). Each board was laid on the topsoil after clearing away the litter layer. Five *Acacia* seeds were placed on each paper disc at once.

Observations were made during two three-hour sessions: morning (starting between 8.00–8.30 am) and afternoon (starting between 2.00–2.30 pm). Observations on the same plots of a site on the same day were avoided. The maximum daily temperatures varied from a minimum of 20 °C to a maximum of 32 °C, and initiation of afternoon observations was purposely delayed on the hottest days until ant activity was observed. The fate of seeds was recorded in a manner similar to that of Harris and Standish (2008), i.e. as either dispersed (seed not visible within 3 cm of the paper disc) or not-dispersed (seed within 3 cm of the paper disc). When seed removal by an ant was observed, it was followed until it reached the nest; the dispersal distance was then measured, and an ant specimen from the nest was sampled for identification. Ants seen feeding on elaiosome, but not dispersing the seed were also sampled for identification (Harris and Standish, 2008). If any seed dispersal occurred without the dispersal agent being seen, it was assumed that those seeds were dispersed by ants rather than other seed dispersers (i.e. other invertebrates, rodents or birds) as no other dispersers were observed at the feeding stations during the experiment.

4.3.2   Ant assemblages

Surface-active ant communities were sampled using pitfall traps and hand-collection. Pitfall traps were operated in September 2013 and March 2014, covering both wet and dry seasons. At each plot, six pitfall traps were established (as described in Chapter 3.3.2), consisting of 40 mm diameter (120 ml) plastic specimen vials, one-third filled with ethylene glycol. Pitfall traps were left open for seven days, and the samples were sorted in 85 % ethanol after collection. Further, while monitoring feeding boards in April and November 2014, surface-active ants

were sampled using a pair of forceps and an ethanol dipped artist's paintbrush. Ants removing seeds to nests and thieving the elaiosome on feeding board were also sampled. The number of ant species collected—i.e. hand collected and collected from pitfall traps—were combined to obtain the total species richness of the ants collected from each site.

All ants were identified to species level using the Heterick (2009) dichotomous key and the ant collection held at the Western Australian Museum. Additionally, ants were classified into myrmecochorous functional groups based on the results of feeding board observations.

4.3.3 Data analysis

As there were no seed dispersal events recorded from residue restoration sites, we focused only on the analysis of data from the mining restoration system. The differences in the number of *Acacia* seeds dispersed and the dispersal distance between mining restoration sites and between time of year (wet vs dry season) were compared using Two-way ANOVA in R studio (version 3.2).

For the pitfall data, the six replicates at each site were combined to obtain the total abundance, richness, and diversity of surface-active ant communities, and these components were then compared between sites within the two restoration systems using Two-way ANOVA in SPSS. Similarly, abundance, richness, and diversity of surface-active seed dispersers and elaiosome thieves in pitfall traps were compared between restoration ages in the mining restoration system using Two-way ANOVA in SPSS. Site ant assemblages from pitfall traps were compared using multivariate analyses performed using PRIMER (version 7). A similarity matrix was constructed on the log-transformed abundance of ants using the Bray-Curtis measures. Principal coordinates (PCO) analysis was performed to produce ordinations of similarity on the Bray–Curtis matrix.

4.4 Results

4.4.1 Ant-mediated seed dispersal

4.4.1.1 Number of seeds dispersed

Of a total of 2275 observed seed dispersal events, 2239 (98.4%) were recorded at the mining restoration system; most seeds (n=2184) were taken into ant nests, while the remainder (n=55) were "dispersed" at least 5 cm away from feeding boards. On four occasions, within 10 min of the seed entering the nest, workers of *Iridomyrmex purpureus* brought an intact seed (without elaiosome removal) out from the nest and deposited it with coarse sand particles closer to the nest opening.

Seeds were rapidly removed during the first 1.5 h (Figure 4.1). The seed removal rate was markedly different between restoration ages. The highest numbers of seeds were removed from the two-year-old restoration site (192.3 ± 20.7 mean number of seeds removed per site). The number of seeds removed declined as age since restoration increased, and was similar between the 20-year-old site (1.9 ± 0.3) and the remnant site (2 ± 0.4) (Figure 4.1).

Number of seeds dispersed varied significantly with age since restoration ($F_{4, 30}$ = 298.2, P < 0.001) and sampling season ($F_{1, 30}$ = 9.6, P < 0.01), with no interaction effect between the two ($F_{4, 30}$ = 2.3, P = 0.08). Significantly more seeds were dispersed in November (mean = 71 ± 21.1) than in March (mean = 41 ± 11.7). The number of dispersal events decreased as restoration age increased, with similar numbers between the jarrah remnant and the 20-year-old mining restoration site (Figure 4.1).

The 36 seeds removed from feeding-boards in the residue restoration system, all from the tuart remnant site, were taken to nests.
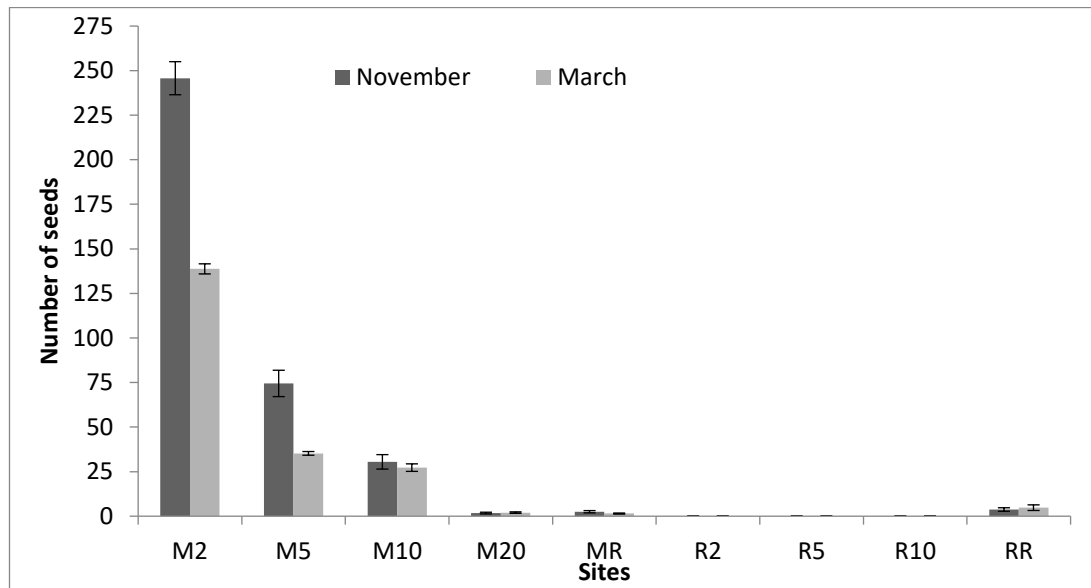
Figure 4.1. Mean number of seeds dispersed in each sampling month per site ± SE (out of 1538 seeds) Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

4.4.1.2 Seed dispersal distance per site by ant species

Mean seed dispersal distance for all mining restoration sites was 4.06 m (ranging from 0.55 m to 40.5 m) and most of the seed dispersal events (~ 94 %) were < 4 m (Figure 4-2). At the mining restoration sites, seed dispersal distances (logx+1) varied significantly with age since restoration ($F_{4, 30} = 8.041$, $P < 0.001$). There was no significant effect of sampling season ($F_{1, 30} = 0.238$, $P < 0.1$) and no evidence of an interaction effect between restoration age and sampling season ($F_{4, 30} = 0.741$, $P < 0.1$). Seed dispersal distance of the 10-year-old restoration site was significantly greater than that of all other restoration sites (Tukey post-hoc test p < 0.05). The maximum dispersal distances (20–40.5 m) were all recorded in the 10-year-old restored site (Figure 4-2a), due to the presence of *I. purpureus* (F.Smith) in all plots of this restoration age (Figure 4-2). Both the 20-year-old site and the jarrah remnant site had limited dispersal in comparison (maximum distance of < 6 m).

Dispersal distance varied between species. *Iridomyrmex purpureus* (average 18.2 m, max 40.5 m) and *Rhytidoponera metallica* (average 8 m, max 16.6 m) were the

long-distance seed dispersers (Figure 4-2b). *Iridomyrmex bicknelli*, *I. mjobergi*, *I. splendens*, and *Camponotus terebrans* all dispersed seeds to a distance of < 5 m from the feeding station. Seeds from a single feeding-board were taken to multiple ant nests and seeds were not taken to the nearest ant nest. Most of the ant colonies were responsible for more than one seed dispersal event. While some feeding boards were regularly visited by ants, some remained un-attendant regardless of the distance to the nearest ant nest. On three occasions, *I. purpureus* displayed dominant behaviour over other ants by attacking and seizing seeds.
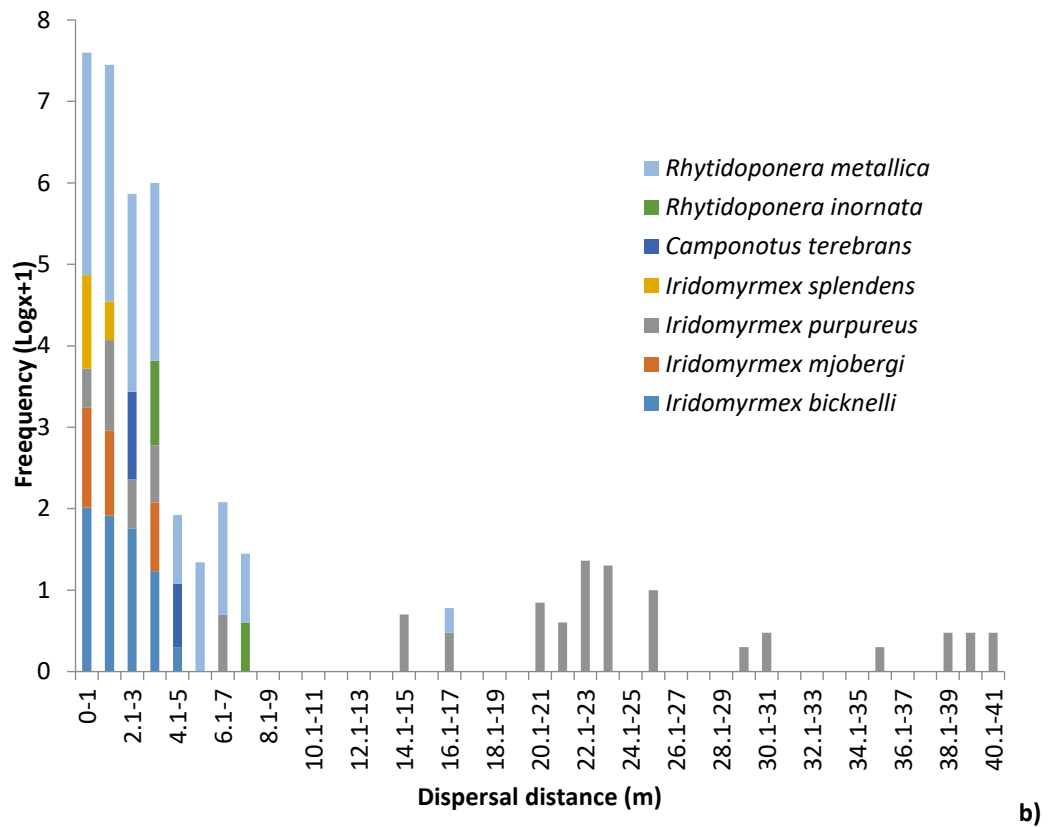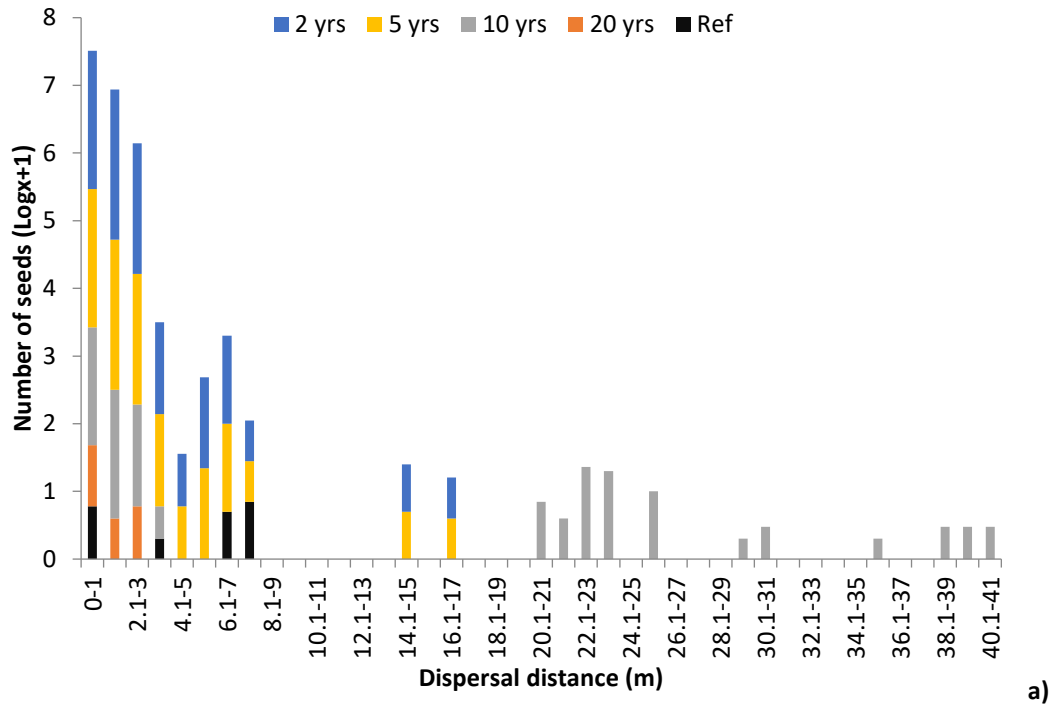
Figure 4-2. Seed dispersal distances (m) from feeding-board a) according to restoration site b) according to ant species in the mining restoration system.

4.4.2　Ant assemblages

4.4.2.1　Total ant community composition in pitfall traps

Over the sampling period, a total of 70 ant species from 24 genera and eight subfamilies were recorded. Forty-eight species were recorded from the mining restoration system and 46 from the residue restoration system, with 24 species common to both systems (Appendix B, Table 4.1). There was a significant difference in ant diversity ($F_{7,18}$ = 10.019, P < 0.001), and ant species richness ($F_{7,18}$ = 15.407, P < 0.01) between sites. At both restoration systems, ant diversity was higher in 5- and 10-year-old restoration sites than adjacent remnant forest sites (Figure 4.3). The 20-year-old mining restoration site had similar diversity to that of the jarrah forest system.
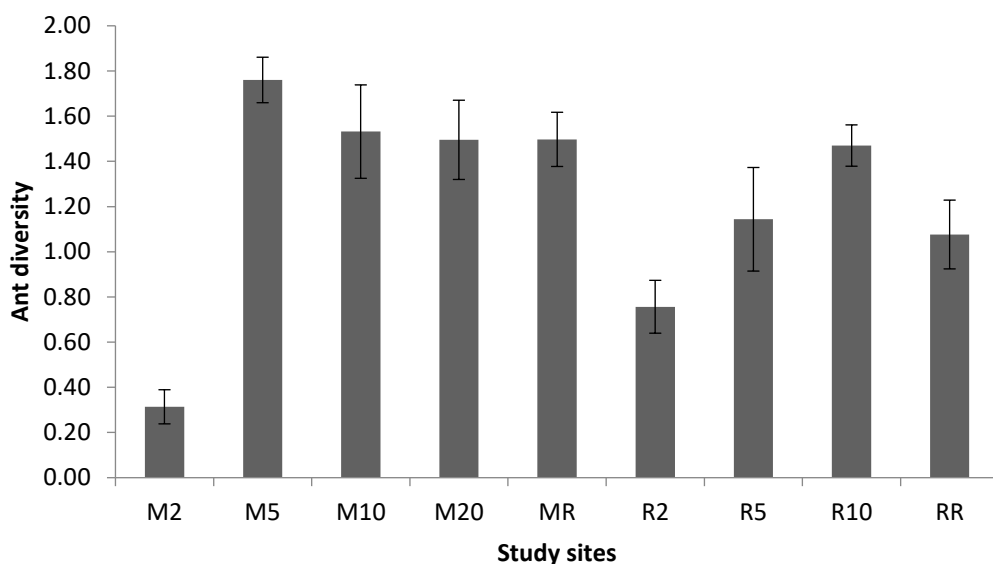
Figure 4.3. Mean ant diversity (±SE) at each site of the two different restoration systems. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

There was a linear upward trend in ant species richness in mining restoration sites as age since restoration increasing; however, ant species richness was not at the same level as that of the undisturbed jarrah forest even at the 20-years-old restoration site (

Figure 4.4). There was no such trend in the residue restoration system; instead, species richness peaked in the five-year-old site and declined in the 10-year-old

site. The tuart remnant had almost twice as many ant species as all younger residue restoration areas. The highest ant species richness, obtained from pitfall trap and hand collection data, was recorded in tuart remnant plots (41 species), followed by jarrah remnant plots (25 species). *Camponotus cinereus notterae*, *Monomorium fieldi*, and *Iridomyrmex splendens* were recorded as seed interacting species during the study, but not sampled in pitfall traps. *Rhytidoponera* spp. were recorded in all mining restoration sites, in the 10-year-old residue restoration site, and in the tuart forest remnant, but were absent in the two- and five-year-old residue restoration sites. *Iridomyrmex bicknelli* was recorded in all sites except in the 20-year-old mining restoration site and the jarrah forest remnant. *Iridomyrmex purpureus* was only recorded in the five- and 10-year-old mining restoration sites, *C. terebrans* was only recorded in the 10- and 20-year-old mining restoration sites, and both species were not recorded in the jarrah and tuart remnants. *Ochetellus glaber* and *Crematogaster laeviceps chasei* were recorded only in the 10- and 20-year-old mining restoration and jarrah forest remnant sites.
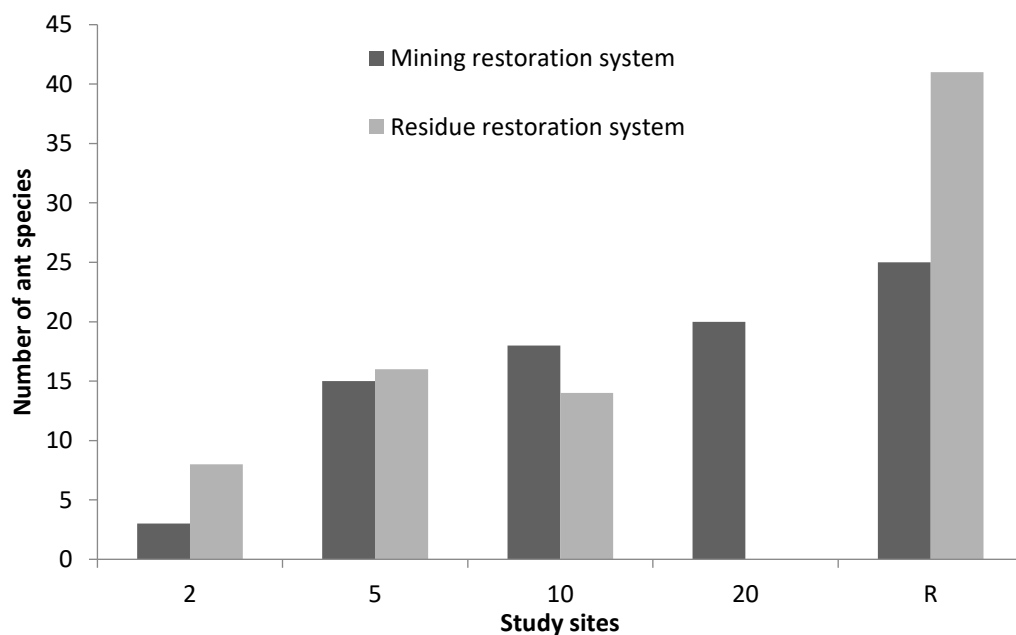
Figure 4.4. Total number of ant species recorded from pitfall traps and hand collection at study sites associated with two restoration systems. Study sites: 2 = two-year-old restoration, 5 = five-year-old restoration, 10 = 10-year-old restoration, 20 = 20-year-old restoration, R = adjacent remnant forest sites.

The Principal coordinates (PCO) analysis based on ant species richness clearly separated the data into four groups by restoration system (mining and residue) and by age since restoration (Figure 4-5), indicating that there was a considerable

difference in ant-assemblage composition between the two restoration systems. Residue restoration sites were clustered together, away from the tuart forest remnant plots, but the older restoration site was closer to the tuart remnant than the younger restoration sites. Jarrah forest remnant plots and 20-years-old mining restoration site plots were clustered together, while two-, five- and 10-years-old mining restoration plots were clearly separated into another group.
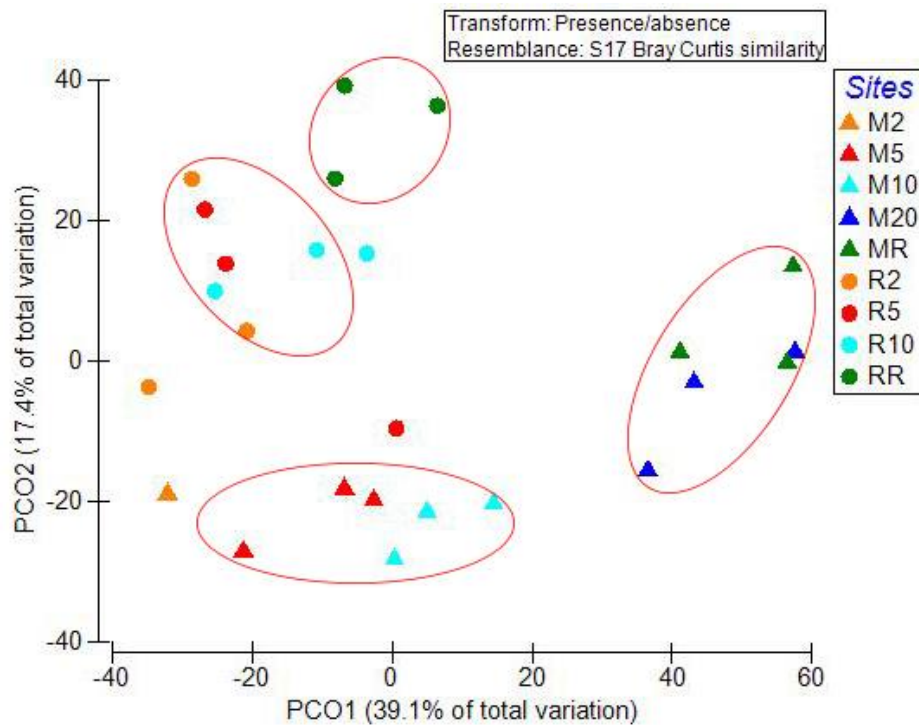
Figure 4-5. Principle Coordinate Analysis of ant species richness at mining and residue restoration system sites. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

4.4.2.2   Seed-interacting ant communities

A total of 20 ant species belonging to 10 genera and four subfamilies were seen interacting with seeds. Twelve seed-dispersing ant species were observed during the study, with seven seed-disperser ant species recorded in each restoration system (Table 4.1). *Rhytidoponera metallica* (> 1800 seed dispersal events) and *I. bicknelli* (256 dispersal events) were observed dispersing seeds at mining restoration sites and tuart remnant sites.

Table 4.1. Seed-interacting ants, collected from each restoration systems by pitfall trapping and hand collection, and their myrmecochorous behaviour towards *Acacia* seeds. Behaviour: T = elaiosome thieves, N= nest seed-dispersers, (1 = presence, 0 = absence).

Sub-family	Species	Mode of behaviour	Presence/ Absent								
			Mining					Residue			
			M2	M5	M10	M20	MR	R2	R5	R10	RR
Dolichoderinae	*Doleromyrma darwiniana fida* (Forel)	T	0	0	1	1	1	0	0	0	0
	Iridomyrmex bicknelli Emery	N	1	1	1	1	1	1	1	1	1
	Iridomyrmex chasei Forel	N	0	0	0	0	0	1	1	1	1
	Iridomyrmex discors Forel	N	0	0	0	0	0	1	1	1	1
	Iridomyrmex mjovergi Shattuck	N	0	1	1	0	0	0	0	0	0
	Iridomyrmex purpureus (F.Smith)	N	1	1	1	0	0	0	0	0	0
	Iridomyrmex splendens Forel	N	0	1	0	1	0	0	0	0	0
	Ochetellus glaber JDM19	T	0	1	1	1	1	0	0	0	0
Ectatomminae	*Rhytidoponera metallica* F.Smith	N	1	1	1	1	1	0	1	1	1
	Rhytidoponera inornata Crawley	N	0	1	0	1	1	0	0	0	0
	Rhytidoponera violacea (Forel)	N	0	0	0	0	0	0	0	0	1
Formicinae	*Camponotus cinereus notterae* Forel	T	0	0	0	0	1	0	0	0	0
	Camponotus terebrans (Lowene)	N	0	1	1	1	0	0	0	0	0
	Melophorus JDM 230	N	0	0	0	0	0	0	0	0	1

Sub-family	Species	Mode of behaviour	Presence/ Absent								
			Mining					Residue			
			M2	M5	M10	M20	MR	R2	R5	R10	RR
Myrmicinae	*Melophorus ludius* Forel	T	0	0	0	0	0	0	0	0	1
	Monomorium fieldi Forel	N	0	0	0	0	0	0	0	0	1
	Monomorium sordidum Forel	T	0	1	1	1	1	0	0	0	0
	Meranoplus rugosus Crawley	T	0	0	0	0	0	0	0	0	1
	Cardiocondyla atalanta Forel	T	1	1	1	1	1	0	0	0	0
	Crematogaster laeviceps chasei Forel	T	0	0	1	1	1	0	0	0	1

Six seed-dispersing ants were observed: *I. bicknelli* Emery*, I. chasei* Forel, *I. discors* Forel, *Melophorus* JDM 230, *R. metallica* (F. Smith), and *Monomorium fieldi* Forel. No visits to seed feeding-boards were observed by dispersers or thieves, and no seeds were dispersed from any of the residue restoration sites, despite foraging ants being observed during the study period; seed-dispersing species and elaiosome stealing species were sampled in pitfall traps or by hand at these sites. Ants (predominantly *Iridomyrmex* spp. and *Rhytidoponera* spp.) were observed carrying dead arthropods or body parts of dead invertebrates, but not seeds.

At the mining restoration sites, *R. metallica* was observed removing seeds regularly (> 1800 seed dispersal events). Of the total observed seed-dispersing events, 81% were by *R. metallica* while *Iridomyrmex* spp. dispersed 17 % of seeds (Figure 4.4).
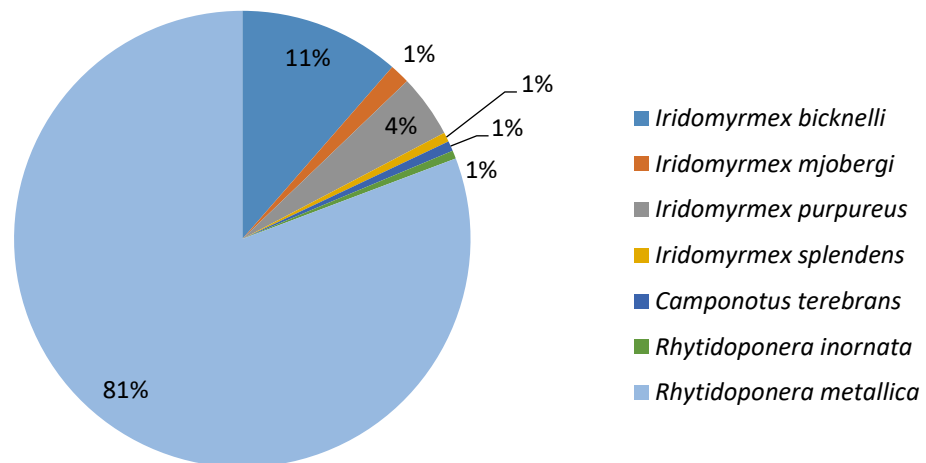
Figure 4.6. Total percentages of seed dispersals by each ant species in the mining restoration system

Species richness of seed-disperser ant species collected from pitfall traps ($F_{8,27}$ = 9.265, P < 0.001), their abundance ($F_{8,27}$ = 8.561, P < 0.001), diversity ($F_{8,27}$ = 17.669, P < 0.001), and dominance ($F_{8,27}$ = 16.433, P < 0.001) all varied significantly between study sites. The highest seed-disperser ant species richness was observed

in the five-year-old restoration site in the mining restoration system, and in the 10-year-old restoration site in the residue restoration system (

Figure 4.7a); the highest species dominance was recorded in remnant sites (

Figure 4.7d). The abundance of seed-disperser ants was highest in the five-year-old mining restoration site, while the 20-year-old mining restoration site and the jarrah and tuart remnant sites had a similar abundance of seed-disperser ants (

Figure 4.7b). Seed-disperser ant diversity was lower in the two-year-old restoration site than in the older restoration sites in both restoration systems (

Figure 4.7c). At the mining system, seed-disperser diversity was higher in the five- and 10-year-old restoration sites than the 20-years-old restoration site and jarrah remnant (

Figure 4.7c). Similarly, at the residue restoration system, the 10-year-old restoration site had higher seed-disperser ant diversity than tuart remnant plots. The lowest seed-disperser ant diversity was recorded at the jarrah remnant plots.
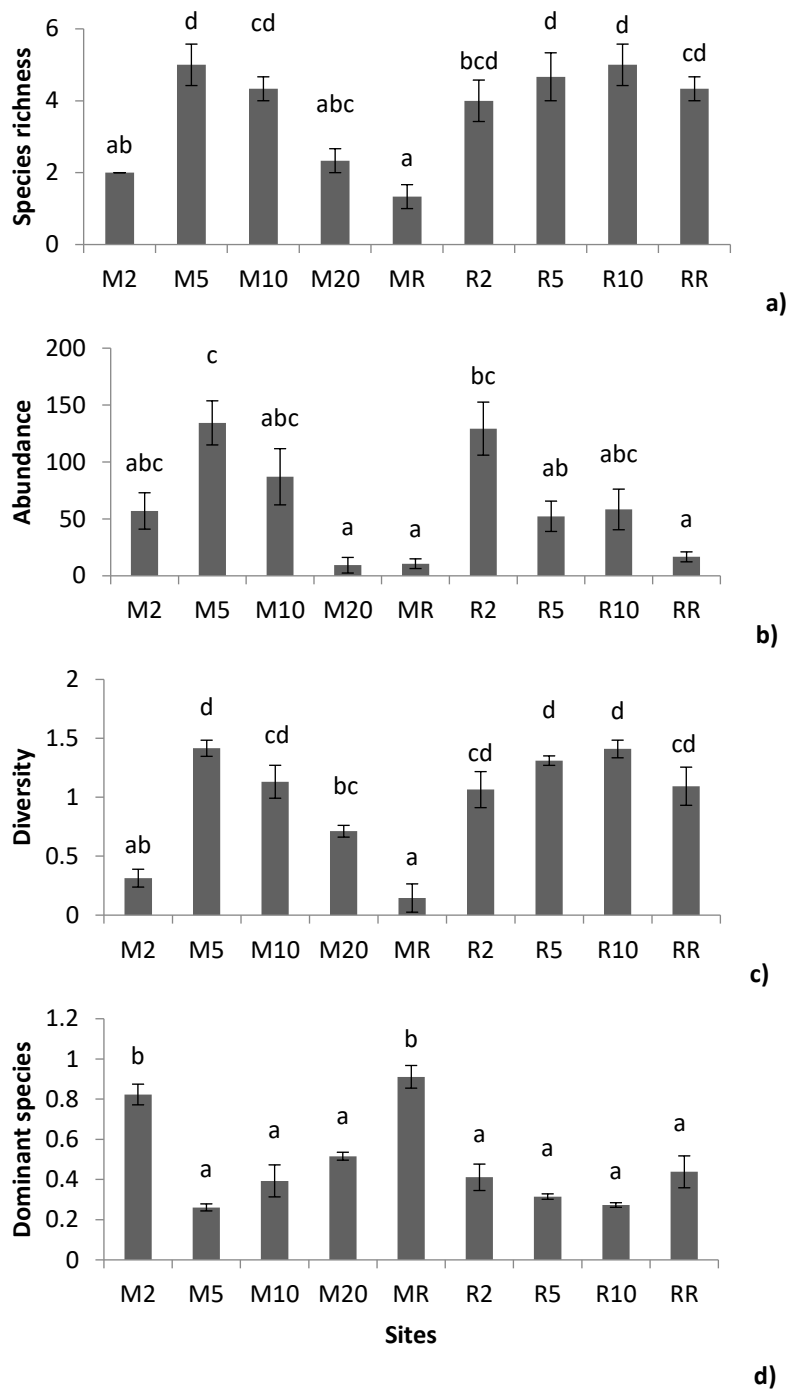
Figure 4.7. a) Species richness, b) abundance, c) diversity, and d) dominant species of seed-disperser ants at each site of the two different restoration systems, as sampled via pitfall traps. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

Seed disperser ant abundance recorded from pitfall traps significantly differed between sites ($F_{7,18}$ = 9.760, P < 0.001), with the highest number recorded at the 10-year-old restoration site followed by the five- and two-year-old restoration sites (Figure 4.8). Ant disperser richness was similar in the jarrah remnant and the two- and 20-year-old mining sites, but the species composition of the two-year-

old restoration site was different to that of the 20-year-old restoration and jarrah remnant sites (Figure 4.8 and Table 4.1). Seed disperser ant richness was higher in all residue restoration sites than in tuart remnant plots (Figure 4.8).

Eight ant species from three subfamilies were observed feeding on the elaiosome, but not dispersing the seed (Table 4.1), and the elaiosomes of 46 seeds were removed *in-situ.* Of the eight taxa, six were recorded at the mining sites, and two were recorded at the residue restoration sites. Elaiosome 'thief' species richness was numerically higher in jarrah forest remnant plots and in the older (20-year-old) restoration site than in younger restoration sites (Figure 4.8). Seeds became unattractive once the elaiosome was removed by thieves. No seed thieves were recorded in pitfall traps at the two-year-old mining site and the tuart remnant site (Figure 4.8). Even though seed thieving ant species were sampled in pitfall traps, no seed thieving events were observed at the five- and 10-year-old mining restoration sites or in any of the residue restoration sites.
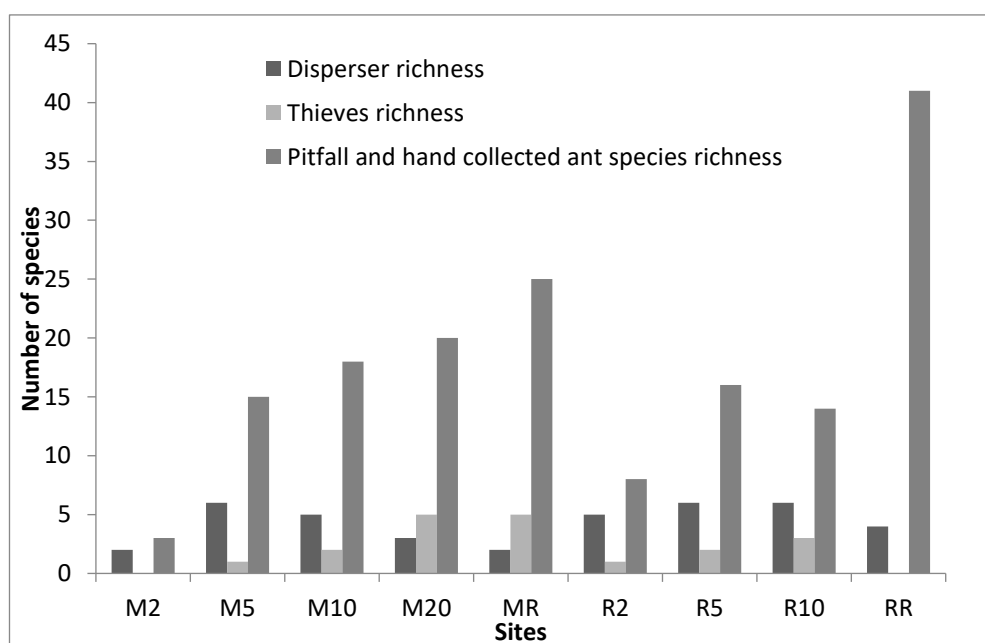
Figure 4.8. Ant species richness of seed dispersers (this study), elaiosome thieves (this study) and total species collected from pitfall traps at each site (Chapter 3). Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

Seed interacting ant species assemblages clearly separated by restoration system in the Principal Coordinate Analysis. Jarrah remnant plots and 20-year-old mining restoration plots were clustered together, indicating similar ant

composition. All other younger mining restoration sites (two-, five-, and 10-year-old restoration sites) and the residue restoration plots were clustered together, with 50 % similarity in assemblages (Figure 4.9). Within this grouping, younger residue sites (two- and five-year-old) and younger mining restoration sites (two-, five-, and 10-year-old) were clustered together and distinct from tuart forest remnant plots and 10-year-old residue restoration plots.
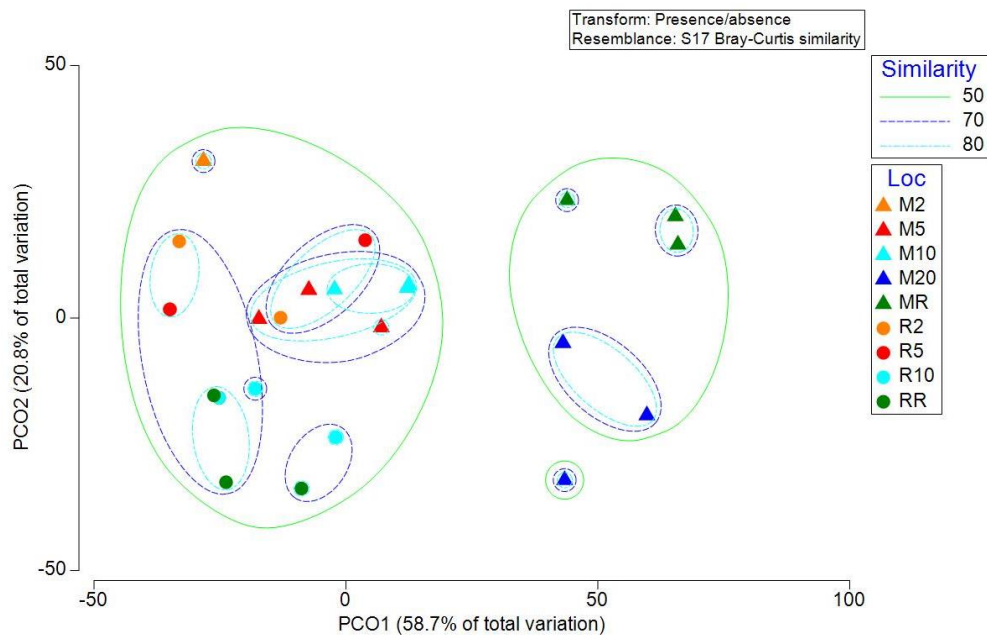
Figure 4.9. Principle Coordinate Analysis of seed-interacting ant species richness at mining and residue restoration system sites. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

4.5   Discussion

This study assessed the re-establishment of myrmecochorous ants and seed dispersal along a chronosequence associated with two distinctly different restoration systems—Alcoa's bauxite mining restoration and refinery residue sand revegetation—and compared these restored sites to adjacent remnant vegetation. Myrmecochorous events in the mining system declined as age since restoration increased and were relatively similar between the 20-year-old restoration and jarrah remnant sites. This might be due to lower abundance of myrmechorous ants in older (20-year-old) mining restoration and jarrah remnant sites than in younger (two-, five-, 10-year-old) restoration sites. This observation agreed with the results obtained by Gove et al. (2007), suggesting that the lower the abundance of myrmechorous ants, the lower the occurrence of myrmecochorous events.

Seed dispersal events were minimal in the residue restoration system; no myrmecochorous events were recorded at restored sites despite dispersal events in adjacent tuart remnants and seed-dispersing ant taxa being present at all the sites. The most abundant species at each site were responsible for the majority of seed dispersal events. Nevertheless, in the mining restoration system, seed dispersal events were not totally reliant on particular ant genera or species and varied between sites. In some sites, seed-dispersing ant assemblages were present, but seed dispersal was relatively rare, particularly at residue restoration sites. For example, species like *Iridomyrmex discors* and *I. chasei* are recorded in the literature as being long-distance efficient seed dispersers (Harris and Standish, 2008); however, even though these species were present, no dispersals were observed in the residue restoration system. The reason for this disparity is unclear. The residue habitat may not support as many myrmecochorous ants. However, seed disperser abundance in pitfall traps was similar between the residue and mining systems. Further, there were no indications of any climatic differences on sampling days that could account for these differences between the systems. The five- and 10-year-old residue restoration sites had thick groundcover that might affect ant activity and discovery of the feeding stations. However, the two-year-old residue restoration plots were open and had higher seed-disperser ant

richness and abundance than two-year-old mining restoration plots. Higher ant species richness at the residue restoration system might increase the competition for available resources, leading to ants being attracted towards more animal protein source, as suggested by Gove et al. (2007). The species present could also be less effective at finding seeds, or there could be an abundance of food, making the seeds less attractive at the residue restoration sites. Gove et al. (2007) also reported lower seed removal rates in high ant diversity plots, suggesting a case of diffuse mutualism that depends on the identity of particular partners and temporal and spatial variation of diffuse mutualists. This link to the variation in the abundance of particular partners is weakly or negatively associated with diversity.

Successful restoration aims to return the abundance and diversity of functional groups (seed dispersers and thieves) to that of the surrounding remnant vegetation. Unsurprisingly, the species composition of the mining and residue restoration systems differed, due perhaps to geographical separation, vegetation community differences, and soil type. In the mining system, the abundance, diversity, and richness of myrmechorous ant communities were observed to be approaching that of the remnant system as restoration age increased, suggesting mining restoration methods were achieving this restoration goal. Though the abundance, diversity, and richness of myrmechorous ant communities in the residue restoration system were approaching that of the remnant system as age since restoration increased, the system lacked the seed dispersal activity, suggesting a lack of myrmecochory ecosystem service even if the functional ant community was to be restored fully.

The difference in soil type between mining and residue restoration sites may partially account for the differences in seed dispersal activity. Mossop (1989) found a slight difference in ant seed removal rate on two different soil types (fertile and infertile soils). Ants present in the residue system were ineffective seed dispersers at the time of sampling, and it is worth further investigation of temporal and species-specific dispersal differences to understand why dispersal was not occurring. The lack of seed dispersal at the residue restoration sites might be a

contributing factor to the limited recruitment of native plant species that have been recorded at the residue sites (Dobrowolski et al., 2009).

Seed-interacting ant assemblage richness generally increased with age since restoration; the 10-year-old restoration plots in the mining restoration system had relatively similar species richness to the 20-year-old restoration site and jarrah remnant plots. This observation suggests that 10 years since restoration may be the transition stage from a rehabilitating ant community to one more representative of the pre-disturbance state. This pattern is similar to the findings of Bisevac and Majer (1999) in mineral sand mine restorations at Eneabba, where the species richness of older restorations (> 11 years) was higher than adjacent remnant forest sites. The higher seed removal rates at the youngest mining restoration site might be severely depleting the broadcasted seeds during the early phase of restoration. However, it is difficult to conclude whether the seed dispersal events at other younger restoration sites in the mining restoration system are favourable or not as the actual effect on seedling recruitment is unclear.

Rhytidoponera species are common seed-disperser ants in most Australian ant-seed removal studies (Gove et al., 2007). Gove et al. (2007) suggested the occurrence of *Rhytidoponera* species to be the best predictor of seed removal rate rather than ant diversity. This contradicts Garrido et al. (2002) and Rey et al. (2002) who found that seed dispersal rates were correlated to ant diversity. The mining system supported the results of Gove et al. (2007), which stated that the seed removal rate was influenced by the occurrence of *Rhytidoponera* species. However, the residue system did not support either theory due to a lack of dispersal events. *Rhytidoponera metallica* is one of the most active elaiosome collectors (Hughes et al., 1994) and plays a significant role as a keystone seed dispersal agent (Hughes and Westoby, 1992b; Hughes et al., 1994; Gove et al., 2007). The species collects large amounts of insect materials, rarely collects plant material other than elaiosomes (Hughes et al., 1994), and sometimes uses seeds

as decorative nest objects or playthings (Russell et al., 1967); there is no evidence that they predate on the seed.

Rhytidoponera metallica tends to change in population size as a response to disturbance type, severity, and time since disturbance (Hoffmann and Andersen (2003) and the genus is classified as an opportunist ant functional group, with unspecialized, "weedy" species that typically occur in disturbed habitats (Andersen, 1997). In this study, the abundance of *R. metallica* was higher in younger mining restoration sites (two-, five- and 10-year-old) than the 20-year-old mining restoration sites and the jarrah remnants. This fits with Andersen's classification and indicates a decline in the level of disturbance. As compared to the mining restoration sites, *Rhytidoponera metallica* was recorded from pitfall traps to be in higher abundance in the tuart remnant and the residue restoration sites; however, despite this, seed dispersal events were absent in residue restoration sites and rare in tuart remnants (three dispersal events only).

The fate of elaiosome bearing plant seeds is highly dependent on seed-removing ant species (Andersen, 1991b; Hughes and Westoby, 1992a; Hughes et al., 1994). All ant species recorded moving seeds had mean dispersal distances exceeding that of the reported global mean (0.96 m; Gómez and Espadaler (1998) and exceeding the mean seed dispersal distance of 2.1 m of ants in south-eastern Australia (Andersen, 1988a). *Iridomyrmex bicknelli* is reported as being an early coloniser of rehabilitated mine-site plots (Heterick, 2009) and was only recorded in younger (< 10 years old) restoration sites. This species was found to be a short-distance seed-disperser by Andersen (1988a) in south-east Australian sclerophyllous vegetation. However, in this study, *I. purpureus* was responsible for most of the long-distance dispersals. *Iridomyrmex purpureus* is large compared to the other seed-dispersing ant species and tends to have large colonies and well-used trails extending far from its nest. In this study, *I. purpureus* nests were very patchy and only found in the middle-aged restoration sites; hence, the largest dispersal distance occurred in the 10-year-old mining restoration site. Further, *I. purpureus* was observed to deposit intact seeds just outside the nest openings,

confirming the findings of Russell et al. (1967) and Majer (1982) that it occasionally use seeds as building blocks during nest construction.

Myrmecochorous seed dispersal events decreased at the mining restoration system as age since restoration increased and was relatively similar between the 20-year-old restoration site and the jarrah remnant site, indicating myrmecochory may be returning to pre-disturbance levels. This may be due to the structure of the habitat, with the community of ants in hot open areas being quite different to that in an area of understory vegetation and deep litter (Andersen, 1995; Hoffmann and Andersen, 2003). Grimbacher and Hughes (2002) compared ground-dwelling ant communities and rates of seed removal by ants in weed-infested, regenerating, and undamaged urban bushland in northern Sydney where, in contrast to this study, seed removal rates were significantly lower in disturbed sites than in undisturbed sites.

Ants need to have high nutrient diets during re-colonization as they have to re-establish the colony within a short period (Dussutour and Simpson, 2009). Elaiosome is rich in nutrients, proteins, lipids, and carbohydrates (Hughes et al., 1994; Hilley, 2014) and high nutrient demand during re-colonization might be the trigger factor for ants in younger restoration sites to be attracted to seeds more extensively than in older sites and remnant plots. Ants are one of the most diverse feeders, and they feed on a wide range of food. However, naturally, they rely on honeydew from aphids and other hemipterans (Helms and Vinson, 2008), small dead invertebrates, plant and fruit saps, flower nectar, and sometimes colony maintained fungus gardens (Cahan and Julian, 1999) for food. Compared to older restoration and remnant plots, vegetation cover is relatively low in younger restorations, with barely any flowering or fruiting (author's observation). This lower vegetation cover may result in less food production by other invertebrates like aphids, which consequently results in food scarcity for ants. This might explain the intensive motivation of ants to be attracted to available seeds in younger restoration sites as an alternative food source. However, this assumption is not valid for the residue restoration system as no seed removal was observed there. Ants (predominantly *Iridomyrmex* species and *Rhytidoponera* species) were observed carrying dead arthropods or body parts of dead invertebrates, but not

seeds. Lack of interest to seed stations might explain that ants in residue restoration sites had readily available food resources than those in mining restoration sites.

Intensive seed removal at younger restoration sites might be a disadvantage for plant recruitment if these seeds are subjected to predation, or deposited in deep unfavourable galleries within ant nests. Andersen (1988b) reported that *Aphaenogaster longiceps* nest-mound soils increase the growth of seedlings by 50 % than control soils. Frouz and Jilková (2008) stated that nutrient content (mostly nitrogen and phosphorous), temperature, and moisture content were increased in ant nests than in the external environment, which provides ideal conditions for seed germination. It might be worth investigating if there is a difference in seed deposition depth due to restoration age or ant species.

All recorded elaiosome thieves were small ants that were unable to carry or drag the seeds to their nest, as found in other studies (Bas et al., 2009). Seed-thieving species richness and abundance were higher in the 20-year-old mining restoration site and the jarrah remnant than in younger mining restoration sites, and this corresponded with higher elaiosome thieving events. Elaiosome thieving occurred on the feeding-board itself, and the *Acacia* seeds were unattractive to these myrmecochorous ants, as reported previously (Espadaler and Gómez, 1997; Bas et al., 2009). For seed-feeding ants, such as *Melophorus* and *Pheidole,* their interest in seed did not depend on the presence or absence of elaiosome. Similar to Harris and Standish (2008), it was found that one *Monomorium* species and all recorded *Iridomyrmex* species dispersed seed. Further, similar to this study, Hughes et al. (1994) observed occasional elaiosome-bearing seed removal by *Iridomyrmex* species. The relative diversity and abundance of seed-feeding species recorded in this study were lower compared to the findings of Harris and Standish (2008) in the Wheatbelt, which had a lot of *Melophorus* species, a high-temperature active species that can disperse seeds relatively long distances.

In conclusion, seed removal rates at the mining restoration system and the residue restoration system differed markedly. As the age since restoration increased, seed removal rates and dispersal distances in the mining restoration system became

similar to those of the jarrah remnant. In contrast, no seed dispersal events were recorded in the residue restoration sites—with only some dispersal events observed in the tuart remnant—despite the presence of seed-dispersing ants.

Chapter 5 Recovery of Ecosystem Engineers: Influence of Ground-Dwelling Invertebrates and Microbes on Litter Decomposition in relation to Bauxite Mining Restoration Age

5.1 Abstract

Re-establishment of litter decomposition and nutrient cycling is essential for the development of self-sustaining ecosystems following mine site restoration. Ground-dwelling fauna plays a vital role in litter decomposition, with microorganisms decomposing litter to release nutrients and invertebrates modifying litter to enhance microbial activity. However, the influence of ground-dwelling invertebrates as a decomposer functional group on litter decomposition is typically less considered in mining restoration than in agricultural practices. This study investigated the influence of both the ground-dwelling invertebrate community and microbes in litter decomposition and nutrient cycling in bauxite mine restoration and residue sand storage area restoration. The mine site restoration system comprised five treatments: 20-, 10-, five-, and two-year-old restoration sites, and an adjacent jarrah forest remnant (undisturbed) site; the residue restoration system comprised four treatments: 10-, five-, and two-year-old restoration sites, and an adjacent tuart forest remnant (undisturbed) site. The weight loss of dry litter (*Hardenbergia comptoniana* leaves) in three types of nylon/polyester mesh bags—fine (50 μm; permeable to microorganisms only), medium (2 mm; permeable to small invertebrates), and coarse (2 cm; permeable to macroinvertebrates)—was measured after 3, 6, 9, and 12 months. Overall litter decomposition was increased as the incubation period increased, and decomposition was higher in the residue restoration system than in the mining restoration system. Microbial decomposition increased as restoration age increased. The presence of ground-dwelling macroinvertebrates increased the rate of decomposition in both restoration systems, and as the restoration progressed, the rate of decomposition was on the trajectory to return to pre-

disturbance levels. The decomposer invertebrate functional group diversity was higher in the mining restoration system compared to the residue restoration system regardless of restoration age; however, the abundance was lower in mining restoration compared to residue restoration. In conclusion, litter decomposition was shown to be progressing in both restoration systems; this study provided a better understanding of the role of ground-dwelling invertebrates as decomposers in mining restoration projects.

5.2   Introduction

Forest loss and degradation is a significant threat to biodiversity, not only affecting species composition and abundance, but ecosystem processes such as litter decomposition (Walker, 1992; Hooper et al., 2005; Cagnolo et al., 2009; Bihn et al., 2010; Tscharntke et al., 2012; Bernaschini et al., 2016). Biogeochemical cycling and energy transfer are key ecosystem processes to maintain a healthy, sustainable ecosystem (Banning et al., 2011a; Kollmann et al., 2016). Species diversity, composition, relative abundance, and interactions between species have a functional consequence on ecosystem processes (Chapin et al., 2000). For example, the interactions between soil biological, physical, and chemical components maintain the equilibrium of nutrient cycling (Banning et al., 2011a). Soil management practices and human use of soil to yield goods and services can alter the soil ecosystem structure and functions, and impact the interaction between the soil physiochemical environment and soil fauna (Vitousek et al., 1997; Casimiro et al., 2019). For example, mining can result in the loss or homogenization of natural soil from forest floors and, hence, disrupts soil ecosystem structure and functions.

The decomposition of litter is a vital function within a healthy ecosystem, and contributes to soil formation, nutrient cycling, and succession of soil fauna. The breakdown of the litter layer and the accumulation of organic matter are vital components of soil formation (Sayer, 2006; Frouz et al., 2007). Litter decomposition is crucial for energy and nutrient transfer through ecosystems (Irmler, 2000; Kollmann et al., 2016). Decomposition of leaf litter returns more than 50 % of net primary production to the soils in terrestrial ecosystems and is

identified as a catabolic complement of photosynthesis (Barrios, 2007). When litter breakdown occurs, it facilitates the movement of carbon (C) from dead organic matter back to the ecosystem (Shaver et al., 1992; Sayer, 2006) and is responsible for about 70 % of the total annual C flux (Raich and Schlesinger, 1992).

Litter decomposition is a two-stage process: stage one is the breakdown of litter into small fractions by detritivores, and stage two is the mineralization of fragmented litter into basic inorganic molecules by microorganisms (Aerts, 1997). Several factors control litter decomposition, including climate, litter chemistry, and soil fauna (Rodríguez Pleguezuelo et al., 2009; Frouz et al., 2015). Climate is vital to litter decomposition as soil biological processes depend on environmental factors such as temperature and rainfall (García-Palacios et al., 2013). Many studies suggest a positive linear relationship between temperature and litter decomposition (He et al., 2010; Salah and Scholes, 2011; Bothwell et al., 2014). At large spatial scales, climate indirectly affects litter decomposition by altering soil fauna distribution and modifying litter chemistry (Wardle et al., 2004; García-Palacios et al., 2013).

Previous studies considered climate to have a more significant effect on litter decomposition than litter chemistry, and litter chemistry to have a more significant effect than soil fauna (Lavelle et al., 1993). However, García-Palacios et al. (2013) suggested that soil fauna was the principal determinant factor of litter decomposition as it was the ultimate actor of litter decomposition. Assessment of ecosystem processes such as nutrient cycling and decomposition provides information about the resilience of restored ecosystems (Ruiz-Jaen and Aide, 2005); however, the verification of whether these processes lead to resilience would require testing under different environmental challenges.

Many studies have identified the positive influence of ground-dwelling invertebrates on the litter decomposition process (Zimmer, 2002; Yang and Chen, 2009; Yoriko Suzuki et al., 2013; Donald et al., 2017). Ground-dwelling invertebrates occupy multiple trophic levels; for example, millipedes and earthworms are detritivores, while Mesostigmata and Collembola are predators and fungivores (Lavelle et al., 1993; Lavelle and Spain, 2001). This trophic level

diversity influences the decomposition process through litter fragmentation, digestion, and stimulation of microorganism activities (Lavelle et al., 1993; Lavelle and Spain, 2001; Yang and Chen, 2009).

There is vast diversity within soil organisms, and most decomposer communities appear to be trophic generalists suggesting there is considerable overlap for food resources (Anderson, 1975; Maraun et al., 2003) and that there may be functional redundancy within communities (Bihn et al., 2010; Baiser and Lockwood, 2011). If there is redundancy, then a species-poor community may function equally as well as a diverse community in terms of litter decomposition. Therefore, decomposer abundance may be as important as diversity in determining decomposition rate, although the relatively species poor the ecosystem is functioning in the measured set of parameters. but this may not be the case in all conditions.

Invertebrates can be classified by body size as micro-invertebrates, meso-invertebrates, and macro-invertebrates. Some of the micro-invertebrates—less than 200 μm in size, including Protozoa and Nematodes—live in water-filled soil pores (Lavelle et al., 2006). Many groups of meso-invertebrates (0.2–2 mm) and a few micro-invertebrate live in air-filled soil pores (Lavelle et al., 2006). Macro-invertebrate (> 2 mm)—including, Annelida, Mollusca, and Crustacea—live in the surface of soil and litter, and either burrow or create their nests in the soil (Lavelle et al., 2006).

Saprophagous macro-arthropods—i.e. millipedes, land-hoppers, woodlice or any insect or life stage of an insect—contribute to the decomposition process directly through enzymatic degradation or indirectly through the promotion of microbial activity (Zimmer, 2002). Thus, saprophagous macro-arthropods are known as "litter transformers" as they consume a large amount of decaying plant material within an ecosystem (Lavelle and Spain, 2001; David, 2014). However, a significant amount of un-decomposed plant material is egested in feces (Gillon and David, 2001). Therefore, saprophagous macro-arthropods have a direct impact on mass loss of litter, while their influence on decomposition is low (Coulis et al., 2013). Ground-dwelling invertebrates also modify the structure and function of soil microbial activities (Hattenschwiler et al., 2005). Millipedes and Isopods affect

litter decomposition through litter fragmentation via assimilation and respiration, which only has a minor direct impact on decomposition (Lavelle and Spain, 2001); however, they have a significant indirect effect on decomposition by increasing the surface area for microbial decomposition (Snyder and Hendrix, 2008). Some invertebrates inoculate litter with microfauna and graze microbial populations to exponential growth levels (Douce and Webb, 1978). For example, faecal pellets of millipedes also act as hot spots for microbial populations (Dangerfield and Milner, 1996). Furthermore, the presence of primary decomposers, such as epigeic earthworms, increases the abundance of microbial communities for litter decomposition (Seeber et al., 2006).

Following the disturbance of land by mining activities, several researchers have reported different sequences of succession within invertebrate soil communities (Majer et al., 1982; Postle et al., 1991; Greenslade and Majer, 1993; Majer et al., 2013). Decomposer communities are specialized in particular decomposition processes and stages, which determines the successional sequence of decomposing communities (Anderson, 1975). Bastow (2013) suggests that succession is determined by the changes in resources and habitat quality over time, rate of colonization and competition, and development rates of decomposer organisms. Generally, fungi and bacteria are known as the first colonizers of litter substrate, and they interact with one another through a series of successional processes to share the litter resource (Bardgett and Walker, 2004; Sayer, 2006). Litter priming by microorganisms appears to be crucial for subsequent litter invertebrate colonization (Sadaka-Laulan et al., 1998; Hedde et al., 2007).

In terms of post-mining restoration, successional changes of ground-dwelling invertebrate communities have been demonstrated by several researchers. For example, changes in composition and increases in diversity of earthworms, (Mudrák et al., 2012), millipedes, isopods, and centipedes (Pizˇl, 2001; Berg and Hemerik, 2004) have been recorded, improving litter quality as age since restoration increases and enhancing the productivity of late-successional plants in post-mining restoration sites. Saprophagous macro-arthropod succession in terrestrial Mediterranean ecosystems demonstrated a shrub-litter consumption preference shift (Coulis et al., 2013) and succession of termites in Australian

savanna restoration was linked to enhanced soil carbon and nitrogen levels, litter decomposition, soil water storage, and plant cover (Dawes, 2010).

Litter decomposition and nutrient cycling are vital ecosystem process components that need to be restored during mine restoration (Courtney et al., 2010). However, the importance of meso- and macro-fauna in re-establishing decomposition and nutrient cycling functions is poorly studied (Cristescu et al., 2012). Most empirical studies on the effect of ground-dwelling invertebrates on litter decomposition have focused on the effect under different climatic conditions (Bokhorst and Wardle, 2013; García-Palacios et al., 2013) and litter chemistries (Frouz et al., 2007; Haitao et al., 2007; García-Palacios et al., 2013; Zhang et al., 2013).

This study aims to investigate the relationship between ground-dwelling invertebrates and decomposition of leaf litter in bauxite post-mining restoration and residue restoration. It is hypothesized that;

- As the age since rehabilitation increases, litter decomposition rates and litter decomposition invertebrate communities will closely resemble undisturbed remnant (reference) sites.
- Invertebrate assemblages will change over time (succession) and will differ between the mining and residue restoration systems.

5.3   Methods

5.3.1   Study sites

Two restoration systems, mining restoration and residue restoration, were chosen. The mining restoration system had five sites (20-, 10-, five-, and two-year-old restoration sites, and an adjacent jarrah forest remnant site) and the residue restoration system had four sites (10-, five-, and two-year-old restoration sites,

and an adjacent tuart forest remnant), with three replicate plots for each treatment (details of study sites provided in Chapter 2).

5.3.2   Experimental design

The relationship between ground-dwelling invertebrates and leaf litter decomposition was investigated with the use of three different leaf litter bags: coarse, medium, and fine (see section 5.3.3 for litter bag specification). The relationship between soil microbial respiration rate (see Chapter 2 for more details of soil microbial respiration) and leaf litter decomposition was also investigated.

5.3.3   Leaf collection

A composite sample of whole fresh leaves of *Hardenbergia comptoniana*, a native Australian ground-cover legume present in the restoration areas, was collected over four weeks from several individual plants at Kwinana in August and September 2014. Leaves were dried at 30 °C in the laboratory oven for 21 days. Only one plant species was selected for the study to reduce the effect of mixed litter (Meyer et al., 2011) and allow a direct comparison of decomposition rates by soil fauna (Gartner and Cardon, 2004).

The rate of litter decomposition was assessed by measuring percentage loss from a known mass of leaf litter in 20 cm × 20 cm black nylon/ polyester litter bags (Ward et al., 1991; Meyer et al., 2011). Three different litter bag designs were used: fine litter bags of 50 μm × 50 μm mesh size (to only allow bacteria, fungi, and other microorganisms to enter), medium litter bags of 2 mm × 2 mm mesh size (to also allow small invertebrates, i.e. meso-invertebrates, such as collembolans and mites to enter), and coarse litter bags of 2 mm × 2 mm mesh with six 20 mm × 20 mm access holes cut in the upper surfaces (to allow macro-invertebrate access). Dry leaf litter (4.0 ± 0.02 g) was added to each bag, and the bags were subsequently stitched closed with nylon/ polyester thread.

In February 2015, the litter bags were placed in the three replicate plots of each mining and residue restoration site. The locations where the bags were placed were the same as used in Chapter 2.3.1, except for two plots in the 10-year-old residue restoration site and two plots in the residue restoration reference site due

to an unplanned fire burning these sites in December 2015. For these locations, plots were moved to new positions within the same restoration site outside the burn footprint. Fifteen litter bags of each mesh size, collectively 45 litter bags, were randomly placed within each 20 m × 20 m quadrats (plots); the bags were placed at least 0.5 m apart from each other and pegged to the ground with 4 mm diameter bamboo sticks. Any litter on the ground was removed before placing the litter bags.

5.3.4   Litter decomposition

Three bags of each mesh size were collected from each quadrat (plot) at four sampling times (3, 6, 9 and 12 months). Upon collection, litter bags were placed individually into calico bags and kept in cool conditions (below 15 °C).

5.3.5   Invertebrate extraction

Large invertebrates were collected by hand from each litter bag within 6 h of collection; the litter was then exposed to heat extraction in Berlese funnel to extract remaining invertebrates within 12 hours of collection. All extracted invertebrates were sorted to the ordinal level, identified to the morphospecies level where possible, and counted.

5.3.6   Invertebrate successional trend and calculation of the rate of litter mass loss

The successional trend of invertebrates between restoration systems was quantified across sampling efforts at 3, 6, 9 and 12 months, by calculating the mean temporal occurrence (T_i) of species (Equation 5.1) according to Irmler (2000).

Equation 5.1.

$$T_i = \sum_{t=1}^{T=mi} n_i \cdot m_i / N$$

where, T_i is temporal occurrence index, n_i is number of individuals at time mi, m_i is months after commencement of experiment, and N is total abundance of species.

After invertebrate extraction, litter samples were dried at 60 °C for 48 h and weighed to the nearest 0.0001 g. The mass-loss rate in different mesh size litter bags was calculated using Olson's formula (Equation 5.2; Olson, 1963):

Equation 5.2.

$$x_t = x_0 \cdot e^{-Kt}$$

where, x_t is mass at time t, x_0 is mass at starting time t_0, and K is mass-loss rate.

The contribution of micro-invertebrates to the mass-loss rate (K_{mf}) was calculated by subtracting the microbial mass-loss rate (K_m) (total mass-loss rate in the litter bags with fine mesh size) from the overall mass-loss rate in the litter bags with medium mesh size. The impact of macro-invertebrates to the mass-loss rate (K_f) was calculated by subtracting the microbial mass-loss rate (K_m) and micro-invertebrate mass-loss rate (K_{mf}) from the total mass-loss rate in the litter bags with coarse mesh size.

5.3.7   Data analysis

Two-way ANOVA in SPSS (version 25) was used to compare the mass-loss rate of litter, between the different mesh size litter bags for each site and between restoration systems. Nested ANOVA in SPSS (version 25) was used to compare the mass-loss rate at each restoration age between the two restoration systems, with bag type and sampling month as random variables, such that significant variations in means (mean mass-loss rate) among groups (two restoration systems) and

among subgroups (restoration age, bag type, sampling month) within groups could be investigated.

Differences in the mass-loss rate of litter between the two restoration systems and sites were tested using two-way ANOVA in SPSS version 25. The interaction effect between sites × sampling month × bag type on litter mass loss rate was determined using the generalized linear model in SPSS.

The relationship between mass-loss rates in fine litter bags and soil microbial respiration (see Chapter 2 for more details of soil microbial respiration) was analysed with Pearson's correlations. The R- statistic was considered significant at $P < 0.05$.

Two-way ANOVA in SPSS version 21 was performed to determine the differences in the abundance of ground-dwelling invertebrates between litter bags and sampling intervals. Ground-dwelling invertebrate successional trend was quantified by calculating the mean temporal occurrence (Irmler, 2000).

Data were normally distributed, and no transformations were necessary.

5.4 Results

5.4.1 Mass-loss rates of leaf litter and litter decomposition

The presence of macro-invertebrates increased the mass-loss rate by between 10 % (jarrah remnant) to 51 % (two-year-old residue site) after 12 months of leaf litter exposure in the field. Overall invertebrate litter decomposition rates after 12 months of exposure were higher in the residue (over 90 %) than in the mining (60–75 %) restoration system (Figure 5.1). After 12 months, the highest decomposition rates by invertebrates (coarse bag – fine bag) were recorded at the 10-year-old and five-year-old restoration sites (21 %) in the mining system. At the residue restoration system, the highest decomposition rate by invertebrates was recorded at the two-year-old restoration site (51.5 %) (Figure 5.1). Further, coarse bags of all restored residue sites had higher mass loss than the tuart remnant and the highest loss was recorded at the five-year-old residue restoration site (Figure 5.1). Medium bag mass loss was also higher in all residue restoration sites than in the tuart remnant, with the two-year-old residue restoration site having the highest mass loss (Figure 5.1).

Generally, in both restoration systems, mass loss of the coarse litter bag was increased as the field incubation period increased (Figure 5.3). In both restoration systems, after three months in the field, coarse litter bags, which allowed access to ground-dwelling invertebrates, lost more than 40 % of dry mass (Figure 5.3a). In the residue restoration system, after three months of field incubation, coarse bags lost between 30–40 % of their mass (Figure 5.3a). In the residue restoration system, there was a drop in fine bag mass loss (weight gain) in 9 months.

In all study sites, mass loss was highest for coarse bags ($F_{2,216}$= 449.527, P < 0.0001) permeable to macroinvertebrates (Figure 5.1). In both restoration systems, the mass loss of fine bags increased as age since restoration increased. Decomposition of leaf litter at the mining system increased with age since restoration and was similar to that of the jarrah forest remnant at the 10-year-old restoration site. In the mining restoration system, decomposition of leaf litter in the coarse bag also increased as age since restoration increased until the restoration age reached 10-

year-old, and the 20-year-old mine-pit restoration and thee jarrah remnant had lower mass loss than the 10-year-old restoration.
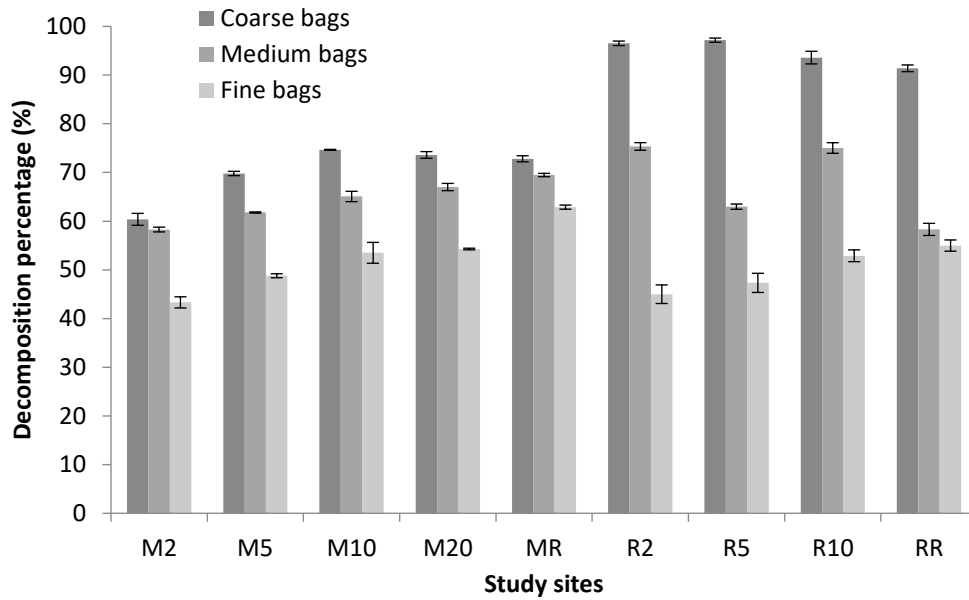
Figure 5.1. Cumulative mean mass loss of litter (%) (± SE) for each litter-bag type at each study site after 12 months. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

There was a significant difference in decomposition rate for all factors—restoration system, restoration age, sampling month and bag type—and significant interactions between factors (Table 5.1).

Table 5.1. Effect of restoration system, restoration age, sampling month and bag type on the decomposition rate of mining and residue sites over 12 months.

Factors	Mass loss rate	
	F - statistics	P – values
restoration system	$F_{1,216}= 52.985$	< 0.0001
restoration age	$F_{8,216}= 40.586$	< 0.0001
sampling month	$F_{3,216}= 181.647$	< 0.0001
bag type	$F_{2,216}= 449.527$	< 0.0001
restoration system* restoration age	$F_{3,216}=58.908$	< 0.0001
restoration system* restoration age * bag type	$F_{6, 216} =11.363$	< 0.0001
restoration system* sampling month * bag type	$F_{6,216}= 36.134$	< 0.0001
restoration system* restoration age * sampling month	$F_{9, 216}= 3.311$	< 0.001
restoration system* restoration age *sampling months*bag type	$F_{18,216}= 2.977$	< 0.0001

Presence of macro-invertebrates significantly increased litter decomposition in both restoration systems ($F_{1,107}= 31.023$, $P < 0.001$). Litter decomposition rates were higher in restored sites than in remnant sites. Litter sampled at nine months was lighter than those collected at 12 months, especially at residue restoration sites (

Figure 5.2).
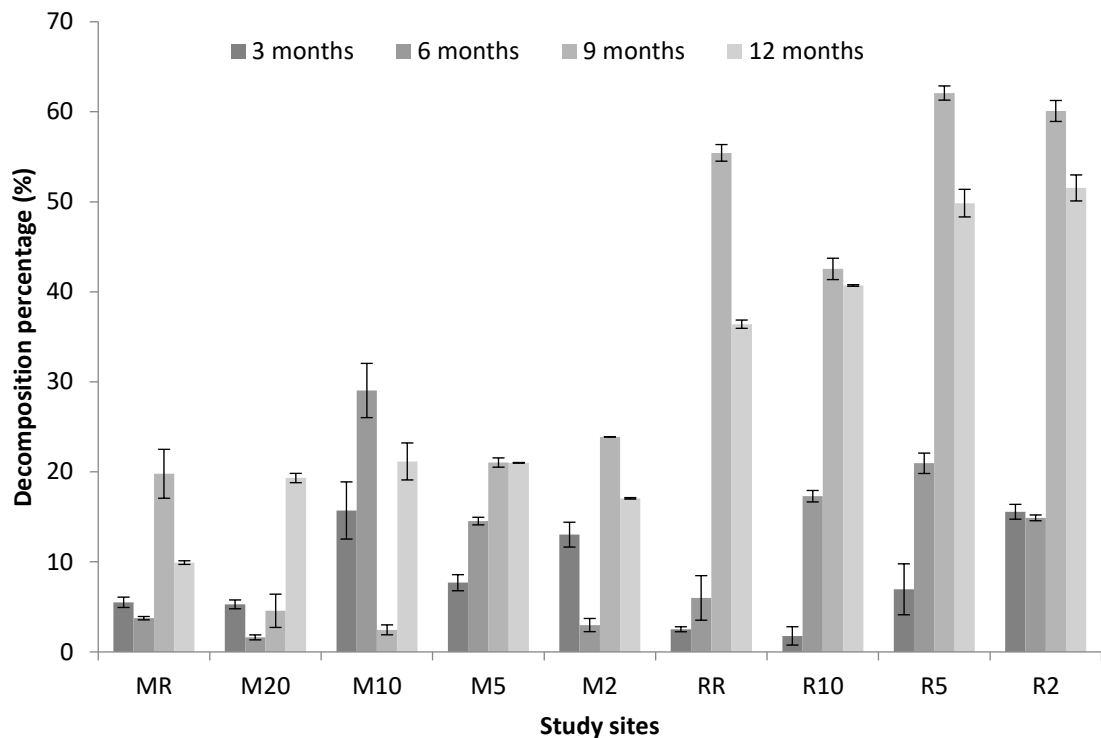
Figure 5.2. Mean decomposition (%) of leaf litter by macro-invertebrates (coarse bag – fine bag) after 3, 6, 9, and 12 months of field exposure. Sites: MR = jarrah remnant, M20 = 20-year-old mining restoration, M10 = 10-year-old mining restoration, M5 = five-year-old mining restoration, M2 = two-year-old mining restoration, RR = tuart remnant, R10 = 10-year-old residue restoration, R5 = five-year-old residue restoration, and R2 = two-year-old residue restoration.

The presence of macro-invertebrates and meso-invertebrates significantly increased the litter decomposition rate in both restoration systems ($F_{1,107}$= 9.646, P < 0.001) (Figure 5.3). Microbial litter decomposition was higher than macro-invertebrate and meso-invertebrate litter decomposition in both restoration systems, apart from the two- and five-year-old residue sites and the tuart remnant, after nine months of field incubation (Figure 5.3). The presence of macro-invertebrates significantly increased litter decomposition, more so at the residue than the mining restoration system ($F_{1,94}$= 30.744, P < 0.001) (Figure 5.3a). At the mining restoration system, as age since restoration increased, the decomposition by macro-invertebrates gradually increased till the restoration sites reached 10 years of age after 3, 6, and 12 months of field incubation (Figure 5.3a). In both restoration systems, the highest decomposition by meso-invertebrates was recorded in the younger restoration sites and the lowest in the remnants, with decomposition by meso-invertebrates higher in the residue than the mining system (Figure 5.3b). Decomposition by macro-invertebrates was consistently higher than decomposition by meso-invertebrates at residue

restoration, but similar at mining restoration (Figure 5.3a, b). In both restoration systems, decomposition by microorganisms increased as age since restoration increased (Figure 5.3c).
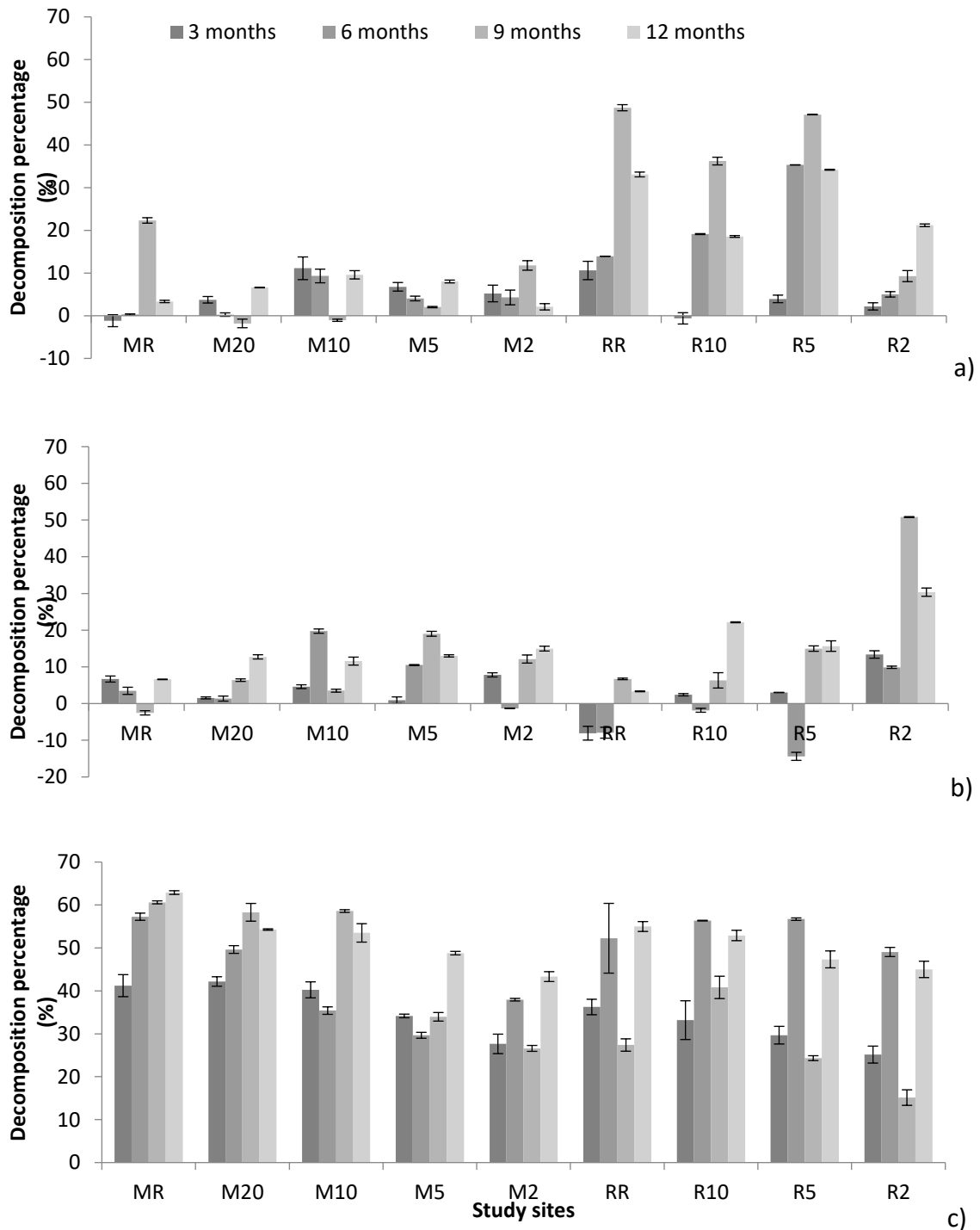
Figure 5.3. Average contribution to decomposition (%) of leaf litter by a) macro-invertebrates (loss of coarse bag - medium bag), b) meso-invertebrates (loss of medium bag - fine bag) and c) microorganisms (fine bag) after 3, 6, 9 and 12 months of incubation at the fields in each site. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

5.4.2   Litter Invertebrates

A total of 2312 ground-dwelling invertebrates belonging to 18 orders and classes were collected at the mining restoration system, and a total of 2779 ground-dwelling invertebrates belonging to 18 orders and classes were collected at the residue restoration system. Collembolans were the most abundant group in both restoration systems (Figure 5.4). Acari, psocopterans, and coleopterans were the next most abundant groups at mining restoration while Acari, diplopods (millipedes), and neuropterans were the next most abundant at the residue restoration system.

Dermaptera, Blattodea, and Orthoptera were present in the coarse bags, but were excluded from the medium litter bags (Figure 5.4). Isopods were recorded in the medium and coarse bags at the mining system while Orthopterans were only recorded in coarse bags at the residue restoration system. Millipedes were not recorded in medium bags at the mining system but were present in both bag-types at the residue restoration system (Figure 5.4).

The abundance of invertebrates was significantly different between sampling months (i.e. time since litter bags were placed at the sites) (Table 5.2). Invertebrate diversity was significantly different between restoration systems, restoration age, and sampling month (Table 5.2). Invertebrate species richness was significantly different between study sites (restoration age) and sampling month (Table 5.2). There was no significant interaction between the study site and sampling month for litter invertebrate abundance, diversity, and richness.

Time since litter bag exposure in the field was positively correlated with invertebrate diversity ($r = 0.289$, $P < 0.05$) and negatively correlated with invertebrate abundance ($r = -0.537$, $P < 0.001$). Age since restoration showed a significant positive correlation to species richness ($r = 0.437$, $P < 0.0001$) and diversity ($r = 0.306$, $P = 0.009$).

The time since the litter bags were in the field ($r = 0.630$, $P < 0.001$), invertebrate diversity ($r = 0.257$, $P = 0.029$), and age since restoration ($r = 0.238$, $P = 0.044$) showed a positive, significant correlation to decomposition rate. A significant negative correlation was observed between invertebrate abundance and decomposition rate ($r = -0.412$, P

< 0.0001). There was no significant correlation between invertebrate species richness and decomposition rate.

Table 5.2. Effect of restoration system, restoration age, and sampling month on invertebrate abundance, diversity, and species richness. Bold text indicates a significant difference (ANOVA, P < 0.05). N.S. indicates non-significance.

Factor	Invertebrate abundance		Invertebrate diversity		Species richness	
	F - values	P - values	F - statistics	P - values	F - statistics	P - values
restoration system	$F_{1,72}=$ 4.540	N.S.	$F_{1,72}=$ 4.540	**0.04**	$F_{1,72}=$ 0.054	N.S.
restoration age	$F_{7,72}=$ 0.897	N.S.	$F_{7,72}=$ 3.362	**0.007**	$F_{4,72}=$ 6.707	**< 0.001**
sampling months	$F_{3,72}=$ 17.606	**< 0.001**	$F_{3,72}=$ 11.872	**< 0.001**	$F_{3,72}=$ 17.031	**< 0.001**
restoration system* restoration age	$F_{3,72}=$ 3.425	**0.027**	$F_{3,72}=$ 1.202	N.S.	$F_{3,72}=$ 4.835	**0.006**
restoration system* sampling month	$F_{3,72}=$ 17.606	N.S.	$F_{3,72}=$ 1.283	N.S.	$F_{3,72}=$ 3.006	**0.043**
restoration age * sampling month	$F_{21,72}=$ 1.276	N.S.	$F_{21,72}=$ 1.465	N.S.	$F_{21,72}=$ 1.104	N.S.
restoration system* restoration age * sampling months	$F_{9,72}=$ 2.273	**0.039**	$F_{9,72}=$ 1.016	N.S.	$F_{9,72}=$ 0.364	N.S.
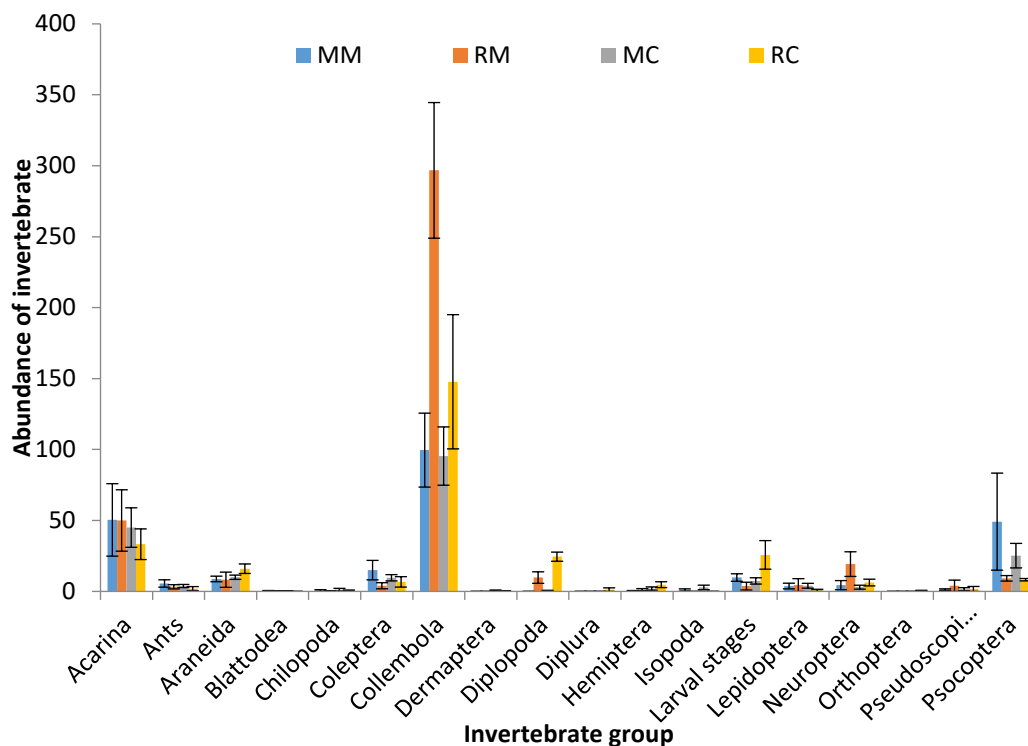
Figure 5.4. Mean abundance of macro-invertebrates collected in medium and coarse litter bags from mining and residue restoration systems. MM = mining restoration medium litter bags, MC = mining restoration coarse litter bags, RM = residue restoration medium litter bags, and RC = residue restoration coarse litter bags. * indicates significant differences in the number of individual invertebrate groups collected from each restoration system.

5.4.3 Invertebrate succession and decomposition rate in leaf litter

Study site had a significant effect on succession of Chilopoda ($F_{8,70}$= 4.603, P < 0.01), Dermaptera ($F_{8,70}$= 2.278, P < 0.05), Isopoda ($F_{8,70}$= 3.758, P < 0.01), and Pseudoscopionidae ($F_{8,70}$= 3.157, P < 0.01). Month of collection had a significant effect on Acarina ($F_{3,70}$= 8.104, P < 0.001), Chilopoda ($F_{3,70}$= 12.829, P < 0.001), Coleptera ($F_{3,70}$= 8.104, P < 0.001), Collembola ($F_{3,70}$= 3.352, P < 0.05), Dermaptera ($F_{3,70}$= 2.959, P < 0.05), Diplopoda ($F_{3,70}$= 4.347, P < 0.05), Isopoda ($F_{3,70}$= 3.375, P < 0.05), and Lepidoptera ($F_{3,70}$= 4.814, P < 0.01). There was a significant interaction effect of study site and time of collection on Chilopoda ($F_{24,70}$= 4.645, P < 0.001), Isopoda ($F_{24,70}$= 2.278, P < 0.05), and Pseudoscopionidae ($F_{24,70}$= 3.758, P < 0.01). Succession of Collembola (r = 0.308, P <

0.001) and Diplopoda (r = 0.373, P < 0.01) were positively correlated with decomposition rate.

5.4.4 Microbial activity and mass-loss rate in leaf litter

The mass-loss rate in fine litter bags (only permeable to microbes) was not significantly different between the two restoration systems, but was significantly different between restoration age ($F_{7,18}$= 24.192, P < 0.001), with an increase in mass-loss rate as age since restoration increased. There was no significant interaction effect between restoration system and sites on fine bag mass-loss rate. There was a positive correlation between fine bag mass-loss and microbial respiration (r_{25} = 0.424, P =0.001).

5.5 Discussion

The ultimate goal of restoration is to establish a self-sustaining ecosystem, which produces its fertilizer by recycling nutrients and supports flora and fauna communities (Society for Ecological Restoration, 2004; Oktavia et al., 2015). This study assessed the influence of litter-dwelling invertebrates on litter decomposition and nutrient cycling. The use of litter bags with three different mesh sizes, which permitted or excluded the access of invertebrates to litter, allowed the influence of invertebrates on litter decomposition to be estimated. Total ground-dwelling invertebrate richness and abundance at each site was positively correlated to litter decomposition. However, consistent with other studies (Irmler, 2000; Seeber et al., 2006; Niemelä et al., 2007; Meyer et al., 2011), invertebrates sampled within litter bags at the time of collection had no significant correlation on the rate of litter decomposition. In general, restoration age and restoration system had a significant effect on litter decomposition.

In this study, the litter bags that allowed the access of invertebrates decomposed litter about 10–50 % faster than those without invertebrates; these results were similar to the findings of Meyer et al. (2011), in which treatments that included macro-invertebrates had litter decomposition occurring 17 % more quickly than treatments that excluded macro-invertebrate. Both macro- and meso-invertebrates played an important role during litter decomposition. The exclusion of invertebrates decreased litter decomposition, as measured in fine litter bags compared to coarse litter bags, similar to the results of Paris et al. (2008) where the lack of macro- and meso-invertebrates

decreased decomposition in the sown pastures of north-eastern Argentina. Zimmer (2002) also found a positive correlation between leaf-litter decomposition and the abundance of invertebrates at woodland sites. Berg and Ekbohm (1993) suggested that the presence of soil macro-invertebrates is essential to complete the mass-loss of litter. Donald et al. (2017) confirmed that the presence of decomposer mesofauna increased the ability of litter decomposition during rain forest restoration. The presence of Collembola enhanced the mass loss of leaf-litter in a soil microcosm experiment (Yang et al., 2012). However, inhabitant invertebrates at the time of litter bag collection or recorded ground-dwelling invertebrates had no significant effect on litter decomposition, consistence with some previous studies (Paris et al., 2008; Meyer et al., 2011; Niemeyer et al., 2012).

In the mining restoration system, litter decomposition rate was significantly different between the four restoration ages, similar to the findings of Ward et al. (1991) in another bauxite mining restoration project. Residue restoration sites had a significantly higher ground-dwelling invertebrate decomposition rate than mining restoration sites. Collembola and millipedes were more abundant at the residue sites compared to the mining restoration sites and both remnant sites. The high abundance of Collembola and millipedes may be responsible for the higher litter decomposition rates at the residue restoration sites. In both restoration systems, the overall litter decomposition by ground-dwelling invertebrates decreased as the age since restoration increased and invertebrate abundance decreased. The litter decomposition rate in both restoration systems was moving in the trajectory towards that found in remnant reference sites; however, even after 20 years since restoration, the rate of litter decomposition had not been achieved to the same level as that of the remnant. This study focused on early litter decomposition rate and the role of ground-dwelling invertebrates as an influencing factor. However, the long-term effect (> 12 months) of ground-dwelling invertebrates on the rate of litter decomposition was not examined during the study. It is recommended that the long-term (>12 months) fate of litter and the effect of ground-dwelling invertebrates be assessed in future studies.

This study found a positive correlation between litter decomposition and soil microbial respiration, similar to the results of Niemeyer et al. (2012) who found litter breakdown to be a highly sensitive ecological parameter that informed on microorganism status in

contaminated sites. Zimmer (2002) also found a positive correlation between litter decomposition and microbial activity in flood-plain woodland sites. Microbial respiration, as opposed to soil microbial respiration, could be measured in each litter bag to investigate the rate of microbial activity with and without macro-invertebrates; this method may be worth considering during future studies.

In natural forests, leaf litter usually consists of a mixture of different species. This study, however, used the leaves of one species to minimize the effect of mixed litter on decomposition. Some studies have used single-species litter to monitor decomposition rates in a given habitat (Ward et al., 1991; Paris et al., 2008; Niemeyer et al., 2012; Donald et al., 2017) while others have used mixed-species litter (Yang and Chen, 2009; Meyer et al., 2011; Zhang et al., 2013). Although Blair et al. (1990) found no difference between decomposition rates in litter from single-species and mixed-species, many other studies have found an influence of litter mixture on decomposition rates (Schädler and Brandl, 2005; Seeber et al., 2006; Zhang et al., 2013). Since the rate of decomposition is determined by the chemical composition of leaf litter (Zhang et al., 2013) and the distribution of litter-decomposing soil fauna determined by the quality of plant litter (Yang and Chen, 2009), the influence of ground-dwelling invertebrates on mixed litter decomposition in restored sites is worth investigating in future studies.

In conclusion, this study indicated that the presence of ground-dwelling invertebrates contributed significantly to litter decomposition in both restoration systems. The mass loss of leaves in coarse litter bags, which allowed ground-dwelling invertebrates access to the litter, was higher than in invertebrate-excluded litter bags and may explain the higher rate of litter decomposition. The study highlights the importance of re-establishing a good diversity of ground-dwelling invertebrates to restoration sites so as to enable the process of litter decomposition and, hence, nutrient cycling.

Chapter 6 Does woody debris addition influence the recolonization of ground-dwelling invertebrate community in restored bauxite mines?

6.1 Abstract

Woody debris is a critical structural and functional component of forest ecosystems, providing habitats for many ground-dwelling invertebrate species. It is slow to develop naturally, after clearing, and its use in mine site restoration has been limited. Snipped wood-waste spread onto restored mine pits prior to seeding has been trialled at Alcoa's Huntly mine site in the northern jarrah forest of south-west Western Australia. This study investigated if this application of snipped wood treatment promoted the return of ground-dwelling invertebrates. In March 2016, the invertebrate fauna was sampled from experimental plots—established in April 2008—which had been treated with 0, 100, or 300 t/ha of snipped wood-waste. Ground-dwelling invertebrates in each treatment were compared to those found in adjacent undisturbed jarrah forest plots. Ground-dwelling invertebrates were sampled using pitfall traps. All invertebrates were sorted to ordinal level, apart from ants (family Formicidae), which were classified to the species level. A total of 2235 individuals from 19 orders were recorded. Acarina (53 % of the total), Coleoptera (19 %), and Collembola (11 %) were the most abundant groups. Invertebrate group richness and abundance differed significantly between treated plots and adjacent jarrah forest plots; invertebrate abundance was significantly lower in the 0 t/ha treatment than in other woody-debris treatments, but invertebrate richness was the same in all treatments. Ant species richness, abundance, and functional group diversity differed significantly between experimental plots and remnant plots; among the experimental plots, only ant diversity differed significantly, with higher diversity in the 100 t/ha plots than in the 300 t/ha plots. In conclusion, the use of snipped wood treatment at the lowest rate of 100 t/ha enhanced the abundance of invertebrates in restored areas and is recommended for further application trials. Encouraging a diverse

invertebrate fauna to recolonise restoration could help speed up the return of key ecosystem functions to pre-disturbance levels.

6.2   Introduction

In forest ecosystems, woody debris—made up of fallen dead trees, sticks and branches, logs, and dead standing wood—is a critical structural and functional component. Wood debris can be categorised as coarse (>10 cm diameter), fine (<10 cm diameter), or litter (any material <1 cm diameter) (Harmon and Sexton, 1996), and its volume varies spatially and temporally; for example, older forests with senescing trees tend to have more woody debris accumulation than younger forests (Killey et al. 2010). Its presence influences the amount of nutrient turnover, mediates plant germination and growth, reduces soil erosion, and influences decomposer community composition and the abundance and diversity of ground-dwelling fauna, including invertebrates, by providing habitat structures, nesting sites, and refuge from environmental stresses (Harmon and Sexton, 1996; Lindenmayer et al., 2002; Woldendorp and Keenan, 2005; Bowman and Facelli, 2013; Manning et al., 2013; Bassett et al., 2015).

The importance of woody debris as a habitat for invertebrates varies with the density, availability, stage of decay, orientation, location, wood debris type, and dimension of debris (Grove and Meggs, 2003). The occurrence of invertebrates, promoted by the presence of woody debris, is vital for decomposition and nutrient cycling (Hattenschwiler et al., 2005). The diversity of ground-dwelling invertebrates—such as ants, beetles, cockroaches, and termites—and saproxylic invertebrates has been shown to positively correlate with the variety and amount of woody debris on forest floors (Grove, 2002; Barton et al., 2011; Seibold et al., 2016).

Destruction and alteration to forest floor woody debris cause a decline of ground-dwelling invertebrate fauna (Blaustein and Wake, 1990; Jonsell et al., 1998; Moseley et al., 2004). Blattodea, Coleoptera, Diptera, Hemiptera, and Orthoptera were found to be sensitive to habitat disturbance, showing immediate responses (Strehlow et al., 2002). For example, Blattodea abundance has been demonstrated to be vulnerable to logging (Strehlow et al., 2002), burning (Abbott, 1984), habitat fragmentation (Abensperg-Traun et al., 1996), and grazing (Bromham et al., 1999). Likewise, changes in the abundance of Coleoptera, Diptera, Hemiptera, and Orthoptera have been reported as a result of

logging (Watt et al., 1997; Niemelä et al., 2007), grazing (Bromham et al., 1999), and fire (Abbott, 1984). It is unknown if ants are sensitive to forest floor woody debris changes.

Compared to remnant vegetation, newly restored ecosystems often lack habitat heterogeneity and structural diversity, including woody-debris components. The lack of this habitat heterogeneity will likely limit invertebrate recolonization into newly restored ecosystems (Lythe, 2012). Therefore, application of woody debris during restoration will likely positively encourage decomposer activity by retaining moisture, creating a cooler microclimate (Ward et al., 1991), and providing additional microhabitats.

Alcoa has been restoring bauxite mine pits since 1966 in the northern jarrah forest of south-west Western Australia, with evolving restoration techniques (see Chapter 1.5 for more details of restoration history). In the 1980s, Alcoa implemented returning coarse woody debris (approximately one habitat pile per ha) to restored areas so as to provide habitats for both vertebrates and invertebrates (Koch, 2007a). Without the addition of woody debris, its natural formation will likely take decades (Whitford, 2002; Koch, 2007a) and invertebrate fauna dependent on debris would likely remain absent until then (Christie et al., 2012).

Whereas, previously, large logs would be piled in one location with no other wood debris addition, in 2008, Alcoa trialled a new technique of woody-debris application—that of snipping and breaking down larger (coarse) wood logs to fine wood-debris or litter, and applying it throughout the landscape following topsoil replacement but prior to seeding (Lythe et al., 2017). Three years after the establishment of woody-debris trialled plots, Lythe (2012) sampled invertebrates recolonizing these trial plots and found that woody debris treatment increased both richness and abundance of invertebrates. However, longer-term changes have not been quantified.

This chapter investigated the longer-term effect (8 years) of woody debris addition, during mine site restoration, on invertebrate communities. The main objective was to examine whether or not snipped woody debris addition attracted a more diverse

invertebrate community than a restoration with no additional woody-debris application. The following research questions were addressed:

- Do ground-dwelling invertebrate abundance, composition, and diversity differ between woody-debris treated and untreated sites?
- Does the amount of woody debris added affect ground-dwelling invertebrate community composition and diversity?
- Do ground-dwelling invertebrate abundance, composition, and diversity more closely resemble remnant plots in woody-debris treated sites than in untreated sites?

6.3   Methods

6.3.1   Study sites

The study area was situated at Huntly mine site, mined and restored in 2008 following Alcoa's standard restoration treatments (see Chapter 1.5 for restoration methods). Approximately 80 m north and east of the experimental area was remnant jarrah forest, to the west was a haul road (40 m away) and to the south (~160 m away) were other restored areas. In April 2008, finely snipped wood (sizes ranging in diameter from 10 to 100 mm) was applied to the experimental plot (2.7 ha in size) following topsoil application. Application of snipped wood was done before contour ripping and seeding.

6.3.2   Experimental design and pitfall trapping

The total experimental area was 150 m × 180 m (2.7 ha). The area was divided into three 40 m wide rows, with a 15 m buffer between each row. Each row was subdivided into three 40 m × 60 m (0.24 ha) treatment plots (Figure 6.1). Into each treatment plot, snipped woody debris treatment was applied at 0, 100, and 300 t/ha with 3 replicates of each (Figure 6.1).

In March 2016, i.e. eight years after the experimental setup, invertebrate sampling was conducted via pitfall traps using methods similar to those of Lythe (2012), who sampled the site after three years. A total of eight pitfall traps (100 mm deep and 30 mm diameter) were laid in two 60 m parallel line transects. Pitfall traps were established at least 15 m apart in each plot with 30 ml of ethylene glycol added to each. The traps were

left open for seven consecutive days. Invertebrate samples were transferred to an 85 % ethanol solution for preservation. At the same time, invertebrates were sampled in the nearby jarrah forest remnant (north of the experimental plot) identical methods. All invertebrates were sorted, counted, and identified to at least the ordinal level. Within Hymenoptera, Ants (Family: Formicidae) were counted separately. Ants were identified to the species level and assigned to functional groups based on their ecological interaction, niches, and response to habitat disturbance (Andersen, 1995, 1997; Andersen et al., 2002; Andersen and Majer, 2004; Heterick, 2009) (see Chapter 3.3.2 for more details).
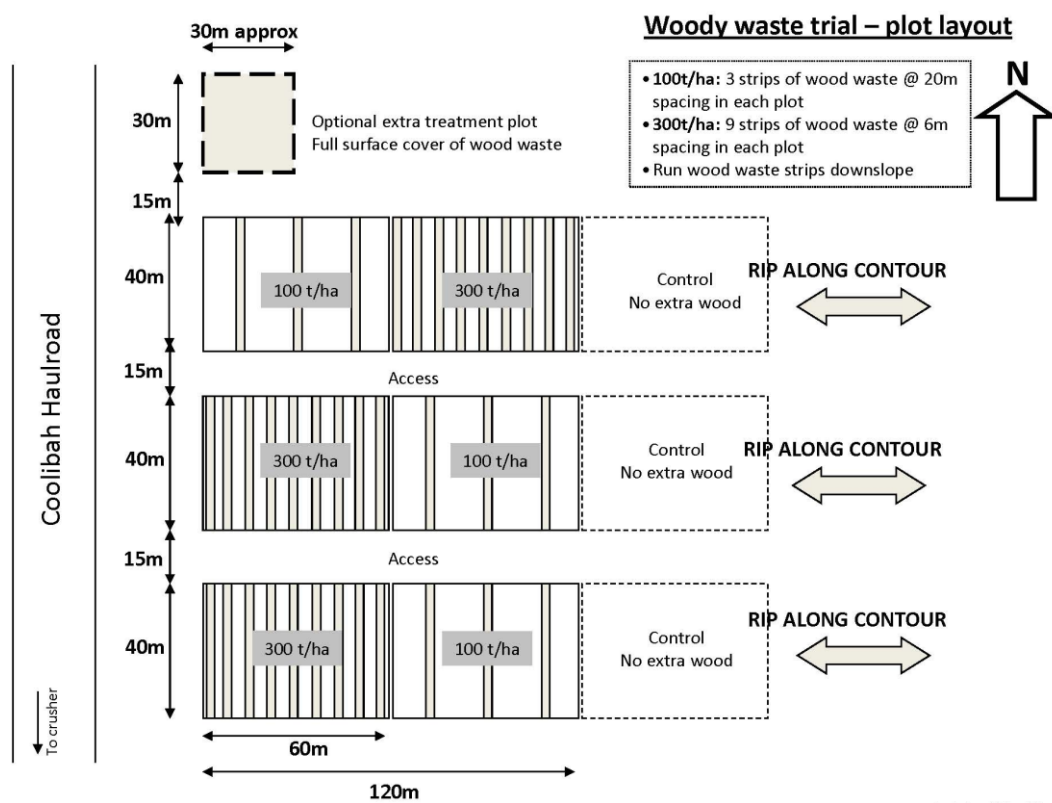
Figure 6.1. Snipped woody debris trial plot layout on restoration area (restored in 2008) at Huntly mine site (Grigg, 2008)

6.3.3 Data analysis

All abundance data were log (x+1) transformed to minimize the effect of very abundant orders/species. Shannon diversity index (H'), Shannon evenness (e^H'/S), and taxonomic richness were obtained for each treatment using Paleontological Statistics (PAST) version 3.14. Shapiro-Wilk tests were performed using SPSS version 21 to check the normality of diversity indices. Differences in invertebrate species abundance, species

richness, and diversity indices between snipped wood treatments and adjacent jarrah forest remnant site were compared using one-way analysis of variance (ANOVA) in SPSS. A Tukey's HSD posthoc test was used to compare treatments when a significant difference was detected in the ANOVA.

Multivariate analyses were performed on the abundance of invertebrate taxa and ant diversity using PRIMER (version 6). A similarity matrix was constructed using Bray-Curtis measures from the log-transformed abundance of a) all invertebrates and b) ants. Principle Coordinate Analysis (PCO) and Hierarchical Cluster analysis were performed to investigate plot similarities on the Bray–Curtis matrix using Primer (version 7). The resultant Hierarchical Cluster analysis plot was overlayed on the nMDS plots at 60 %, 70 %, 80 %, and 90 % resemblance levels. Experimental plots positioned closer together in the ordination space were more similar to each other.

6.4   Results

6.4.1   Invertebrate richness and abundance

A total of 2235 specimens from 19 orders were sampled. Ground-dwelling invertebrates, except Collembola, were less abundant in the remnant site than in any of the restored plots. Overall, Formicidae was the most abundant group (55 % of the total), followed by Collembola (28 %) and Coleoptera (7 %). Acarina, Dermaptera, Diplopoda, Isopoda, and Phasmatodea were only recorded in the jarrah forest remnant plots. Blattodea was recorded at the jarrah forest remnant plots and the 0 t/ha treatment plots, but was absent in both 100 t/ha and 300 t/ha treatment plots (Figure 6.2;Table 6.1).
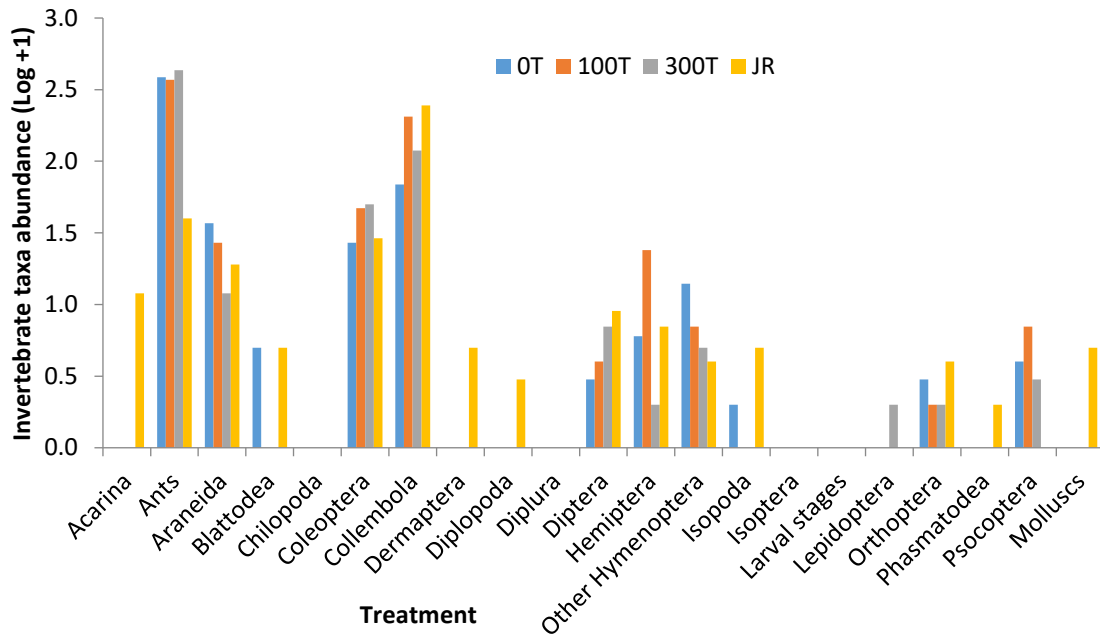
Figure 6.2. Total invertebrate abundance (log (x+1) transformed) for each experimental treatment (mean of three replicates per treatment). Treatments: 0T= 0 t/ha, 100T= 100 t/ha, and 300T= 300 t/ha of snipped woody-debris application, and JR= jarrah forest remnant.

Table 6.1 The mean ± SE invertebrate taxa abundance of three replicates for each treatment. Treatments: 0T= 0 t/ha, 100T= 100 t/ha, and 300T= 300 t/ha of snipped woody-debris application, and JR= jarrah forest remnant.

Invertebrate taxa	0T	100T	300T	JR
Acarina	0 ± 0	0 ± 0	0 ± 0	3. 7 ± 0.9
Ants	128. 7 ± 22.8	123 ± 16.6	144 ± 26	13 ± 3.5
Araneida	12 ± 5.7	8. 7 ± 3.2	3.7 ± 1.2	6 ± 1.5
Blattodea	1.3 ± 0.9	0 ± 0	0 ± 0	1.3 ± 0.3
Coleoptera	8.7 ± 0.3	15.3 ± 10.9	16.3 ± 5.6	9.3 ± 4.5
Collembola	22.7 ± 3.3	68 ± 12.7	39.3 ± 5.5	81.7 ± 8.3
Dermaptera	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0.3
Diplopoda	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.3
Diptera	0.7 ± 0.3	1 ± 0.6	2 ± 1	2.7 ± 1.7
Hemiptera	1.7 ± 0.3	7.7 ± 1.8	0.3 ± 0.3	2 ± 1.1
Hymenoptera other than Formicidae	4.3 ± 0.9	2 ± 0. 6	1.3 ± 0.3	1 ± 0.6
Isopoda	0.3 ± 0.3	0 ± 0	0 ± 0	1.3 ± 0.3
Lepidoptera	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0
Orthoptera	0.7 ± 0.7	0.3 ± 0.3	0.3± 0.3	1 ± 0
Phasmatodea	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3
Psocoptera	1 ± 0.6	2 ± 1.1	0.7 ± 0.7	0 ± 0
Molluscs	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0.3

6.4.2 Effect of snipped-wood cover on invertebrate richness and abundance

Invertebrate taxa richness differed significantly between treatments ($F_{3,11}$ = 31, P < 0.05). The jarrah forest remnant site had higher invertebrate taxa richness (mean 12.7 ± 0.3) than the restored plots; there was no difference in taxa richness between the restored plots, regardless of woody-debris application rate (0 t/ha, 100 t/ha, and 300 t/ha) (Figure 6.3a). The abundance of invertebrates also differed significantly between treatments ($F_{3,11}$ = 6.191, P < 0.05). The remnant plots had the lowest abundance (mean 126.7 ± 14.1), the 0t/ha plots had intermediate abundance, and the 100t/ha and 300t/ha treatment plots had the highest

abundance (Figure 6.3b). Invertebrate diversity indices were similar between treatments (P > 0.05).

Principle Coordinate Analysis (PCO) based on the invertebrate taxa abundance clearly separated restored plots and remnant jarrah forest plots into two distinct groups along axis 1 (Figure 6.4a). Cluster analysis based on invertebrate taxa abundance supported these groupings (Figure 6.4b). Woody-debris treated plots (100 t/ha and 300 t/ha) were more similar to untreated plots (0 t/ha) than remnant plots.
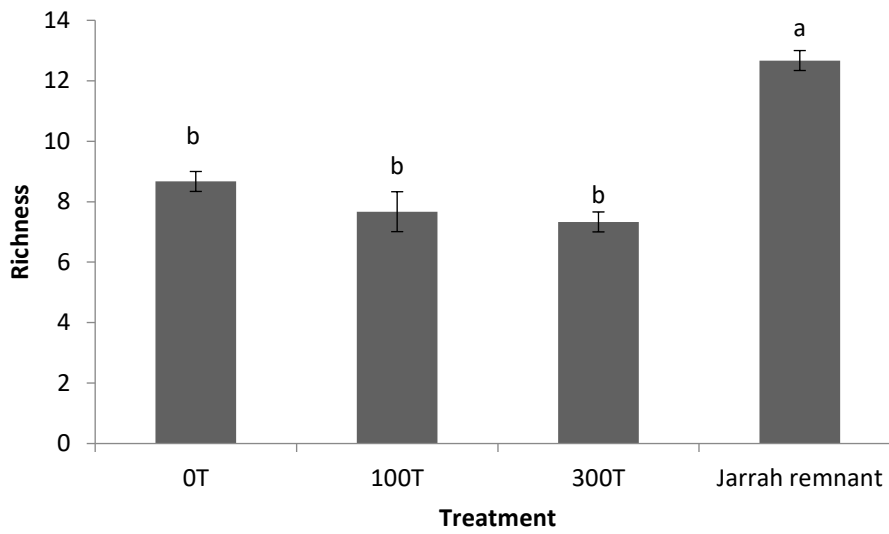
a)
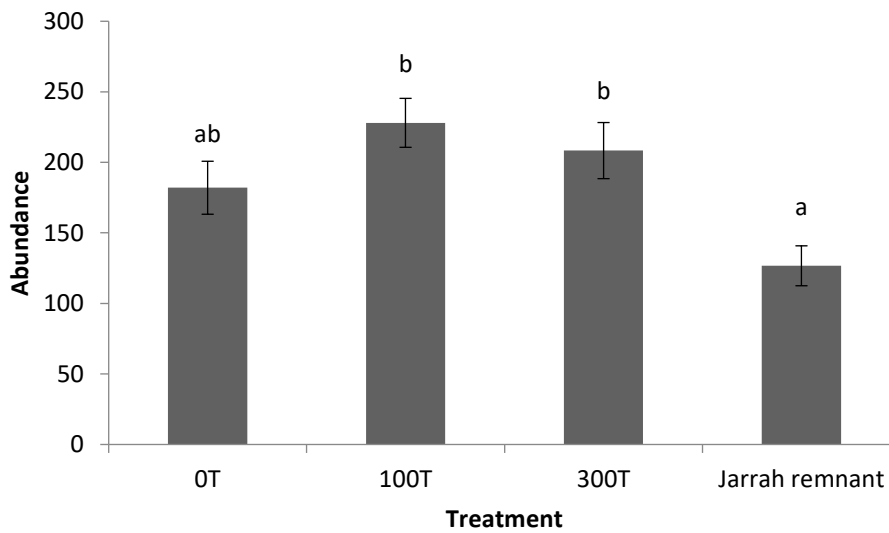
b)

Figure 6.3. Order-level comparisons (mean ± SE) of a) invertebrate richness and b) abundance between different snipped woody-debris treatments (three replicate plots per treatment). Plots with different letters indicate significantly different means (Tukey HSD, p < 0.05). Treatments: 0T= 0 t/ha, 100T= 100 t/ha, and 300T= 300 t/ha of snipped woody-debris application.
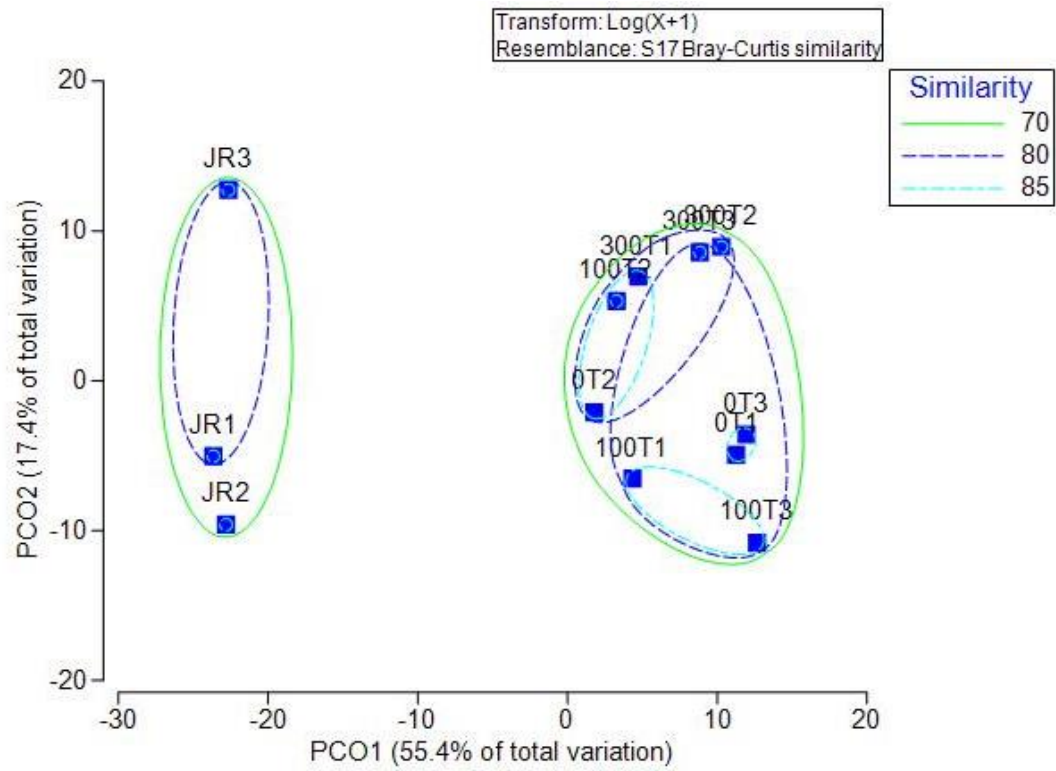
a)
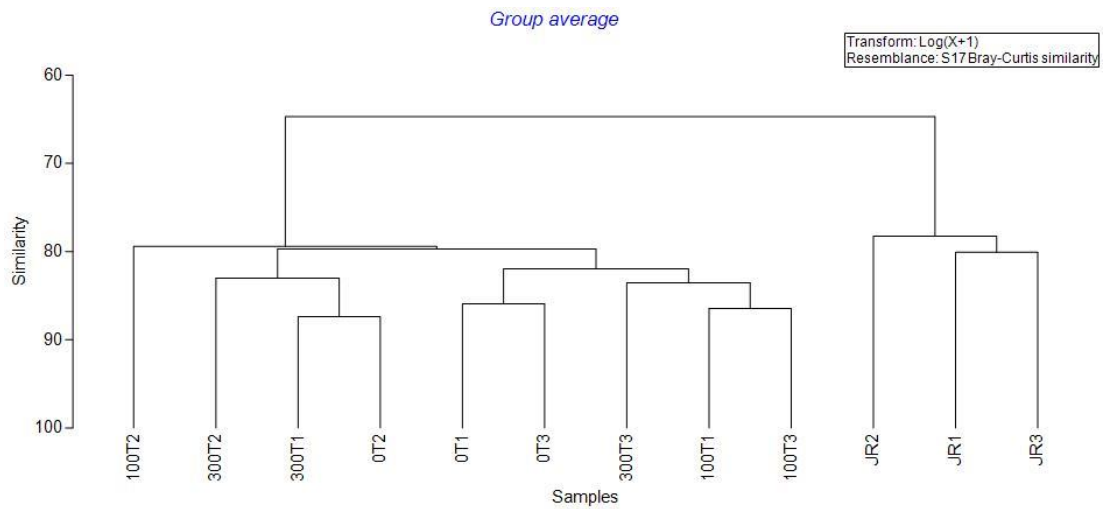
b)

Figure 6.4. a) Principal Coordinate Analysis based on invertebrate taxa abundance (log (x+1) transformed), and b) Bray-Curtis cluster similarity at 60 %, 70 %, 80 %, and 90 % of each site overlayed for each treatment. Treatments: 0T= 0 t/ha, 100T= 100 t/ha, and 300T= 300 t/ha of snipped woody-debris application, and JR= jarrah forest remnant.

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6.4.3 Ant species richness, abundance, and functional group diversity

A total of 1226 ant individuals, representing four families, nine genera, and 11 species, were recorded. Collectively, five species were recorded at the restored plots and eight species were recorded at the jarrah forest remnant plots. Ant abundance was significantly different between plots ($F_{3,11}$ = 2.867, P < 0.05), with the highest ant abundance recorded in the 100 t/ha treatment plots and the lowest in the remnant plots (Figure 6.5a); ant abundance between 0, 100, and 300 t/ha woody-debris application plots was similar (Figure 6.5a). Ant diversity also differed significantly between treatments ($F_{3,11}$ = 6.686, P < 0.05); the highest ant diversity was recorded in the jarrah remnant plots, while the lowest was recorded at the 300 t/ha plots (Figure 6.5b). The mean dominance index (D) of ants significantly differed between treatments ($F_{3,11}$ = 6.974, P < 0.05), with the highest recorded in 300 t/ha treatment plots and the lowest in jarrah remnant plots (Figure 6.5c).
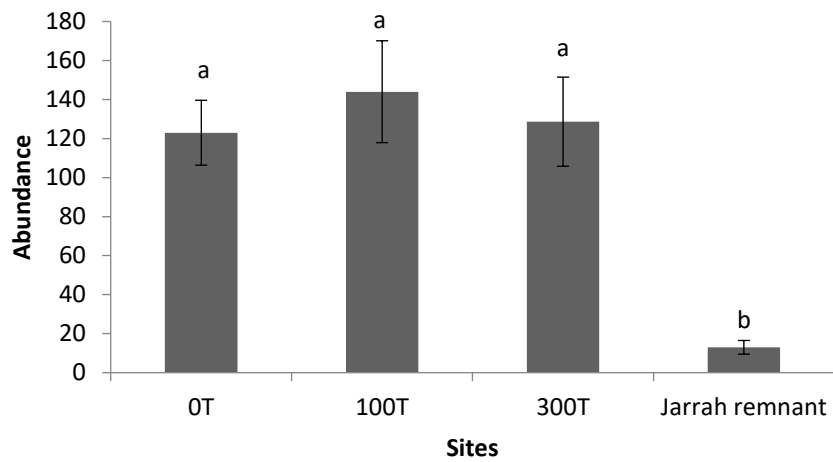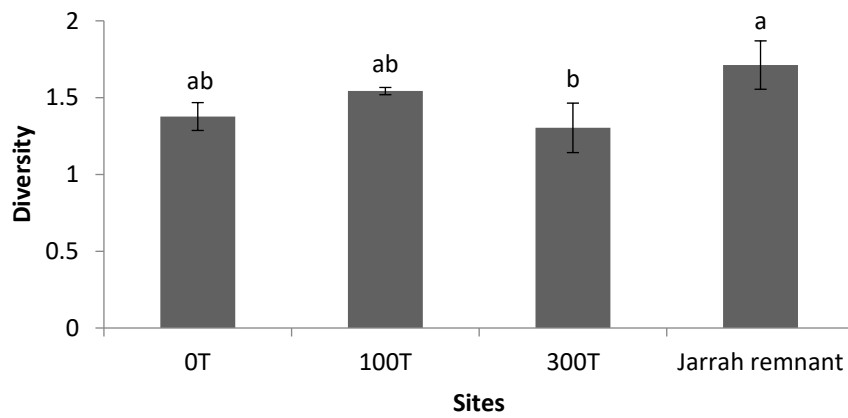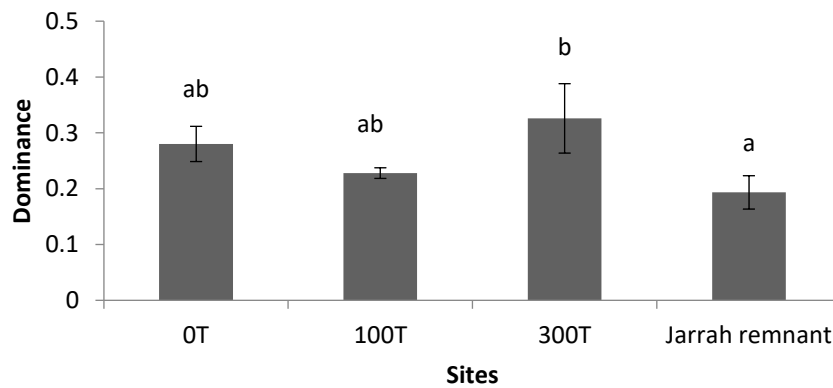
Figure 6.5. Comparisons of ant a) abundance, b) diversity, and c) dominance (mean ± SE) between different snipped woody-debris treatments and jarrah remnant plots (three replicates per treatment). Plots with different letters indicate significant differences between means (Tukey HSD, $p < 0.05$). Treatments: 0T= 0 t/ha, 100T= 100 t/ha, and 300T= 300 t/ha of snipped woody-debris application.

Ant diversity separated into two clusters according to jarrah forest remnant and restored plots (Figure 6.6).
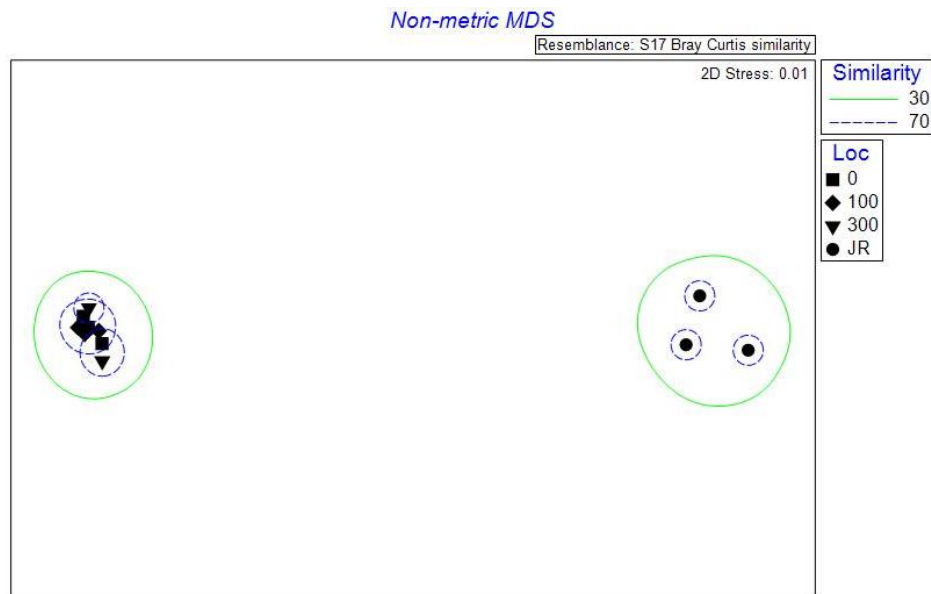
Figure 6.6. Non-metric Multi-Dimensional Scaling (nMDS) of ant diversity across different treatments. Treatments: 0= 0 t/ha, 100= 100 t/ha, and 300= 300 t/ha of snipped woody-debris application, and JR= jarrah forest remnant.

A total of six ant functional groups were recorded (Figure 6.7). Four functional groups were common to all restored plots (0, 100, and 300 t/ha woody-debris application treatments); Cryptic Species and Cold-climate Specialists were absent from these plots. Jarrah remnant plots also had four ant functional groups; Subordinate Camponotini and Generalized Myrmicinae functional groups were, however, absent from the remnant plots (Figure 6.7a). Dominant Dolichoderinae was the most abundant ant functional group in all three restored plots, while Cryptic Species was the most abundant in jarrah remnant plots (Figure 6.7b).
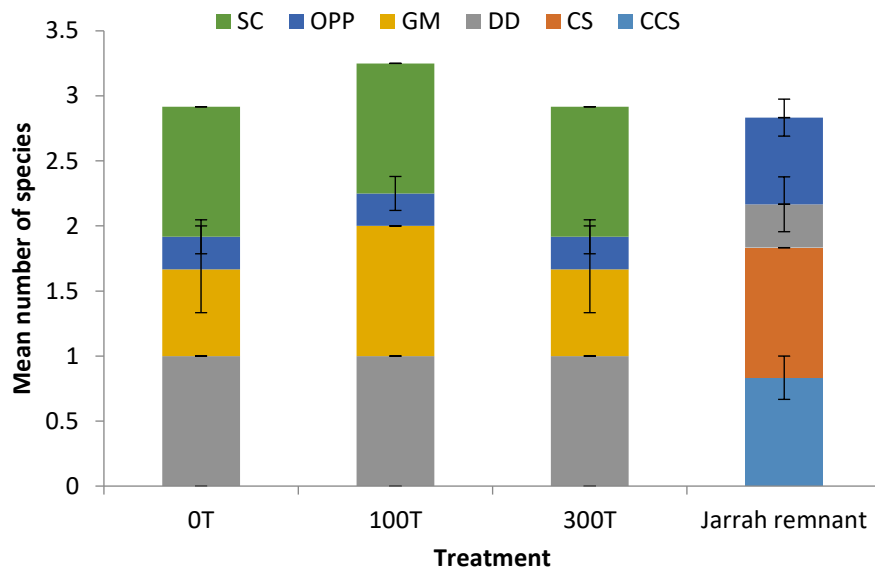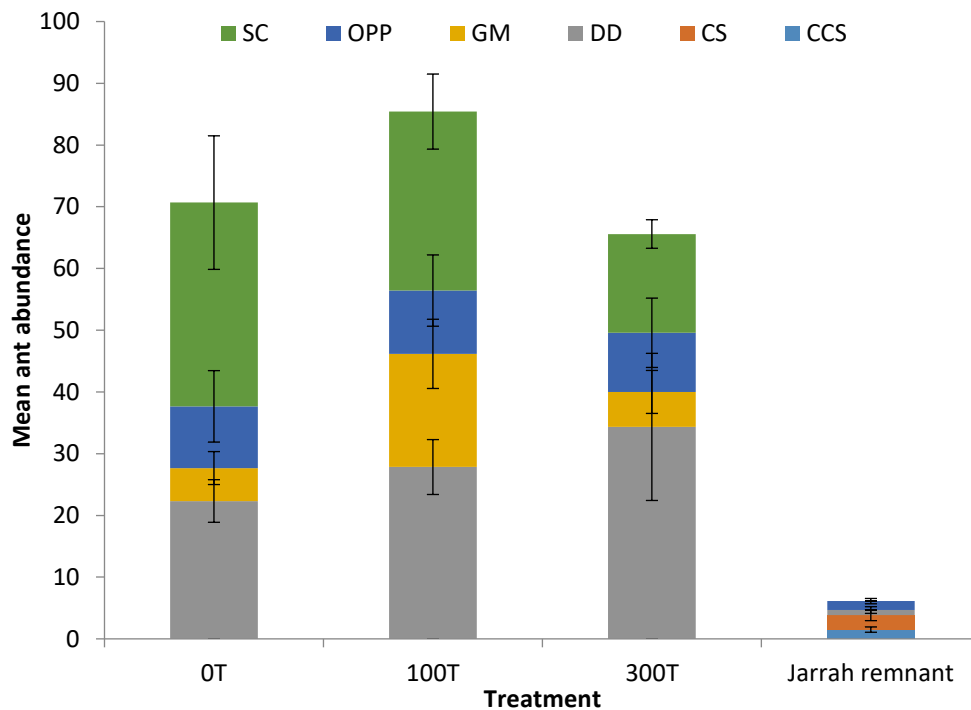
Figure 6.7. The mean (± SE) ant functional group a) species richness and b) abundance in each treatment. Treatments: 0= 0 t/ha, 100= 100 t/ha, and 300= 300 t/ha of snipped woody-debris application, and JR= jarrah forest remnant. SC, Subordinate Camponotini; OPP, Opportunists; GM, Generalized Myrmicinae; DD, Dominant Dolichoderinae; CS, Cryptic Species; CCS, Cold-climate Specialists.

6.5 Discussion

For restored ecosystems to become self-sustaining, many demands must be met, one of which is the production of woody debris materials (Hasegawa et al., 2012) that provide habitats for vital ground-dwelling soil fauna and conserve their biodiversity (Lindenmayer et al., 2002). One possible way to meet this requirement is the addition of woody debris as the restoration work commences. This study assessed the effect—after eight years—of snipped woody-debris addition during bauxite mining restoration. The study was carried out in autumn as Lythe et al. (2017) reported the prevalence of more invertebrate species in autumn than in winter or spring. The results of this study suggested that the application of snipped woody-debris during restoration encouraged invertebrate recolonization. Overall, addition of woody debris tented to increase invertebrate species abundance. It is suspected that perhaps the lack of significance was due to a lack of statistical power, rather than a real lack of statistical difference in invertebrate abundance.

Despite woody-debris addition, all restored sites were missing some invertebrate taxa like Acarina, Dermaptera, Diplopoda, Molluscs, and Phasmatodea. This observation contradicts that made by Lythe et al. (2017) after three years of woody-debris treatment plot establishment. Lythe et al. (2017) observed high numbers of Acarina in treatment plots. These Acarina might have gotten "free rides" to treatment plots with snipped woody debris and perhaps did not survive in the long-term (eight years) due to unfavourable environmental conditions in treatment plots. Further, this observation contradicts the results of Chapter 3 of this thesis, where Acarina was recorded after two years of restoration establishment and was present in all subsequent ages. However, Cuccovia and Kinnear (1999)'s study in restored jarrah forest only recorded Acarina 20 years after initial restoration. Acarina, Dermaptera, Molluscs, and Diplopoda are categorized as decomposer communities, and decomposers are the fundamental group in an ecosystem that facilitate litter decomposition and nutrient release, subsequently encouraging soil development (Hasegawa et al., 2012; Astor, 2014);

the absence of the above mentioned decomposer communities in restored mine pits might obstruct these ecosystem functions.

Addition of snipped woody-debris encouraged recolonization by some invertebrate taxa. For example, Psocoptera was positively encouraged. This observation is in line with the recolonization pattern reported in Chapter 3 of this thesis, where Psocoptera was observed after two years since initial restoration. Psocoptera is designated as a detritivore or scavenger in an ecosystem and is known to perform vital soil ecosystem processes like decomposition and nutrient release (Thornton, 1985). Therefore, the presence of Psocoptera will positively affect decomposition and nutrient release in treatment plots. However, wood debris treatment appeared to negatively affect particular invertebrate taxa such as Blattodea and Isopoda. Isopoda and Blattodea are also known as litter transformers (Hasegawa et al., 2012), facilitating nutrient release and litter fragmentation during decomposition. However, this observation contradicts the results of Chapter 3 of this study where Isopoda and Blattodea were recorded in five- and 10-year-old restoration plots. Similar to Lythe et al. (2017), the abundance and density of ants were also lower in the 300 t/ha treatment than the 100 t/ha treatment plots. This may be because woody debris interrupts ant foraging and nesting activities. However, there is no evidence that adding woody debris is promoting restoration towards remnant invertebrate assemblage as is occurring over time (Chapter 3).

Woody debris addition limited the occurrence of some ant functional groups. The absence of Cryptic Species in woody-debris addition plots might be due to the additon of woody debris creating an unfavourable habitat for them (Majer et al., 1982). The higher abundance of Dominant Dolichoderinae recorded in woody-debris addition plots might be the cause for the absence of Cold-climate Specialists in such plots (Hoffmann and Andersen, 2003), as they occur in cool environments where the abundance of Dominant Dolichoderinae is low.

A limitation of this study was the taxonomic discrimination, which was mostly done only to ordinal level. It is important to sort invertebrate groups to species/morphospecies level in future studies by characterising environmental

DNA (eDNA) in order to identify more invertebrate indicator species and differentiate between assemblages. Further, the effect of snipped woody-debris addition on plant cover and plant diversity needs to be assessed in future studies. The long-term impact of the addition of snipped woody debris on soil physiochemical and microbiological properties also needs to be evaluated in future research to understand the overall negative/positive effect of woody-debris addition. No evidence of restoration benefits through woody-debris addition were obtained from this study, but a future study at a bigger scale needs to be performed to eliminate local site effects and fully ascertain this finding.

Statement of authors' contributions: Experimental set up established by Lythe et al. (2017) used for this experimental chapter. Data collection, data analysis and data interpretation for long term effect of woody debris addition was done by D. Mihindukulasooriya.

Chapter 7   Conclusions   and recommendations

Open cut bauxite mining, which subsequently needs to be repaired, disturbs the land and disrupts its biodiversity. Ecosystem restoration is a multidimensional activity involving repair of the physical, biological, and chemical components of the environment (Society for Ecological Restoration, 2004) and restoration projects require long-term monitoring and evaluation. The ultimate goal of restoration is the attainment of a self-sustaining ecosystem that blends with its natural landscape and requires minimal ongoing intervention.

This thesis investigated soil invertebrate succession and the development of key ecosystem processes along a chronosequence of restored sites within two distinctly different restoration projects associated with Alcoa's bauxite mining operation: 1) jarrah forest restoration after bauxite mining (mining restoration), and 2) restoration of a bauxite residue sand storage area, i.e. establishment of habitat on residue sand generated during the refining of bauxite to alumina (residue restoration). Mining restoration has a well-documented and long history, with adjacent jarrah forest remnants as a reference system for restoration targets (Nichols et al., 1985; Koch and Samsa, 2007; Banning et al., 2011a; Craig et al., 2014). In contrast, residue sand restoration does not have identifiable restoration requirements. It is a nascent environment containing sterile soil with high alkalinity and salinity, devoid of organic matter, which leads to limited plant recruitment (Jasper et al., 2000; Alcoa, 2013). There is no historical reference system for the residue restoration system, but this study used adjacent tuart remnant habitat as a surrogate reference ecosystem.

Five key questions are discussed here in regard to the findings of this project: 1) Do invertebrate communities in restored systems resemble the ones in remnant ecosystems?; 2) Is ecosystem function improving?; 3) Is there support for functional redundancy?; 4) Does adding wood debris improve the process?; and

5) What can be learnt from this research in terms of restoration management and what direction could future research take?

7.1 Are invertebrate communities in restored systems developing a resemblance to the ones in remnant ecosystems?

This is the first study to compare ground-dwelling invertebrate recolonization patterns between bauxite mining and residue restoration systems. Both restoration methods promoted the return of ground-dwelling invertebrate community composition. Unsurprisingly, upon performing multivariate analyses, invertebrate communities could be primarily separated according to restoration system. Within each restoration system, community structure changed as age since initial restoration increased. The recovery of ground-dwelling invertebrate diversity (measured by pitfall trapping and litter invertebrate extraction) increased with restoration age, with the 20-year-old restoration site having invertebrate diversity with 91 % similarity to that of the remnant site. Furthermore, ground-dwelling invertebrate composition (measured by pitfall trapping and litter invertebrate extraction) in the 20-year-old site resembled remnant composition closely, with 70 % similarity to that of the remnant site composition. The invertebrate community composition in this study was similar to that of previous studies in the jarrah forest (Majer, 1983; Postle et al., 1986; Greenslade and Majer, 1993; Majer et al., 2007b; Majer et al., 2013). Even though the residue restoration practice encouraged the recolonization of invertebrate taxa, there were still components missing; possibly, the community composition may remain distinct and may never closely resemble that of the neighbouring remnant habit for a long time into the future.

The ground-dwelling invertebrate diversity was significantly higher in bauxite mining restoration (Shannon diversity indices ranging from 1.05 in the two-year-old site to 1.55 in the jarrah remnant site) than in residue restoration (diversity ranging from 0.62 in the two-year-old site and 0.74 in the 10-year-old site). Isopterans were missing from the residue restoration system while Acarina, Formicidae, Collembola, and Diplopoda were recorded in higher numbers,

indicating high habitat disturbance (Burbidge et al., 1992; Hoffmann and Andersen, 2003). Therefore, the occurrence of Isopterans and decreasing numbers of Acarina, Formicidae, Collembola, and Diplopoda can be used as indicators to assess the progress of residue restoration systems.

The mining restoration system likely provides more suitable soil conditions for invertebrate re-establishment than the residue restoration system. Residue sand has challenging physical and chemical characteristics (Gräfe et al., 2011) which many components of the invertebrate community may not be adapted to living in. Additional time may be required for natural modification of the abiotic environment to allow greater colonisation or physical conditions may need to be modified to support greater diversity.

It is concluded that although restoration practices in both systems are restoring diversity, the original invertebrate composition has still not been achieved after 20 and 10 years since mining and residue restoration, respectively. The succession of invertebrates in both restoration systems was mainly determined by age since restoration. Though there were distinct genera and species in both restoration systems, the invertebrate composition also overlapped between the systems, making identification of specific indicator taxa challenging. Focusing on the ant taxa, the disappearance of *Iridomyrmex purpureus* and *I. suchieri* from younger restoration sites indicates that these sites have habitat features that are unfavourable for disturbed-area or open-habitat preference species. The occurrence of *Leptogenys neutralis, Strumigenys quinquedentata*, *Tapinoma minutum broomense*, and Myrmicinae and Ponerinae subfamilies (except *Cardiocondyla atalanta*) in older mining restoration sites likely indicates that restored sites have improved habitat suitability for species which occur in undisturbed, shaded forest conditions. Similar to other studies, *Rhytidoponera metallica* was identified as an indicator species of anthropogenic disturbance (Hoffmann and Andersen, 2003; Andersen and Majer, 2004).

7.2 Is ecosystem function improving?

Successful ecosystem restoration requires re-establishment of fundamental ecosystem processes and interactions among existing biological communities.

Therefore, restoring ecosystem functions can be considered one of the most important focuses of restoration as it encompasses the entire ecosystem and evolutionary processes that occur in a particular environment (Grimbacher and Hughes, 2002). One such ecosystem function, seed dispersal, is a vital animal-plant interaction to restore in order to effectively develop a self-sustainable ecosystem that needs minimal ongoing anthropogenic management. This study compared seed dispersers, seed removal rate, and seed dispersal distances to assess the reestablishment of this function in restored areas.

Seed dispersal rates differed markedly between the two restoration systems, with a total of 2239 seed dispersal events at the mining restoration system and only 36 events at the residue restoration system; this limited seed dispersal might be the contributing factor to the limited recruitment of native plant species recorded within the residue restoration system (Alcoa, 2013; Banning et al., 2014). This observation was correlated with the low abundance of myrmechorous ants in residue sand restoration sites. Conversely, at the mining restoration system, as age since restoration progressed (0–20 years), rate of seed dispersal began to closely resembled that of the remnant system (with 94 % of the dispersal rate to that of the remnant in the 20-year-old site). Myrmecochorous events at mining restoration sites declined as age since restoration increased and were relatively similar between the 20-year-old restoration site and the jarrah remnant site. This observation was in accordance with the high abundance of myrmechorous ants in younger mining restoration sites, and is in agreement with the results of a previous study (Gove et al., 2007).

Litter decomposition is a vital ecosystem function to re-establish during restoration to improve soil characteristics and promote nutrient recycling to sustain the ecosystem (Oktavia et al., 2015). This study compared the litter decomposition rate between the two restoration systems (mining and residue) to determine changes along the chronosequence and to assess the role of soil invertebrates in this ecosystem function. Both restoration methods encouraged the acceleration of litter decomposition towards that of the remnant systems. Recovery was more rapid in bauxite mining restoration than in residue restoration, which may reflect that topsoil addition—likely containing microorganisms and

some invertebrates (Banning et al., 2011a)—is essential to initiate recovery. At mining restoration, the litter decomposition rate in the 20-year-old restoration site was only 82 % to that of the remnant system. Alternatively, litter decomposition in the residue restoration system was higher than in the tuart remnant and was positively correlated with ground-dwelling invertebrate abundance and richness. As age since restoration increased, litter decomposition rates decreased towards the value of the adjacent tuart remnant; after 10 years since restoration, the rate of litter decomposition was 85 % to that of the tuart remnant. A decrease in decomposition rate could be used as an indicator to assess the progress of residue restoration, as decreasing decomposition rates indicate lower invertebrate abundance, which is in agreement with the results of other studies where undisturbed sites have lower invertebrate abundance than disturbed sites (Bisevac and Majer, 1999; Xavier et al., 2009). The initial higher rate of decomposition might be due to wood mulch introducing invertebrates to the residue sites, promoting litter decomposition. The presence of ground-dwelling invertebrates improved the rate of litter decomposition in both restoration systems by at least 23 % over that achieved by microorganisms alone.

Ants significantly improved the rate of seed dispersal in the restored systems (90 % of the dispersal rate of the remnant), and ground-dwelling invertebrates improved litter decomposition by at least 23 %; however, the influence of invertebrates differed between restoration systems and age since restoration. In both restoration systems, the re-establishment of ecosystem functions was not fully achieved due to limited re-establishment of invertebrate diversity. This study highlights that efforts to recolonise the disperser and decomposition communities will speed-up the succession and return the ecosystem functions of restored sites to pre-disturbance levels.

Soil physiochemical properties of mining restoration sites resembled those of the jarrah remnant, with the 20-year-old restoration site closely approximating the soil properties of the remnant site, in agreement with the result of a previous study (Ward, 2000). Soil microbial respiration of mining restoration was moving in the direction of that observed in the jarrah remnant across the chronosequence; however, the respiration rates did not achieve levels recorded for the remnant

(after 20 years, microbial activity was only 21 % of that recorded from the jarrah remnant). The soil physiochemical properties of residue restoration sites gradually improved along the chronosequence; however, they remained distinct from the properties of the tuart remnant. Microbial activity in residue restoration sites was low and was correlated with lower levels of soil organic matter compared to the tuart remnant and the mining restoration sites.

Soil physiochemical and microbiological properties are vital indicators to assess the success of restoration; soil invertebrate abundance is related to the development of important soil physiochemical (negative correlation to organic C, total nitrogen, ammonium nitrogen, C:N ratio, and cation exchange capacity) and structural characteristics (negative correlation to bulk density and positive correlation to porosity). Therefore, efforts to re-establish a diverse ground-dwelling invertebrate community to restored sites will likely accelerate the return of soil physiochemical and microbiological properties to pre-disturbance levels.

7.3 Is there support for functional redundancy?

Functional group richness was generally lower in restored sites compared to remnant vegetation. After 10 years of restoration, only 83 % and 51 % of remnant ant functional group richness was achieved at mining and residue restoration, respectively; ant functional groups overlapped in the two restoration systems, but functional diversity was lower in the residue system than in the mining system. This indicates that the functional groups present in the residue restoration system were not performing all ecological functions (such as seed dispersal) of other ant species. The abundance of Dominant Dolichoderinae and Hot-climate Specialists is known to reduce when the restoration develops dense shade with a thick litter layer, leading to an increase in the abundance of Generalized Myrmicinae and Opportunists (Andersen, 1993; Andersen, 1995; Hoffmann and Andersen, 2003). Cryptic Species abundance was reduced as age since restoration increased, the development of a litter layer creating an unfavourable habitat for them (Majer et al., 1982; Andersen, 1991a). Species richness of Specialist Predators increased with restoration age in mining restoration; typically this group is the last to recolonise after mine site restoration (Hoffmann and Andersen, 2003), indicating

that the mining restoration sites were indeed on the restoration trajectory towards the remnant system. Generalized Myrmicinae and Opportunists richness increased with age since restoration; however, both groups showed differing responses to disturbance. As the two functional groups are known to have different habitat preferences and competitive abilities with other groups, these varying responses were expected (Andersen, 1995; Andersen et al., 2002). Similar to the conclusions of Casimiro et al. (2019), ant functional group analysis of this study suggested the advantage of proving a community response rather than relying on a single species response.

Ant functional groups can be used as sensitive and robust indicators to assess soil functionality in both bauxite mining and bauxite residue restorations. The stability of the ecosystem is enhanced if each important functional group necessary for maintaining ecosystem function and structure is present (Walker, 1995). This study suggests an approach to the "functional group" concept during restoration, based on the use of functional groups defined according to ecosystem processes (litter decomposition and seed dispersal). Functional groups are vital for the re-establishment of ecosystem processes during restoration and warrant priority in terms of management efforts.

7.4 Does adding wood debris improve the process?

Adding additional woody debris increased the recolonization of invertebrate communities in the mining restoration system. Encouraging a diverse ground-dwelling invertebrate community to return to restored sites by topsoil and woody debris application likely accelerates recovery. If these processes are followed, the invertebrate community of restored sites will return to pre-disturbance levels more quickly, as also shown by Lythe et al. (2017) in the same system. However, several challenges remain. Woody debris used for restoration is likely to be in early stages of decaying, which attracts stage specialist decomposition species (Gibb et al., 2006). This might exclude a range of other decomposer species, resulting in many decades before the later-stage decomposer specialists are recruited in the system (Greenslade and Majer, 1993). Therefore, future research into the effect of woody debris on restoration could examine the decomposer community

composition at a finer scale. The second challenge is the cost associated with woody-debris addition. However, increasing the amount of additional woody debris (100 t/ha to 300 t/ha) did not appear to accelerate the recolonization of invertebrate, so smaller amounts are recommended.

7.5 What can be learnt from this research in terms of restoration management and what direction could future research take?

Restoration strategies for bauxite mining restoration and residue restoration used by Alcoa over the last 20 years support ground-dwelling invertebrate re-colonization, increasing soil microbial activities and developing soil ecosystem functions like seed dispersal and litter decomposition. Improving soil ecosystem functionality is critical during restoration and is the key to re-establishing a degraded ecosystem. Considering the results of this study, the following can be identified in terms of restoration management:

- Ground-dwelling invertebrates can be used as indicators of soil ecosystem functioning in degraded ecosystems and can be incorporated in management practises during mine site soil management to assess soil ecosystem functions. Ant species and ant functional group changes can be used as indicators to assess restoration success. The recovery invertebrates and ecosystem processes mirroring the recovery of the vegetation.

- Mining restoration using natural topsoil is resulting in a system whose ecosystem process are track towards those of the remnant system; however, the addition of woody debris (at the rate of 100 t/ha) will likely speed up recovery bracket at the rate of would improve recovery. Instead of applying a higher rate (300 t/ha) of woody debris in restoration practices, a lower rate (100 t/ha) can achieve better results.

- Residue restoration has limited recovery because of the hostile residue sand. During residue restoration, woody-debris addition, topsoil

application, and use of organic amendments should be trialled to provide additional inputs of organic matter to accelerate soil ecosystem functions.

- There is a lack of monitoring of invertebrate fauna and ecosystem processes within the residue system. Residue restoration does not recover ecosystem processes and diverse invertebrate communities. Further trials need to improve invertebrate colonisation and soil properties.

- Soil microbial respiration is a sensitive and robust indicator to assess soil functionality during restoration. The 1-day Solvita test to measure soil microbial respiration is a prompt assessment tool to define soil quality and functionality during restoration.

This study indicates that the return of ground-dwelling invertebrate communities, ant functional groups, and species will accelerate ecosystem functions (decomposition and seed dispersal). However, the exact combination of invertebrate functional groups that maintain a similar system to that of undisturbed native forest sites is still unclear, and needs to be investigate in future studies. A challenge to invertebrate monitoring is the taxonomic expertise required to identify taxa to sufficient taxonomic levels so as to make detailed comparisons between sites. However, it is time consuming to assess taxa in this manner, and it is expensive to hire taxonomists. Future investigations involving metabarcoding of invertebrate DNA (Fernandes et al., 2018) may overcome these impediments.

Future studies need to use litter from different plant species and/or use a mixture of litter, and measure decomposition over a longer period to assess the effect of mixed leaf litter during litter decomposition. Due to a limited budget, this study only measured the loss of dry mass and did not consider the rate of nutrient release (i.e. N, P, K, etc.) during decomposition. Therefore, future studies need to examine the loss of nutrients due to ground-dwelling invertebrate activity and leaching by rainfall to assess the rate of nutrient release over time. Although this study could not explain the specific relationship between ground-dwelling invertebrates and microbial decomposition, results suggested that mutualism between ground-dwelling invertebrates and soil microbes increase the rate of litter decomposition. Future research could also investigate the re-establishment

of soil ecosystem functions with woody-debris addition to determine if there are additional benefits and if higher woody-debris addition has any other benefits.

This study found that secondary seed dispersal by ants was lacking in the residue restoration system despite it being frequent in younger mining restoration sites. This will likely affect the distribution of seeds within soil seed banks and potentially affect the impact of fire on seed survival. This study only focused on seed dispersal rate and seed dispersal distance; thus, the fate of seeds after dispersal in each system is unclear. Seed germination and seedling recruitment from or close to ant nests need to be monitored over a longer period to assess the long-term fate of nest dispersed seeds. Additionally, further investigation, by way of seed germination experiments, is required to see if seeds are being spread or if the seed bank is being predated upon and depleted.

The physiochemical and microbiological characteristics of soil from mining restoration sites were consistent with the characteristics of the native analogue ecosystem (jarrah forest); however, in contrast, residue restoration site soils are unlikely to resemble the soil of the chosen reference ecosystem (tuart forest). Therefore, restoration success criteria will also depend on the functional ecosystem characteristics of the study systems. This study measured soil microbial respiration using the Solvita test as a function of microbiological activity. Future studies need to investigate the recovery of soil microbial species richness and microbial biomass during the reestablishment of soil ecosystem functions. Additionally, further studies need to focus on metabarcoding of soil environmental DNA (Gellie et al., 2017) in order to identify the soil microbial community composition and recognize the missing microbial species from restored sites.

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Appendix A

Ant abundance in each study site (mean ± SE). Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

Plot ID	M2	M5	M10	M20	MR	R2	R5	R10	RR
Arnoldius scissor (Crawley)	0±0	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Anonychomyrma itinerans perthensis (Forel)	0±0	0±0	1.33±0.33	1.33±0.33	9.33±6.83	0±0	0±0	0±0	36.66±29.48
Doleromyrma darwiniana fida (Forel)	0±0	0±0	0.66±0.66	2±0.57	20.33±8.37	0±0	0±0	0±0	0±0
Dolichoderus semiorbis Shattuck & Marsden	0±0	16.66±15.67	0.33±0.33	1.66±0.66	0±0	0±0	0±0	0±0	0.66±0.66
Iridomyrmex bicknelli Emery	109±19.13	44.66±8.19	54.66±39.5	6.33±4.48	0.66±0.33	51±21.51	11±6.55	25±23.5	5.66±3.84
Iridomyrmex chasei Forel	0±0	0±0	0±0	0±0	0±0	359.33±276.85	217.33±54.57	91.33±30.94	114±67.11
Iridomyrmex discors Forel	0±0	0±0	0±0	0±0	0±0	133.33±28.95	252.66±215	21.33±7.21	9.66±9.17
Iridomyrmex dromus Clark	0±0	8.33±7.35	0±0	0±0	0±0	1.66±0.33	6±1.52	2.33±1.85	0±0
Iridomyrmex gracilis spurcus Wheeler	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.66±1.66
Iridomyrmex notialis Shattuck	0±0	0±0	0±0	0±0	0±0	0±0	1.33±1.33	0±0	0±0

Species									
Iridomyrmex mjobergi Forel	0±0	19±7.21	5±3	0±0	0±0	0±0	1.33±0.88	1.33±0.33	0.33±0.33
Iridomyrmex purpureus (F.Smith)	0.33±0.33	35±20.64	22.66±13.01	0±0	0±0	0±0	0±0	0±0	0±0
Iridomyrmex rufoniger suchieri Forel	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Iridomyrmex splendens Forel	0±0	7.33±4.37	0±0	1±0.57	0±0	0±0	0±0	0±0	0±0
Iridomyrmex suchieri Forel	0±0	0±0	0±0	0±0	0±0	15.66±15.66	0±0	0±0	0±0
Iridomyrmex suchieroides Heterick & Shattuck	0±0	0±0	0±0	0.33±0.33	0±0	0±0	0±0	19.33±18.34	12±6.55
Nebothriomyrmex majeri Dubovikov	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Ochetellus glaber (Mayr)	0±0	0.33±0.33	3.33±2.02	4.33±1.45	2±0.57	0±0	0±0	0±0	0±0
Tapinoma minutum broomense Forel	0±0	4.66±2.9	2.33±0.88	2.66±1.66	4.66±2.72	0±0	0.66±0.66	0±0	1±1
Camponotus JDM 63	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.33
Camponotus claripes minimus Crawley	0±0	0±0	0±0	0±0	1±0.57	0±0	0±0	1±0	0.33±0.33
Camponotus claripes JDM 25	0±0	0±0	0.33±0.33	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Camponotus scratius Forel	0±0	1±0.57	0±0	0±0	0±0	0±0	1±1	0±0	0±0
Camponotus terebrans (Lowne)	0±0	10.66±5.36	36.66±8.66	9.33±7.33	0±0	0±0	9±8.5	2±2	0±0
Camponotus walkeri Forel	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.66	0±0	0±0
Melophorus aeneovirens (Lowne)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33

Melophorus JDM 230	0±0	0±0	0±0	0±0	0±0	0.66±0.66	0±0	0±0	5.66±1.76
Melophorus JDM 520	0±0	0±0	0±0	0±0	0±0	2±2	0.33±0.33	1.66±1.2	0±0
Melophorus JDM 783	0±0	0±0	0±0	0±0	0±0	0±0	3.66±2.66	16.33±6.64	3±2.51
Melophorus ludius sulla Forel	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	10.66±4.33
Melophorus turneri perthensis Wheeler	0±0	11±10	7.33±1.2	0±0	0±0	1±0.57	2.66±1.2	3±2.08	2.66±1.76
Notoncus enormis Szabo`	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Notoncus gilberti Forel	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33	3.33±1.85
Notoncus hickmani Clark	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.66±0.88
Prolasius JDM109	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Prolasius antennatus McAreavey	0±0	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Stigmacros aemula Forel	0±0	0.33±0.33	0.66±0.66	1±0.57	2.66±1.2	0.33±0.33	1.33±0.33	1±1	0±0
Stigmacros bosii (Forel)	0±0	0±0	0±0	2.33±0.33	1.66±1.2	0±0	0±0	0±0	0±0
Stigmacros brachytera McAreavey	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Stigmacros epinotalis McAreavey	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Stigmacros glauerti McAreavey	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1±0.57
Stigmacros JDM 115	0±0	0±0	0.66±0.66	0±0	0.33±0.33	0±0	0±0	0±0	0±0
Myrmecia urens complex sp. JDM 1	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.33

Species									
Brachyponera lutea (Mayr)	0±0	0±0	0.33±0.33	0.66±0.33	1±0.57	0±0	0.33±0.33	1±1	0.66±0.66
Hypoponera congrua (Forel)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Hypoponera eduardi (Forel)	0±0	0±0	0.33±0.33	0±0	0±0	0±0	0.66±0.66	0±0	0±0
Leptogenys neutralis Forel	0±0	0±0	0±0	1±0.57	1.66±1.66	0±0	0±0	0±0	0±0
Austroponera rufonigra (Clark)	0±0	0±0	0.66±0.66	0.33±0.33	0.33±0.33	0±0	0±0	0±0	0±0
Rhytidoponera inornata Crawley	0±0	14.33±14.33	0±0	1.33±1.33	1.66±1.2	0±0	0±0	0±0	1±0.57
Rhytidoponera metallica (F.Smith)	57.66±19.93	76±13.52	35.33±3.17	11±9.53	10±10	0±0	0.33±0.33	7.33±0.88	12±4
Rhytidoponera violacea (Forel)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Discothyrea turtoni Clark	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.33±0.33
Austromorium flavigaster (Clark)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Cardiocondyla atalanta Forel	4±4	1±0.57	0.33±0.33	2.66±0.88	3±2.51	36±16.52	0±0	0.66±0.33	0.33±0.33
Crematogaster disper Forel	0±0	0±0	0±0	0.66±0.66	2.33±0.88	0±0	0±0	0±0	0±0
Crematogaster laeviceps chasei Forel	0±0	0±0	1.33±0.33	2±0.57	1.33±0.33	0±0	0±0	0±0	6±2.64
Meranoplus ferrugineus Crawley	0±0	0±0	0.66±0.66	1.33±0.88	0±0	0±0	0±0	0.33±0.33	17.66±5.2

Meranoplus rugosus Crawley	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0	0.33±0.33
Meranoplus JDM 74	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Meranoplus JDM 968	0±0	0±0	1±0.57	0±0	0±0	0±0	0±0	0±0	0±0
Monomorium JDM 101	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Monomorium lacunosum Heterick	0±0	0±0	0±0	0±0	0±0	0±0	0.66±0.66	0.33±0.33	0.66±0.66
Monomorium leae Forel	0±0	0.66±0.66	0.66±0.66	1.33±0.66	2.66±1.2	0±0	0±0	0±0	5.33±5.33
Monomorium sordidum Forel	0±0	1±1	1.33±0.33	2±1	1.66±0.33	3±3	127.66±53.07	45.33±16.73	29.66±12.41
Pheidole JDM 164	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.33±1.33	2.66±0.88
Pheidole ampla perthensis Crawley	0.33±0.33	0±0	0±0	0.33±0.33	1±0.57	0±0	0.33±0.33	0±0	7.33±3.52
Solenopsis clarki Crawley	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	21.66±16.67
Strumigenys perplexa (F.Smith)	0±0	0±0	0±0	1±0.57	3.33±2.33	0±0	0±0	0±0	0±0
Tetramorium impressum (Viehmeyer)	0±0	0±0	0±0	0±0	0±0	34.33±9.59	26.33±6.35	6.66±1.85	0.33±0.33
Tetramorium simillimum (F. Smith)	0±0	0±0	0±0	0±0	0.66±0.66	6±3.78	3.33±3.33	0±0	0.66±0.66
Myrmecia clarki Crawley	0±0	0±0	0±0	0±0	0.66±0.33	0±0	0±0	0±0	0±0
Myrmecia tepperi Emery	0±0	0±0	0.33±0.33	0±0	0±0	0±0	0±0	0±0	0±0

Myrmecia mandibulari F.Smith	0±0	0±0	0±0	0.33±0.33	0.66±0.33	0±0	0±0	0±0	0±0
Myrmecia vindex F.Smith	0±0	0±0	0±0	0.33±0.33	0.33±0.33	0±0	0±0	0±0	0±0
Stigmatomma panctulatum (Clark)	0±0	0±0	0±0	0.66±0.33	1±0.57	0±0	0±0	0±0	0±0

Appendix B

Summary of ant species sampled via hand collection or by using pitfall traps at each study plot. Species interaction with seeds are highlighted. Species recorded only during hand collection are marked using *. Sites: M2 = two-year-old mining restoration, M5 = five-year-old mining restoration, M10 = 10-year-old mining restoration, M20 = 20-year-old mining restoration, MR = jarrah remnant, R2 = two-year-old residue restoration, R5 = five-year-old residue restoration, R10 = 10-year-old residue restoration, and RR = tuart remnant.

Subfamily	Species	M2	M5	M10	M20	MR	R2	R5	R10	RR	Seed disperser	Elaiosome thief
Dolichoderinae	*Arnoldius scissor* (Crawley)					1						
	Anonychomyrma itinerans perthensis (Forel)			1	2	1				1		
	Doleromyrma darwiniana fida (Forel)				2	2						Y
	Dolichoderus ypsilon Clark									2		
	Dolichoderus semiorbis Shattuck & Marsden				1							
	Iridomyrmex bicknelli Emery	1	1	2			1	1	2	1	Y	
	Iridomyrmex chasei Forel						2	2	2	2	Y	
	Iridomyrmex discors Forel						2	2	2	1	Y	
	Iridomyrmex dromus Clark						1	2	2			
	Iridomyrmex gracilis spurcus Wheeler									1		
	Iridomyrmex notialis Shattuck							1				
	Iridomyrmex mjobergi Forel			1				2	1	1	Y	
	Iridomyrmex purpureus (F.Smith)		2	2							Y	

	Iridomyrmex rufoniger suchieri Forel									1		
	Iridomyrmex splendens Forel				1					2	Y	
	Iridomyrmex suchieri Forel						1					
	Iridomyrmex suchieroides Heterick & Shattuck				1					1		
	Nebothriomyrmex majeri Dubovikov									1		
	Ochetellus glaber (Mayr)			2	1	1						Y
	Tapinoma minutum broomense Forel			1	2	2						
Formicinae	*Camponotus cinereus notterae* Forel									2		Y
	Camponotus claripes minimus Crawley					1			1	1		
	Camponotus claripes JDM 25			1		2						
	Camponotus scratius Forel							1				
	Camponotus terebrans (Lowne)			2	2			2	2		Y	
	Camponotus walkeri Forel							1				
	Melophorus aeneovirens (Lowne)									2		
	Melophorus JDM 230									2	Y	
	Melophorus JDM 783							1	1	1		
	Melophorus ludius sulla Forel									2		Y
	Melophorus turneri perthensis Wheeler		2	2						1		
	Notoncus enormis Szabo`									1		
	Notoncus gilberti Forel									2		

Subfamily	Species										
	Prolasius antennatus McAreavey					1				2		
	Stigmacros aemula Forel						1	1				
	Stigmacros bosii (Forel)				1							
	Stigmacros epinotalis McAreavey									2		
	*Monomorium fieldi Forel											
	Stigmacros glauerti McAreavey									1		
	Stigmacros JDM 115			2								
Myrmeciinae	*Myrmecia urens* complex sp. JDM 1									1		
	Myrmecia clarki Crawley			1		2						
	Myrmecia tepperi Emery					2						
	Myrmecia mandibulari F.Smith									2		
	*Myrmecia vindex F.Smith					2						
Ponerinae	*Brachyponera lutea* (Mayr)			1		1	1	1		2		
	Hypoponera congrua (Forel)									1		
	Hypoponera eduardi (Forel)			1			1					
	Leptogenys neutralis Forel				1	1						
	Austroponera rufonigra (Clark)					1						
Ectatomminae	*Rhytidoponera inornata* Crawley					1				2	Y	
	Rhytidoponera metallica (F.Smith)	2	2	2	2	2		1		2	Y	
	Rhytidoponera violacea (Forel)					1				2	Y	

Proceratiinae	*Discothyrea turtoni* Clark								1				
Myrmicinae	*Cardiocondyla atalanta* Forel			1	1	1	1		1				Y
	Crematogaster laeviceps chasei Forel			2	1	2			2				Y
	Meranoplus JDM 968			1									
	Meranoplus ferrugineus Crawley			1	2				1				
	Meranoplus rugosus Crawley				1				1				Y
	*Monomorium fieldi Forel								1	Y			
	Monomorium lacunosum Heterick						2		1				
	Monomorium leae Forel					1			2				
	Monomorium sordidum Forel			1	1		2	2	1				Y
	Pheidole JDM 164								1				
	Pheidole ampla perthensis Crawley					1			2				
	Solenopsis clarki Crawley								1				
	Strumigenys perplexa (F.Smith)				1	1							
	Strumigenys quinquedentata Crawley					1	2	2	2				
	Tetramorium impressum (Viehmeyer)						1	1	1				
	Tetramorium simillimum (F. Smith)					1							
Amblyoponinae	*Stigmatomma panctulatum* (Clark)				1								