The influence of soil disturbance on seed dispersal by ants in roadside environments - southern NSW, Australia

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Certificate of Authorship

I, Zsofia Palfi,

Hereby declare that this submission is my own work and to the best of my knowledge and belief, understand that it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis [or dissertation, as appropriate]. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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Abstract

Myrmecochory (or seed dispersal by ants) is a common ant-plant mutualistic relationship worldwide. Ants move seeds into their nests to consume the elaiosome, an attractive food reward, and then discard the seed into the surrounding area. Habitat disturbance, such as fire, is known to alter the success of this mutualism by influencing the composition and behaviour of ant communities, however little is known of the effects of soil disturbance. In much of south-eastern Australia, past land-use has resulted in extensive clearing of previous woodlands for agricultural purposes, where remaining patches of remnant vegetation exist mainly in roadside environments. Many roadsides are now of high conservation status, yet regularly disturbed by (soil) disturbances from road management activities.

Field studies were conducted in a typical fragmented agricultural landscape in southern NSW, Australia. The selected area contains a large network of minor rural roads of gravel construction that require periodic management, often associated with soil disturbances. Twenty-four road segments were selected that each contained a non-disturbed (road verge) and a disturbed (by road maintenance) zone. Seeds of Acacia pycnantha, a common myrmecochorous shrub, were offered to ants at multiple seed depots in both zones at each site to conduct observations on ant-seed interactions and subsequent seed dispersal processes.

Ant species richness was greater in the non-disturbed zones, where wide road verges possessed a greater number of species as compared to narrow roadsides. The composition and abundance of individual seed-dispersing ant species varied between disturbed and non-disturbed zones. Overall, 26 ant species were recorded interacting with seeds, however only a few species were responsible for the majority (82%) of ant-seed interactions. In particular, Rhytidoponera metallic and Iridomyrmex purpureus were important contributors in terms of the number of seed removal events. Melophorus bruneus typically performed seed removal events. Smaller bodied
Monomorium and Pheidole almost exclusively performed elaiosome consumption, potentially reducing the success of such seeds.

Greater mean seed dispersal distances were recorded in the disturbed zone (mean 12m, maximum 120m), where large meat ants (Iridomyrmex purpureus) were responsible for most of the occasional, long-distance dispersals. This species appeared to thrive in habitat conditions created as a result of soil disturbances from roadworks. Field observations also recorded secondary dispersal events away from nests, which may further aid in seed dispersal. The occasional long-distance seed dispersal which were recorded in this study may have direct consequences for plant communities, by facilitating colonization into new sites, and providing habitat connectivity in otherwise fragmented and highly modified rural landscapes.

In summary, despite the severity of disturbances from roadworks, certain ant species prefer to nest in such areas owing to foraging or habitat benefits (e.g. cleared substrate) caused by this form of soil disturbance. Given their contrasting responses to disturbance, there is need for studies to further assess the comparable role of Rhytidoponera and Iridomyrmex as dispersal agents, with particular regard to seed survival and future seedling establishment. The results of this study provide new insights into the mechanisms of seed dispersal by ants occurring in roadside environments and other habitats affected by anthropogenic soil disturbance regimes.
Chapter 1 : Introduction


Chapter 1: Introduction

1.1 SEED DISPERSAL

Worldwide, human activities are causing accelerated, novel and substantial changes to ecosystem patterns and processes. Human land-use change and associated climate change are considered the main drivers influencing biodiversity alteration and losses (Vitousek et al. 1997, Sala et al. 2000). In many fragmented or other human dominated landscapes, habitat isolation can lead to dispersal limitations for many species, increasing the risk of inbreeding and eventual population declines (Saunders et al. 1991, Young et al. 1996). Therefore, the maintenance of habitat connectivity is desirable in facilitating seed dispersal for the conservation of many plant species and communities (Taylor et al. 1993, Auffret and Cousins 2013, Suarez-Esteban et al. 2016).

Seed dispersal has profound effects on vegetation structure, ranging from habitat colonization to maintenance of diversity by supporting population spread and genetic connectivity (Wang and Smith 2002, Trakhtenbrot et al. 2005), as it connects the end of the reproductive cycle of an adult plant with the establishment of their offspring (Wang and Smith 2002, Vander Wall and Longland 2004). Plants have evolved an innumerable variety of devices to disperse their reproductive structures (Herrera 2002).
Traditionally, plant species can be classified into different dispersal modes based on their seed morphology (van der Pijl 1982, Howe and Smallwood 1982, Thomson et al. 2010), which in turn is also used to infer the most likely dispersal vector (“standard” vectors, Higgins et al. 2003) and associated dispersal distances (Vittoz and Engler 2007). For example, seeds with elaiosomes (see below) are characterised by limited dispersal, while seeds with wings and plumes, enhanced by dispersal by the wind, usually possess somewhat longer distances (van der Pijl 1982, Willson 1993, Gomez and Espadaler 2013). Nonetheless, seeds may be also dispersed by multiple vectors, so-called “non-standard” vectors, involving mechanisms different from which a seed has adapted to, often resulting in dispersal over very long distances (Higgins et al. 2003).

The vast majority of dispersing seeds undergo short-distance dispersal movements away from the parent plants, while only a slight portion is occasionally moved to distant locations (e.g. Andersen 1988, Pascov et al. 2015), and are often aided by non-standard mechanisms (Harper 1977, Willson 1993, Higgins et al. 2003). Long-distance dispersal (LDD) events are rare and stochastic processes (Nathan et al. 2003, 2008). Although a very small proportion of seeds might be transported for long distances, they can have disproportionate effect on species dynamics, determining large-scale processes such as population spread, gene flow between populations and colonisation of unoccupied habitats (Cain et al. 2000, Levin et al. 2003, Nathan et al. 2003). The importance of LDD was first recognised over a century ago by naturalists such as Darwin (1895), but initial observations were mostly anecdotal, “viewing these events as freak happenings of no
significance” (Carlquist 1981). Even now, efforts to observe and quantify LDD events are particularly difficult, because of the immense complexities and uncertainties involved with the identity of LDD mechanisms (Nathan et al. 2003).

**1.2 SEED DISPERSAL BY ANTS**

Seed dispersal by ants, or myrmecochory, is a significant ant-plant mutualistic relationship that occurs almost worldwide (Berg 1975, Beattie and Culver 1981). In evolutionary terms, seed dispersal by ants has evolved in most of the major angiosperm lineages and it is more frequent in families which originated 70-80 million years ago, than those which diverged earlier (ca. 100 million years ago) (Lengyel et al. 2010). Lengyel et al. (2010) identified at least 101, but possibly up to 147 independent origins of myrmecochory. This aspect is well demonstrated by the broad taxonomic and biogeographical distribution of myrmecochorous species in many habitats (Lengyel et al. 2009, 2010).

Myrmecochory can represent one of the main dispersal modes in communities at regional and local level (Berg 1975, Rice and Westoby 1981, Bond and Slingsby 1984). Myrmecochorous plants dominate communities in many habitats, in terms of both species richness and abundance (Handel et al. 1981). For example, myrmecochores are common in dry sclerophyll shrublands of Australia and South Africa, consisting mostly of woody shrubs occurring in low-nutrient, fire-prone habitats. It is thought that nutrient poverty may favour plants to produce
elaiosomes (which is relatively ‘cheap’ to produce) as compared to producing fleshy fruits adapted for vertebrate dispersal in similar conditions (Westoby et al. 1990, 1991, Orians and Milweski 2007, Lengyel et al. 2010). In contrast, in temperate woodlands of the Northern Hemisphere myrmecochores often occur as herbaceous plants growing in the understorey of deciduous forests (Handel 1981, Beattie and Hughes 2002).

1.2.1 Benefits gained through myrmecochory

The presence of a reward is a common characteristic of mutualistic interactions between plants and animals, especially in the form of food (Beattie 1985, Mayer et al. 2005). To attract ants, many plants produce a specialized outer appendage to their seeds called the elaiosome, forming the total plant dispersal unit called the diaspor (Ridley 1930, Berg 1975, Beattie 1985). The presence or absence of elaiosome is an important factor of diaspor removal, where elaiosome-bearing seeds are generally more attractive for ants than seeds without elaiosomes (Hughes and Westoby 1992, Peters et al. 2003, Reifenrath et al. 2012). The size of seeds and their respective elaiosomes will greatly affect diaspor acceptance by ants, with greater preference shown for high elaiosome/seed-size ratios (Oostermeijer 1989, Hughes and Westoby 1992, Mark and Olesen 1996, Edwards et al. 2006, Ehlers 2012).
Ants benefit from this mutualism by obtaining a ‘free lunch’ of additional energy-rich food (Beattie and Hughes 2002), which is thought to comprise a small portion of their total diet (Morales and Heithaus 1998, Bono and Heithaus 2002, Lubertazzi et al. 2010, Majer et al. 2011). The nutritional requirements of ants vary with the state of the colony (Beattie 1985). Studies have shown that elaiosome-supplemented diet in laboratory conditions affected colony parameters, increasing colony fitness through increased production and weight of larvae (Gammans et al. 2005), or even may have an effect on shifting sex ratios, producing more female reproductives (gynes) (Morales and Heithaus 1998). Therefore, the enhancement of ant diet by elaiosome consumption may play an important role in ant survival and fitness.

Furthermore, various potential benefits to myrmecochorous plants have been reported. Purportedly, the most important advantages for seeds from ant dispersal include reduction in parent-offspring and sibling competition, escape from predators, avoidance of fire, and relocation of seeds to more favourable microsites (i.e. the ant nest) for survival, germination and plant establishment (see Giladi 2006 and Rico-Gray and Oliveira 2007 and references therein). The importance of each expected benefit is ecosystem-specific, related to differences in behaviour of the main seed-dispersing ant species (Giladi 2006).
1.2.2 The fate of ant-dispersed seeds

Seeds deposited into artificial depots generally disappear within the first several hours (Passos and Ferreira 1996, Beaumont et al. 2009). When seeds are removed by ants, most are taken directly to ant nests (Culver and Beattie 1978, Hughes and Westoby 1992, Rowlesand O’Dowd 2009). In the nest ants remove the elaiosome for food, which is mainly given to their larvae (Beattie 1985, Bono and Heithaus 2002, Servigne and Detrain 2010). The intact and still viable seeds are then variously discarded; into underground chambers (i.e. incorporated into the floors and walls of passageways; Auld 1986), onto nest refuse piles, or out into the surrounding area of the nest (e.g. resulting in a secondary seed dispersal; Hughes and Westoby 1990, Beaumont et al. 2013). Seeds found again on the ground, or seeds that were not removed, may also follow several fates: they can germinate in situ, become part of the seed bank, be predated upon by other species (e.g. rodents), or have their elaiosomes robbed by other ants (“cheating”) (Harris and Standish 2008, Boulay et al. 2009, Salazar-Rojas et al. 2012). "Cheater” ants do not transport seeds, but remove the elaiosome in situ. This in turn decreases the chances of removal by true dispersers, and increase the likelihood of seeds being taken away by granivores (seed predators) (Andersen and Morrison 1998, Aranda-Rickert and Fracchia 2011).

Myrmecochory is generally considered to be an “unevenly diffuse” mutualism (Gove et al. 2007), where only a few ant species are excessively important for the seed dispersal process (Boulay et al. 2007, Lubertazzi et al. 2010,
Aranda-Rickert and Fracchia 2011). While many ant species have been observed removing seeds, activities are usually plant and ant specific, where dispersal is performed mainly by one ant species (60%-90%) (Andersen 1988, Gove et al. 2007, Beaumont et al. 2009, Salazar-Rojas et al. 2012) or in some cases by two or three species (Boulay et al. 2007, Parr et al. 2007, Zelikova and Breed 2008). Numerous studies have recorded dispersal distances by following individual ants whilst transporting diaspores from experimental seed depots to their nests (Andersen 1988, Hughes and Westoby 1992, Servigne and Detrain 2010, Warren et al. 2010). Alternate methods include studies of the foraging areas of a given ant species to determine likely seed dispersal distances (Lubertazzi et al. 2010).

Although mean dispersal distances by ants are considered short (1-2m, Gomez and Espadaler 2013), such distances are still considered sufficient to reduce parent-offspring conflict (Rico-Gray and Oliveira 2007, Beaumont et al. 2009). Moreover, the shape of dispersal curves often reveals unique long dispersal events (Andersen 1988). Maximum ant dispersal distances were observed as long as 180m (Whitney et al. 2002), particularly where ant nest density is low at a given area (Andersen 1988). The contribution of different ant species to dispersal is very unequal (Boulay et al. 2007) since the demography and foraging behaviour of seed-dispersing ants can greatly vary (Andersen and Morrison 1998). For example, species with large foraging areas, and consequently longer dispersal distances, typically have larger body sizes than species with smaller foraging ranges (Pudlo et al. 1980, Mitchell et. al. 2002, Ness et al. 2004).
1.2.3 Habitat disturbance effects on myrmecochory

Disturbance regimes occur widely, both by natural and anthropogenic means (Hobbs and Huenneke 1992, Tilman and Lehman 2001). Species loss and biodiversity change are common consequences of environmental stress and disturbance, often as a consequence of altered habitat conditions (Hobbs 1993, Fischer and Lindenmayer 2007, Britton 2013), which in turn ultimately influence ecosystem functioning.

Disturbance events may lead to changes in mutualistic ant-plant interactions (Crist 2009). Shifts in ant species composition and relative abundances have been reported due to fragmentation (Mitchell et al. 2002), edge effects (Ness and Morin 2008, Christianini and Oliveira 2013), invasion (Christian 2001) and other disturbance regimes (Andersen and Morrison 1998, Heithaus and Humes 2003). These shifts in species composition and abundance due to prevailing disturbance regimes are considered the main reasons for varied outcomes in dispersal services (Crist 2009).

Functional redundancy in seed-dispersing ant communities may occur in contrasting habitats (e.g. disturbed versus undisturbed), which may ensure the maintenance of functional roles of ecosystem processes (Rivas-Arancibia et al. 2014). However, the majority of the studies reported a positive association between the increased abundance of the main seed disperser with an increase in species richness and abundance of the myrmecochores. This suggests that seed-
dispersing ants are not functionally redundant in their influence on ant-dispersed plant species (Mitchell et al. 2002, Leal et al. 2013).

Ant-dispersed plant species are more prone to habitat fragmentation as opposed to vertebrate dispersed taxa, and may take longer time to recover after disturbances (McLahan and Bazely 2001). Ness and Morin (2008) linked the rarity of ant-dispersed plants in forest edge habitats with the low abundance and activity of the important local disperser ant species. In Australia, to date, variability in myrmecochory has been largely investigated in the context of fire or mining disturbances (i.e. Majer 1982, Andersen and Morrison 1998, Parr et al. 2007, Beaumont et al. 2013), which are major disturbance regimes throughout the continent (Hobbs 1987, Majer 1984).

However, soil disturbance is another common disturbance regime in Australian and other ecosystems. Native animals have long been recognized as playing vital roles in maintaining ecosystem functioning through their digging and burrowing activities (Eldridge and James 2009, Fleming et al. 2014). The loss of numerous digging mammals (so-called ‘ecosystem engineers’) has resulted in the regression of various ecosystems (Fleming et al. 2014). Soil disturbance, however, may also occur in the form of human activities (Spooner 2005a, Cambi et al. 2015). In many cleared and fragmented agricultural landscapes soil disturbance regimes pervade most remnant ecosystems, via the ongoing use and maintenance of tracks and roads (Bognounou et al. 2009). Some studies have investigated the effects of soil disturbances from roads and tracks on ecosystems, and found varying effects on the surrounding habitat and vegetation structures (e.g. Lugo and Gucinski 2000,
Spooner 2005a). However, the effects of soil disturbance regimes on seed-dispersal mutualism have been poorly studied.

1.3 THESIS OVERVIEW

The thesis examines the influence of soil disturbance on the mechanism of seed dispersal by ants in roadside environments in southern NSW, Australia. Specifically, it aims to determine the influence of soil disturbance on:

- the composition of seed-dispersing ant communities occurring along roadsides;

- ant-seed interactions and other associated ant behaviour; and

- seed removal rates and dispersal distances carried out by the resident ant assemblage.

To address these aims, the thesis consists of the following chapters:

- Chapter 2 begins with a description of the study area, the focus roadside habitats and myrmecochorous plant species, and general methodology used in this study;
• Chapter 3 examines the seed-dispersing ant assemblage in two habitats, disturbed (by soil disturbance) versus non-disturbed (vegetated) zones of roadsides;

• Chapter 4 describes the direct observations on ant-seed interactions at seed depot (‘cafeteria’ style) experiments, the importance of which determine subsequent seed dispersal patterns;

• Chapter 5 investigates the influence of anthropogenic soil disturbances on dispersal distances achieved by respective ant species; and

• Chapter 6 presents the synthesis and general discussion of the overall results. I highlight the overall contribution and significance of the thesis, and provide further recommendations for future research.

1.3.1 Thesis chapter style

Chapters (3–5) were prepared as papers for publication (manuscripts), and contain separate introductions, methods, results and discussion sections. Consequently, this format inevitably leads to some minor repetition between chapters, particularly with introductions and methodological details. Therefore, analogous sections of methods have been removed from the corresponding chapter and are summarized in Chapter 2. To avoid unnecessary duplications, a single reference list is combined at the end of the thesis.
1.3.2 Research outputs from the thesis

Peer-reviewed publication:


Manuscript in review:

Zsofia Palfi, Peter G. Spooner and Wayne Robinson (2017 in review) Seed dispersal distances by ants increase in response to anthropogenic disturbances in Australian roadside environments. *Frontiers*

Conferences (oral presentations):


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Other (short report):

Chapter 2: Research methodology
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2.1 STUDY AREA: LOCKHART SHIRE

Lockhart Shire is a rural local government area located in the Riverina region of southern New South Wales, Australia. The area has a cool temperate climate, with mean annual rainfall ranging from 500–600 mm, and altitude ranging from 200–450 m. Topography consists of low undulating hills and flat riverine plains, with sporadic granite and porphyry outcrops (Lockhart Shire Council State of Environment Report 2013). Much of the region is arable farmland dominated by cropping and grazing farm systems, subdivided by a network of minor and major roads. As much of the landscape is cleared or highly fragmented, these roads often harbour the last vestiges of ‘intact’ remnant woodlands, grasslands and other ecosystems (Lunt and Bennett 2000), which form clearly visible boundaries along most of the roadsides (Figure 2.1.).

Figure 2.1. Lockhart Shire in southern New south Wales.
Habitats of various kinds are under pressure from various anthropogenic disturbances. Roads represent a conspicuous part of many environments and their effects on ecosystems are numerous (Forman and Alexander 1998). The Lockhart study area contains a large network of roads (~1600 km), where minor roads represent almost half of the total road network (750 km; Spooner et al. 2004). The research was confined to this area to ensure that a consistent approach to local government road management was applied across the study area.

The verge adjacent to roads often contains unique remnant vegetation which provide insights into historical vegetation structures (Spooner and Lunt 2004) and constitute important refuges for native flora and fauna. Roadside habitats can provide a conduit for many species, providing connectivity between distant patches, thus acting as corridors (Tikka et al. 2001). However, minor roads are maintained by anthropogenic inputs, which although are deleterious to many plants, are often advantageous to others, depending on their life history and dispersal traits (Lugo and Gucinksi 2000, Gelbard and Belnap 2003, Spooner 2005a). Historical roadwork activities, have been proven to facilitate the recruitment and persistence of roadside plant populations in Australia (Spooner and Lunt 2004, Spooner et al. 2004), especially favouring some *Acacia* species (Spooner 2005a). *Acacias* are important and widespread myrmecochorous genera in Australia and elsewhere (Berg 1975). As such, roadside environments provide an ideal context to
study the impact of soil disturbance on mutualistic interactions, such as myrmecochory.

2.1.2 *Acacia pycnantha* Benth. – a common myrmecochorous plant

*Acacia* is the largest genus in the family Mimosaceae and is the largest genus in the Australian flora, with more than 900 species occurring in the continent. *Acacia pycnantha* (Golden Wattle) occurs in temperate regions across much of Victoria and south-eastern Australia. This species is usually a shrub or small tree, 4-8 m tall, and it grows on a range of soils including clays and shallow, stony loams. *Acacia pycnantha* is commonly found in the understorey of open Eucalypt woodlands and in open scrub formations (Tame 1992). True leaves are absent, instead it has leathery phyllodes of 6-20 cm, which are flattened leaf stalks that have adapted to function as leaves. Flowering mainly occurs during August and October, and dark brown seed pods dehisce and release seeds during November to January (Costermans 1981).

*Acacia* plants often require fire for their seed germination (Auld 1996), however, fire has been largely eliminated from roadsides. Spooner et al. (2004) hypothesised that disturbances from roadworks may act as surrogate for fire, for example through scarification of the hard seed coat of *Acacia* seeds. *Acacia* species are known to be involved into numerous seed-dispersal interactions with ants (Beattie 1985).
2.1.3 Roadside sites selection criteria

Building on an existing roadside vegetation dataset (Bull 1997, Spooner 2005b, Figure 2.2), a random stratified approach was used to select study sites along ‘road reserves’. In Australia, ‘road reserves’ are areas of land which are set aside for road transportation (Spooner 2005b) and can be divided into three zones: (1) the road surface, (2) the ‘road-effect zone’ (sensu Forman and Alexander 1998), which is the area adjacent to the road (1-5 m from the road edge) and is frequently disturbed by road maintenance (grading) operations (hereafter disturbed zone), and (3) the non-disturbed verge of roadside vegetation (hereafter non-disturbed zone). In this study, the width of the entire road reserve varied up to 60 m.

Figure 2.2. Distribution of *Acacia pycnantha* on road sections in the Lockhart Shire, as surveyed by Spooner et al. (2004) (adapted from Spooner 2005c).
Figure 2.3. Typical minor rural road of varying roadside width, wide (top) and narrow (bottom) with remnant Eucalyptus trees, in the Lockhart Shire study area.
Twenty-four sites were randomly selected where roadside Acacia populations occurred (Figure 2.4). An Acacia population was defined as a minimum of 30 individual plants dispersed either evenly, or forming clumped patterns, within a 60m longitudinal section of road verge (density approx. 30-170 stems/ha depending on roadside width). Acacia populations were separated by at least 500m from each other to ensure independence of ant-plant interactions. Sites were stratified into two groups based on the verge width, narrow (3–14m) or wide (15–60m) roadsides.

**Figure 2.4.** Location of the study sites (n = 24) in the Lockhart Shire council, NSW, Australia. Minor roads are omitted for clarity. (Source of map: Spatial Data Analysis (SPAN), Charles Sturt University, https://six.nsw.gov.au/).
2.1.4 Collecting seeds for ant sampling

Prior to commencing the study, *Acacia pycnantha* seeds were collected from the Lockhart Shire area (outside the actual study sites) during the seed ripening period in 2013 (November–December). Seeds were extracted from their legumes (pods) and then stored in glass containers in a cool and dark place until deployed in seed depot experiments (see below). Only undamaged seeds with intact elaiosomes were used. Originally, seeds of the two *Acacia* species that differed in their seed sizes (*A. pycnantha* being somewhat bigger than *A. montana*) were collected. However, the extraction of seeds from *A. montana* pods proved to be problematic due to seed viability issues. A pilot study was performed, which indicated that ants of all sizes were attracted to, and could easily remove *A. pycnantha* seeds. Therefore, use of seeds of a single species of *Acacia* was deemed adequate for this study, as myrmecochorous relationships tend to be site, rather than species or seed specific (Andersen 1986).

2.1.5 ‘Cafeteria’ style experiment

‘Cafeteria’ style experiments were used to identify seed-dispersing ant assemblages in the disturbed and non-disturbed zones. ‘Cafeteria’ experiments normally involve the presentation of a variety of seed-choices (e.g. different seed species/sizes) to determine ant-seed preferences (Culver and Beattie 1978). In this study, the term “cafeteria style experiment” is used to describe the use of this
design, where only a single *Acacia* species seed was offered. At each site, a 60 m transect was first established at the centre of the *Acacia* population parallel to the road. This initial transect was then used to position two main 25 m transects, following a matched pair study design, with the disturbed and non-disturbed areas separated longitudinally by a 10 m gap from each other. Each transect was placed centrally as a function of the width of their respective zones. To observe ants, 6 cafeteria style seed depots were established at 5 m intervals along each transect. Seed depots represented a notional square of approximately 9 x 9 cm on the ground. At each depot, a palm-sized clearing of the vegetation was made (as required) to facilitate ant observations (Hughes and Westoby 1990). Seeds were placed directly on the ground separated by approximately 2 cm from each other.

The research was performed during the summer months of December 2013 to March 2014. Two observational periods were performed in a day: the morning session started from 8:00 am to 12:00 pm and the afternoon session from 16:00 pm to 20:00 pm, covering 4h per session. During midday, observations were curtailed due to the high ambient temperatures (often >38°C) when ants were largely inactive above ground. Ants observed at seed depots during the experiments were identified in the field where possible. If individuals could not be identified, they were followed until a drop point was reached (e.g. nests or *en route*), then captured and placed into vials filled with 70% ethanol for later identification. Collected individuals were later identified in the lab using field guides or expert advice (A. Andersen pers. comm. 2014).
In general, the study design and experimental setup followed preceding similar studies which observed and recorded seed dispersal processes by ants in relation to disturbance regimes (e.g. Zelikova and Breed 2008, Leal et al. 2013).

This study was carried out in accordance with AAC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and Charles Sturt University Animal Care and Ethics Committee policy. This project was exempt from approval requirements because it was deemed 'research with limited impact' (category 4).
Chapter 3: Soil disturbance effects on the composition of seed-dispersing ants in roadside environments
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Abstract

Myrmecochory (the dispersal of seeds by ants) is a significant ecological process in sclerophyll woodlands, and habitat disturbance is known to alter the extent and success of this mutualism. I investigated the influence of soil disturbance on the composition of the seed-dispersing ant community. Surveys were conducted in roadside verges where soils are regularly disturbed by road maintenance activities. Using a cafeteria style experiment approach, I selected 24 road segments of different widths to investigate the seed-dispersing ant composition and abundance in relation to soil disturbance. Ant species richness was greater in non-disturbed than disturbed zones, where road verge width significantly influenced results. The composition and abundance of individual seed-dispersing ant species varied between disturbed and non-disturbed zones. *Rhytidoponera metallica* were more abundant in non-disturbed sites, whereas *Melophorus bruneus* and *Monomorium rothseini* were more frequently recorded in disturbed areas. Commonly found *Iridomyrmex purpureus* was significantly more abundant in disturbed zones in narrow roadsides and vice versa in wide roadsides, and strongly influenced total community composition. Variation in the abundance of commonly recorded *Iridomyrmex* and *Monomorium* genera were related more to site conditions (roadside width and habitat) than soil disturbance. The rich composition of seed-dispersing ants in roadside environments, and the effects of soil disturbances on
these ant communities, provide a key insight to important seed dispersal vectors occurring in fragmented rural landscapes.

**3.1 INTRODUCTION**

Myrmecochory is a significant mutualistic relationship that occurs in many ecosystems worldwide, and is a common form of seed dispersal for many plants (Beattie 1985, Lengyel et al. 2010); especially in sclerophyll vegetation on low-nutrient soils (Berg 1975). Myrmecochory provides important advantages to plants, including a reduction in parent-offspring competition, predator avoidance, and dispersal of seeds to more favourable microsites for subsequent germination and establishment (Giladi 2006, Rico-Gray and Oliveira 2007). However, the extent to which ants may facilitate the distribution of plants largely depends on the composition of the co-occurring ant community, available habitat and the disturbance conditions which prevail (e.g. Lassau and Hochuli 2004, Zelikova and Breed 2008).

Habitat disturbances such as fire, grazing or mining are known to shape ant communities directly by reducing and/or removing ant biomass, and indirectly through changes in vegetation structure, altered food availability and other competitive interactions (e.g. Hoffman and Andersen 2003, Hoffmann 2010). Due to the predictability of responses of ant communities to various stress and disturbances, ants are widely used as bioindicators of ecosystem health (Andersen 1997, Majer et al. 2004). Ant species richness is often greatest under moderate
disturbances (Graham et al. 2009), but an increase in disturbance may induce an increase in the abundance of disturbance tolerant species, which in turn, may result in a reduction of overall species diversity (e.g. Majer and Beeston 1996); although this is not always the case (Gibb and Hochuli 2004). Furthermore, the assessment of habitat structural complexity is often a good predictor of ant species richness and composition, where leaf litter, tree canopy cover and other habitat characteristics are known to regulate ant assemblages (Lassau and Hochuli 2004, Yates et al. 2012).

In Australia and elsewhere, an extensive network of vegetated minor roads exists in an otherwise cleared and fragmented agricultural landscape (e.g. Bennett 1991, Forman and Alexander 1998, Spooner 2015). These road corridors often retain significant tracts of native vegetation adjacent to the road surface (the road verge), in turn providing structural and functional connectivity for many plants and animals (Tewksbury et al. 2002). As a result, minor rural roads constitute an important landscape element which can have important conservation values (e.g. Vasconcelos et al. 2014, Spooner 2015). Roadside verges often possess similar biodiversity values to remnant vegetation in nearby reserves (Bennett 1990, Major et al. 1999, Schabel and Eldridge 2001), and this is especially true for roads with wider verges (Keals and Majer 1991, Spooner and Lunt 2004).

Roadside environments provide an ideal context to study the effects of disturbance on seed-dispersing ant communities. Minor roads are normally maintained by periodic disturbances in the form of road maintenance activities (e.g. mowing and soil grading), which depending on their frequency and intensity, is
known to demote or promote certain plant species (Spooner et al. 2004, Bognounou et al. 2009). For example, soil disturbances from periodic roadwork activities is known to facilitate the recruitment of *Acacia* species in south-eastern Australia (Spooner 2005a), a plant genus which normally relies on ants to disperse their seeds through myrmecochory (Berg 1975). However, the response of mutualistic ant communities to soil disturbances has been poorly studied (but see Keals and Majer 1991, Major et al. 1999).

To help analyse the responses of ant communities to various stress and disturbance regimes (both natural and human-induced disturbances), a functional group scheme was developed for Australian ants (Andersen 1995). The functional group scheme has proved to be especially useful in the context of minesite rehabilitation and other environments where soil changes occur (Hoffmann and Andersen 2003). Using this framework, I undertook studies to investigate the effects of soil disturbance on seed-dispersing ant species in roadside environments in south-eastern Australia. I predicted that there would be greater species richness in non-disturbed versus disturbed roadside environments, and that disturbance tolerant and thermophilic species would characterise ant community composition in disturbed areas. Second, as vegetated roadsides vary in width and resultant habitat conditions (Karim and Mallik 2008), I investigated if these attributes influenced the composition of seed-dispersing ants in disturbed and non-disturbed areas.
3.2 METHODS

3.2.1 Composition of seed-dispersing ants

A complete description of the study sites and study design is presented in Chapter 2. In summary, a stratified random sampling approach was used to select 24 roadside sites located in the Lockhart Shire council area, southern NSW, where common Acacia populations were known to occur. As roadside environments occur at varying widths, which in turn influence habitat and disturbance conditions (Spooner et al. 2004), sites were stratified into two groups: narrow (3–14 m) or wide (15–60m) roadsides.

Cafeteria (Culver and Beattie 1978) style experiments were used to describe the seed-dispersing ant composition in the study sites. Seed depots were placed in a linear fashion along two 25 m transects with 5 m spacing between them, one transect in each zone (2 transects, 12 seed depots/site). Freshly harvested diaspores from Acacia pycnantha plants were used and 10 seeds were placed at each depot.
3.2.2 Sampling vegetation characteristics

To evaluate the influence of microhabitat characteristics on ant community composition, vegetation structure and cover attributes were recorded using a point-intercept method along the 60 m transect for each zone (disturbed vs. non-disturbed). Observations were taken every metre with the use of a pointed wooden pole (60 points per transect/120 points per site). The following habitat characteristics were recorded: the percentage cover of bare soil, litter, perennial plants, annual plants, herbaceous plants, and moss/lichen. Tree and shrub canopy cover was also assessed along the transect using the line intercept method.

3.2.3 Statistical analyses

Differences in seed-dispersing ant species richness and abundance between zones (disturbed vs. non-disturbed), roadside width (narrow vs. wide) and time period (morning vs. afternoon) were analysed using a factorial ANOVA with repeated measures approach, where width was treated as fixed factor, zone and time as repeated measures within each site, and site as a random factor. For abundances, species that occurred at more than 2 sites were included in the analyses. For all models, the residuals were analysed to assess the assumptions of normality and homogeneity of variances (Zar 1999).
Multivariate analysis of variance was used to investigate ant community compositional dissimilarities between zone, width and sampling period for all sites. The number of seed depots each ant species occurred at (max n = 6) was used as an index of abundance at each site. Species with less than two occurrences (or 5%) of sites were not included in analysis, as these species did not contribute to the compositional differences. A Bray-Curtis dissimilarity matrix was constructed on the species occurrences followed by permutational analysis of variance (PERMANOVA, Anderson 2001) to test for ant compositional dissimilarities between zones, roadside widths and time of sampling. Variance components estimates from PERMANOVA were calculated to determine the relative importance of the factors (zone, roadside width and time of sampling) in explaining the ant community variation. PERMANOVA was carried out in Primer software (Clarke and Gorley 2006).

To visualize multivariate patterns in the composition of seed-dispersing ants, non-metric multidimensional analysis (NMDS) was applied on the Bray-Curtis dissimilarity matrix of ant occurrences. Vectors were fitted to the existing NMDS plot to indicate the relationship of species to the ordination space using the envfit() function, and the 'vegan' package was used to create NMDS plot (Oksanen et al. 2015). The ordination treats the 4 observations per site (n = 24) as independent points, which I consider reasonable for the sake of the visualisation, but does not apply statistical tests of significance to the correlations and present only species that had a correlation of 0.4 or more with the space.
Ant species observed during the seed removal experiment were categorised into functional groups according to Andersen (1995). The effect of zone, width, time on the proportion of seed depots occupied by functional groups was analysed using generalized linear mixed model (GLiMM) fitting a binomial distribution. Zone, width and time were treated as fixed factors and sites as independent and random (not paired) in this analysis because of computational limitations within the GLiMM procedure (http://CRAN.R-project.org/package=nlme). Significant interactions were followed up by using separate models for each functional group.

To compare vegetation characteristics across the sites I used a three-factor ANOVA, with zone and width treated as fixed factors and site as random factor. To examine the relationship between ant species richness and the measured habitat variables, regression analysis (linear mixed-effects model) was performed using the ‘nlme’ package (Pinheiro et al. 2014). An Akaike Information Criteria (AIC) approach was used to select the ‘best’ models for predicting ant species richness, by comparing the base model including zone and width as fixed factors, and site as a random factor with new models that included habitat variables as covariates, one at a time. Model fit process followed Burnham and Anderson (2002), where ΔAIC values ≤ 2 were considered to be the models with the most utility, as they have substantial support (Arnold 2010). Statistical tests were carried out using R statistical software (R Development Core team 2013) unless otherwise stated.
3.3 RESULTS

3.3.1 Species richness of seed-dispersing ants

Twenty-six species in 12 genera and 5 subfamilies (*Formicinae*, *Dolichoderinae*, *Ectatomminae*, *Myrmicinae* and *Ponerinae*) were observed in this study (Appendix 1), where more seed-dispersing ants were recorded in wide roadsides than in narrow roadsides. Using an ANOVA approach, there was a significant three-way interaction between zone, roadside width and time of day of sampling ($F = 6.0$, df = 1,22, $p = 0.02$; Table 3.1). Species richness was always greater in the non-disturbed areas of wide roadsides compared to disturbed, regardless of time of day of sampling (Figure 3.1). For narrow roadsides, the number of species was greater in the non-disturbed versus disturbed zones, however this result was only true in the morning sampling period (Figure 3.1).

### Table 3.1. ANOVA testing the effects of (disturbance) zone, (roadside) width and (sampling) time, and their interactions, on total ant species richness in roadside environments.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Error Mean Square (MS)</th>
<th>MS Source</th>
<th>MS Error</th>
<th>F</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone</td>
<td>1, 22</td>
<td>Site (zone)</td>
<td>20.2</td>
<td>3.2</td>
<td>6.4</td>
<td><strong>0.01</strong></td>
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<tr>
<td>Width</td>
<td>1, 22</td>
<td>Site</td>
<td>51.0</td>
<td>3.8</td>
<td>13.3</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Time</td>
<td>1, 22</td>
<td>Site (time)</td>
<td>1.0</td>
<td>2.1</td>
<td>0.5</td>
<td>0.48</td>
</tr>
<tr>
<td>Zone × width</td>
<td>1, 22</td>
<td>Site (zone)</td>
<td>18.4</td>
<td>3.2</td>
<td>5.8</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>Time × width</td>
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<td>Site (time)</td>
<td>6.0</td>
<td>2.1</td>
<td>2.9</td>
<td>0.10</td>
</tr>
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<td>Zone × time</td>
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<td>Site (time × zone)</td>
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<td>2.2</td>
<td>0.0</td>
<td>0.89</td>
</tr>
<tr>
<td>Zone × time × width</td>
<td>1, 22</td>
<td>Site (time × zone)</td>
<td>13.5</td>
<td>2.2</td>
<td>6.0</td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>
Figure 3.1. Ant species richness in relation to zone (D = disturbed and ND = non-disturbed), road verge width (narrow and wide) and sampling time (am = morning, pm = afternoon). The box plots show the minimum and maximum values, boxes are the interquartile range and the bold bar shows the median value. Outliers (> 1.5 times the interquartile range) are annotated with a circle.

In total, 18 species were included in PERMANOVA multivariate analyses, with 8 species excluded as they only occurred at 2 or less sites (Table 3.2). There was a significant difference in the composition of seed-dispersing ant species between disturbance zones and time period, where roadside width had a strong but non-significant effect on results (Table 3.2). 53% of the variation in ant species composition was between disturbance zones (see NMDS analysis; Figure 3.2). Only 29% of the variation in ant species composition was between roadside widths and 11% between the sampling periods.
Table 3.2. Permutational analysis (PERMANOVA) on the composition of seed-dispersing ant species in relation to (disturbance) zone, (roadside) width and (sampling) time. Bold type denotes a significant result.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>Error MeanSquare</th>
<th>MS Source</th>
<th>MS Error</th>
<th>Pseudo-F</th>
<th>P(perms)</th>
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<tbody>
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<td>Zone</td>
<td>1, 22</td>
<td>Site</td>
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<td>4831.8</td>
<td>7.1</td>
<td>&lt;0.001</td>
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<td>10658.0</td>
<td>4831.8</td>
<td>2.2</td>
<td>0.054</td>
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<tr>
<td>Time</td>
<td>1, 22</td>
<td>Site</td>
<td>3140.3</td>
<td>4831.8</td>
<td>3.8</td>
<td>0.01</td>
</tr>
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<td>Zone × width</td>
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<td>Site (zone)</td>
<td>1633.0</td>
<td>1789.6</td>
<td>0.9</td>
<td>0.48</td>
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<td>Zone × time</td>
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<td>Site (zone)</td>
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<td>1789.6</td>
<td>0.6</td>
<td>0.74</td>
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<tr>
<td>Width × time</td>
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<td>Site (time)</td>
<td>1142.2</td>
<td>828.5</td>
<td>1.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Zone × width × time</td>
<td>1, 22</td>
<td>Site (zone × time)</td>
<td>867.5</td>
<td>675.6</td>
<td>1.3</td>
<td>0.26</td>
</tr>
</tbody>
</table>

The multivariate ordination results showed that *Rhytidoponera metallica* was associated with non-disturbed zones, whereas *Melophorus sp. A (bruneus gp.)* and *Monomorium sp. B (rothsteini gp.)* were more associated with disturbed zones (Figure 3.2). Two common *Iridomyrmex* species (*I. purpureus* and *I. rufoniger*), *Rhytidoponera cristata* and *Monomorium sp. A (sordidum gp.)* were positioned at the boundary of the two clusters, suggesting their abundance is better explained by the variation in site conditions rather than the effects of soil disturbance (zone).
Figure 3.2. Non-metric MDS ordination diagram displaying seed-dispersing ant composition in disturbed (D) and non-disturbed (ND) zones. The strength and direction of the correlation of ant species with the NMDS axial scores (P < 0.05) are shown by the fitted vectors. Stress = 0.21. Species abbreviations: Ir.pur = Iridomyrmex purpureus, Ir.ruf = I. rufoniger, Mon.sor = Monomorium sp. A (sordidum gp.), Mon.rot = M. sp. B (rothsteini gp.), Mel.bru = Melophorus sp. A (bruneus gp.), Rh.met = Rhytidoponera metallica, Rh.cri = R. cristata.

3.3.2 Individual species analyses

*Iridomyrmex purpureus* was significantly more abundant in the non-disturbed zones in wide roadsides and vice versa in narrow roadsides, where their abundance varied according to sampling time (Table 3.3). Similarly, the abundance of *I. rufoniger* was significantly influenced by an interaction between the effects of zone × sampling time (Table 3.3) and were more frequently recorded at seed depots in the disturbed
zones in the afternoon while their abundance in non-disturbed zones was very similar in both sampling times.

*Rhytidoponera metallica* was observed at significantly more seed depots in the non-disturbed areas of roadsides (Table 3.3). The abundance of *R. cristata* was significantly influenced by the interaction between time and roadside width (Table 3.3), with it often occurred in narrow roadsides in the morning period, but greater occurrence was recorded in the afternoon of wide roadsides.

In contrast, *Melophorus* sp. A (*bruneus* gp.) was more abundant in the disturbed zones and in wide roadsides, but was also significantly influenced by sampling time (Table 3.3). Similarly, *Monomorium* sp. B (*rothsteini* gp.) was significantly influenced by zone (Table 3.3), where most were recorded only in the disturbed zone. The occurrence of *Monomorium* sp. A (*sordidum* gp.) was also influenced by sampling time, where their abundance at seed depots was significantly greater in the afternoon period (Table 3.3).
Table 3.3. ANOVA of the effects of (disturbance) zone, (roadside) width and (sampling) time, and their interactions, on the abundance of individual ant species correlated with the NMDS plot axes. Bold type denotes a significant result.

<table>
<thead>
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<th>Species</th>
<th>Source</th>
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<th>Time</th>
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<td>1, 22</td>
<td>1, 22</td>
<td>1, 22</td>
<td>1, 22</td>
</tr>
<tr>
<td>Iridomyrmex purpureus</td>
<td>F value</td>
<td>0.06</td>
<td>1.35</td>
<td>0.11</td>
<td>5.04</td>
<td>6.33</td>
<td>0.33</td>
<td>0.30</td>
</tr>
<tr>
<td>Iridomyrmex rufoniger</td>
<td>Pr(&gt;F)</td>
<td>0.81</td>
<td>0.26</td>
<td>0.75</td>
<td><strong>0.04</strong></td>
<td><strong>0.02</strong></td>
<td>0.57</td>
<td>0.10</td>
</tr>
<tr>
<td>Rhytidoponera metallica</td>
<td>Pr(&gt;F)</td>
<td>&lt; 0.001</td>
<td>0.07</td>
<td>0.15</td>
<td>0.57</td>
<td>0.88</td>
<td>0.59</td>
<td>0.72</td>
</tr>
<tr>
<td>Rhytidoponera cristata</td>
<td>Pr(&gt;F)</td>
<td>0.03</td>
<td>0.30</td>
<td>1.64</td>
<td>0.00</td>
<td>9.46</td>
<td>1.25</td>
<td>0.00</td>
</tr>
<tr>
<td>Melophorus sp. A (bruneus gp.)</td>
<td>Pr(&gt;F)</td>
<td><strong>0.02</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.01</strong></td>
<td>0.57</td>
<td>0.41</td>
<td>0.28</td>
<td>0.40</td>
</tr>
<tr>
<td>Monomorium sp. B (rothsteini gp)</td>
<td>Pr(&gt;F)</td>
<td><strong>&lt; 0.001</strong></td>
<td>0.20</td>
<td><strong>0.05</strong></td>
<td>0.50</td>
<td>0.73</td>
<td>0.33</td>
<td>0.52</td>
</tr>
<tr>
<td>Monomorium sp. A (sordidum gp)</td>
<td>Pr(&gt;F)</td>
<td>0.09</td>
<td>1.38</td>
<td>4.96</td>
<td>0.09</td>
<td>0.00</td>
<td>2.43</td>
<td>2.43</td>
</tr>
</tbody>
</table>

3.3.3 Functional group composition

Species were listed into six functional groups (Appendix 1). Most functional groups contained 2–5 species: Dominant Dolichoderinae (2 species), Opportunists (3), Subordinate Camponotini (5), Cold Climate Specialists (2), Hot Climate Specialists (2), and Generalized Myrmecinae were the most species rich group with 9 species. The most frequently distributed functional groups were Dominant Dolichoderinae, Opportunists and Generalized Myrmecinae. Overall, the proportion of seed depots occupied by functional groups was significantly influenced by the effects of
disturbance, roadside width and sampling time period, where there were also significant interactions between zone x FG, time x FG and width x FG (Table 3.4).

Table 3.4. Results of GLiMM on the effects of (disturbance) zone, (roadside) width and (sampling) time, and their interactions, on the proportion of seed-dispersing ants in six functional groups (FG) (calculated at each site). Bold type denotes a significant result.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>χ²</th>
<th>P &gt; χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone</td>
<td>1</td>
<td>1.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>1.1</td>
<td>0.29</td>
</tr>
<tr>
<td>Time x zone</td>
<td>1</td>
<td>0.1</td>
<td>0.78</td>
</tr>
<tr>
<td>Width</td>
<td>1</td>
<td>5.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Zone x width</td>
<td>1</td>
<td>1.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Time x width</td>
<td>1</td>
<td>0.2</td>
<td>0.67</td>
</tr>
<tr>
<td>Time x zone x width</td>
<td>1</td>
<td>2.8</td>
<td>0.10</td>
</tr>
<tr>
<td>FG</td>
<td>4</td>
<td>342.2</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Zone x FG</td>
<td>4</td>
<td>26.2</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Time x FG</td>
<td>4</td>
<td>13.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Time x zone x FG</td>
<td>4</td>
<td>0.7</td>
<td>0.95</td>
</tr>
<tr>
<td>Width x FG</td>
<td>4</td>
<td>35.7</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Zone x width x FG</td>
<td>4</td>
<td>1.0</td>
<td>0.91</td>
</tr>
<tr>
<td>Time x width x FG</td>
<td>4</td>
<td>3.9</td>
<td>0.42</td>
</tr>
<tr>
<td>Time x zone x width x FG</td>
<td>4</td>
<td>2.8</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Further analysis of the percentage of individual functional groups at sites showed that Dominant Dolichoderinae (DD) was significantly affected by an interaction between zone x roadside width (Z = 0.92, p = 0.01), where there was a higher proportion of DD recorded in the disturbed zones of narrow roadsides, however wide roadsides had a greater proportion of DD in the non-disturbed zones (Figure 3.3).
Similarly, there was a greater percentage of Opportunists (OPP) in non-disturbed zones \((Z = 2.49, p < 0.001)\) of both wide and narrow roadsides (Figure 3.3). In contrast, the percentage of Hot Climate Specialists (HCS) was significantly greater in the disturbed zones \((Z = 0.00, p = 0.01)\), and there was a significant interaction effect between roadside width and time of day of sampling \((Z = -0.008, p = 0.01)\); Figure 3.3).

For Generalized Myrmecinae (GM), there was a significant interaction between the effects of disturbance zone, roadside width and sampling time on their percentage values \((Z = 2.98, p = 0.01)\). More seed depots were occupied by GM at wide and narrow roadsides in the disturbed zones in the morning, however this pattern reversed for wide roads in the afternoon. Subordinate Camponotini (SC) were mainly recorded in the non-disturbed zones \((Z = 1.26, p = 0.01)\) in wide roadsides \((Z = 1.70, p < 0.001)\).
Figure 3.3. Proportion of seed depots occupied by respective Functional Groups (FGs) between zones (D = disturbed and ND = non-disturbed), roadside width (narrow and wide) and time of sampling (am = morning and pm = afternoon) in roadside environments in the Lockhart Shire (southern NSW). FGs abbreviations: DD = Dominant Dolichoderinae, PP = Opportunists, GM = Generalized Myrmecinae, SC = Subordinate Camponotini, HCS = Hot Climate Specialists and CCS = Cold Climate Specialists.
3.3.4 Habitat characteristics

Using an ANOVA approach, there was a significant difference between the percentage cover of most structural attributes between disturbed and non-disturbed zones (Table 3.5). In general, the disturbed area had less tree and shrub cover, less understorey plant cover, but more bare ground as compared to non-disturbed road verges.

Further ANOVA analysis of habitat characteristics versus roadside width showed that the percentage cover of litter, perennial grasses and mosses also significantly differed between narrow and wide roadsides (litter: $F = 4.57$, df = 1,22, $p = 0.04$; perennial grass: $F = 6.14$, df = 1,22, $p = 0.02$; mosses: $F = 75.84$, df = 1,22, $p = 0.01$; not shown in Table 3.5). GLiMM analysis showed that the percentage cover of litter was a significant habitat variable in the best fitted model ($\Delta \text{AIC} = -15.4$, df = 10, $p < 0.01$) to predict ant species richness, where perennial grass cover was also a strong predictor variable (Table 3.5).
Table 3.5 Vegetation characteristics (mean percentage ± standard error of the mean percentage) in disturbed and non-disturbed (n =24) zones of roadside environment. Results of ANOVA are shown: degrees of freedom (Df), F-value and p values (P > F), Akaike's Information Criterion (AIC) and Δ AIC are for GliMM models including each covariate compared to the base model (containing disturbance and width) on species richness of seed-dispersing ants.

<table>
<thead>
<tr>
<th></th>
<th>Disturbed</th>
<th>Non-disturbed</th>
<th>Df</th>
<th>F value</th>
<th>P&gt;F</th>
<th>AIC</th>
<th>Δ AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>205.19</td>
<td>15.41</td>
</tr>
<tr>
<td><strong>Canopy cover</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trees</td>
<td>23.80 ± 4.59</td>
<td>41.43 ± 5.92</td>
<td>1, 22</td>
<td>13.2</td>
<td>0.001</td>
<td>206.39</td>
<td>1.2</td>
</tr>
<tr>
<td>Shrubs</td>
<td>3.9 ± 1.66</td>
<td>15.71 ± 3.09</td>
<td>1, 22</td>
<td>11.8</td>
<td>0.01</td>
<td>205.66</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Ground cover</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barge ground</td>
<td>49.11 ± 2.83</td>
<td>10.09 ± 2.22</td>
<td>1, 22</td>
<td>121.9</td>
<td>&lt; 0.001</td>
<td>206.46</td>
<td>1.27</td>
</tr>
<tr>
<td>Litter</td>
<td>17.27 ± 1.70</td>
<td>24.01 ± 2.79</td>
<td>1, 22</td>
<td>4.5</td>
<td>0.04</td>
<td>189.78</td>
<td>-15.4</td>
</tr>
<tr>
<td>Logs</td>
<td>7.84 ± 1.21</td>
<td>16.55 ± 2.05</td>
<td>1, 22</td>
<td>14.1</td>
<td>0.001</td>
<td>205.08</td>
<td>-0.11</td>
</tr>
<tr>
<td>Perennial grass</td>
<td>14.4 ± 1.81</td>
<td>38.16 ± 0.72</td>
<td>1, 22</td>
<td>39.4</td>
<td>&lt; 0.001</td>
<td>199.7</td>
<td>-5.49</td>
</tr>
<tr>
<td>Annual grass</td>
<td>25.58 ± 2.72</td>
<td>45.59 ± 4.73</td>
<td>1, 22</td>
<td>4.4</td>
<td>0.04</td>
<td>205.41</td>
<td>0.22</td>
</tr>
<tr>
<td>Herbs</td>
<td>5.14 ± 1.06</td>
<td>4.63 ± 1.04</td>
<td>1, 22</td>
<td>0.1</td>
<td>0.73</td>
<td>206.3</td>
<td>1.11</td>
</tr>
<tr>
<td>Mosses/lichens</td>
<td>0.07 ± 0.07</td>
<td>5.27 ± 1.07</td>
<td>1, 22</td>
<td>26.9</td>
<td>0.001</td>
<td>206.06</td>
<td>0.87</td>
</tr>
</tbody>
</table>
3.4 DISCUSSION

Soil disturbances associated with roadside grading activities results in the removal of much of the above ground vegetation, creating habitat conditions unsuitable to most ant species. As predicted, I found a greater richness of seed-dispersing ant species in the non-disturbed compared to disturbed areas of roadside environments. Other studies which have investigated the response of ants to disturbance have either recorded a similar reduction in species richness (King et al. 1998, Rivas-Arancibia et al. 2014), an increase (Azcárate and Peco 2012), or no change (e.g. Parr and Andersen 2008, Hoffman and James 2011, Beaumont et al. 2012). These contrary study results are largely explained by differences in sampling methodologies, in conjunction with differences in environmental factors (below).

I found that the composition of ant species was generally similar for both disturbed and non-disturbed zones, however, the abundance of individual species at each site varied greatly in disturbed and non-disturbed zones. Other disturbance contrast studies have also demonstrated this pattern (Beaumont et al. 2012). For example, York (2000) found that frequently burnt and long-unburnt plots greatly differed in the composition and relative abundances of ant assemblages, despite a considerable proportion of species (66%) being identical in both treatments. Differences in the abundance or occurrence of individual ant species are typical in comparisons of burnt or other disturbed habitats, owing to a greater representation of a few common species favoured by these habitats (Beaumont et al. 2012).
This study did not attempt to complete a full enumeration of ant species in roadside environments. Pitfall traps are commonly used for such a purpose, whereas bait techniques are generally used to examine behavioural and foraging patterns (Bestelmeyer et al. 2000). Seed bait cafeteria style experiments were used in this study, which represent a more specialised method designed for seed-dispersing ant species. As a result, comparisons to other studies which have conducted a full enumeration of ant species may result in misleading conclusions. Although our focus was the seed-dispersing ant species, a relatively large array of generalist species was also identified.

3.4.1 Disturbance effects on seed-dispersing ant species

Other studies have described how the intensity, frequency and timing of various disturbance regimes can lead to varied responses of ant species (Majer 1984, Majer et al. 1984, Andersen et al. 2003, Rivas-Arancibia et al. 2014). Fire is the most common form of natural disturbance in Australian temperate communities, the effects of which on ant communities have been well studied (reviewed in Hoffmann and Andersen 2003). Not surprisingly, ants have developed a high resilience to disturbances in many fire prone environments (Rodrigo and Retana 2006, Parr and Andersen 2008, Andersen et al. 2014). As a result, the immediate and short-term effects of burning on ant species richness is often not significant (Parr et al. 2004), nor is the longer time-since-fire period (Rodrigo and Retana 2006). Such findings
are generally attributed to the refuge that ground-dwelling ants obtain from their nests and habitat conditions created post disturbances (Parr and Andersen 2008), which likewise, may have influenced the results of this study.

Often overlooked, soil disturbance is also a naturally re-occurring disturbance event in many ecosystems, e.g. digging and pugging by native animals, wind throw of trees. Anthropogenic activities, in the form of road maintenance activities (grading), represent another form of soil disturbance which continues to influence species composition and function in roadside environments (Spooner et al. 2004) and other contexts. In this study, the width of the roadside was a major factor in explaining the response of ants to soil disturbances, where in general, wide roadsides had greater species richness. As such, wide road verges (>30m) were considered to possess similar conditions and ant assemblages to other natural habitats such as woodland reserves (Major et al. 1999).

In contrast to wide road verges, narrow verges (often only 2–10 m wide) are more strongly influenced by periodic soil disturbances (Spooner et al. 2004, Delgado et al. 2007). In conjunction with edge effects, these highly modified environments could be considered all ‘edge’ habitats. Road verge width also influences habitat conditions in terms of differences in vegetation structure, litter cover, light availability, and microclimate, which in turn plays a major influence on species occurrence (Kaspari et al. 2000). I found that the percentage cover of litter and perennial grasses had a strong influence on species richness in roadside verges. As described by Spooner et al. (2004), grading is likely to impact upon the vegetated areas of narrow roadsides more frequently and severely than wider roads,
therefore habitat conditions will vary greatly with roadside width, and following this, influence the abundance of individual ant species (see below).

3.4.2 Individual species abundance patterns

In this study, species from the genera *Iridomyrmex*, *Rhytidoponera* and *Monomorium* were the most frequently recorded species, which follows the results of other studies in Australia (Nichols and Burrows 1985, Hoffman and James 2011), and of other myrmecochorous studies (Berg 1975, Davidson and Morton 1981, Parr et al. 2007).

*Melophorus*, a Hot Climate Specialist (Andersen 1995), was more prevalent in the disturbed zones of roadsides. Such species are heat-tolerant and are known to forage in the hottest part of the day (Greenslade 1979, Christian and Morton 1992), thus avoiding interference with any other dominant species. Given the sampling approach, I may have underestimated the actual abundance (i.e. sampling was carried out in the morning and afternoon). *Melophorus* was hardly ever recorded in narrow roadsides, whereas they could be regarded as characteristic of the disturbed area of wide roadsides (see NMDS plot, Figure 3.2). It appears *Melophorus* uses the advantages of a disturbed area for nesting, but forages in adjacent non-disturbed habitat where resources are plentiful.
**Monomorium** is a widely distributed genus across many biomes on the Australian continent (Andersen 1995, Gosper et al. 2015). *Monomorium* species, another Hot Climate Specialist, were frequently recorded in both disturbed (91%) and non-disturbed zones (54%) in both wide and narrow road sites, suggesting they also prefer soil disturbed habitats. They have generalized feeding and nesting habits, are able to recruit rapidly in response to availability of food resources, and can readily defend themselves, even against the dominant *Iridomyrmex* (Andersen 1995). These characteristics may explain the co-occurrence of *Monomorium* and *Iridomyrmex* species recorded in this study.

*Iridomyrmex purpureus* is a highly successful, early coloniser of a habitat (Nichols and Burrows 1985), behaviourally and numerically dominant (Greenslade 1976, Andersen and Patel 1994, Gibb 2005), and can favour open, well insolated habitats (Greaves 1971, Andersen 1995). Being from the Dominant Dolichoderinae functional group (Andersen 1995), their greater abundance in disturbed zones was predicted, however, this result was only upheld for narrow roadsides. The dominance of perennial grasses in non-disturbed zones of narrow roadsides (as compared to wide roadsides) is thought to largely explain these findings, as it stands in contrast with habitat preferences of this species. Open areas confer advantages to *I. purpureus* in terms of suitable warmth, open conditions for nesting (Greenslade 1976) and ease of foraging. In addition, field observations suggest that *I. purpureus* uses the graded soil surface as a ‘runway’ to access resources at great distances (Pers. Obs). As complex vegetation structure (e.g. perennial grasses) is known to reduce the foraging speed and discovery of new resources by ants (Gibb
and Parr 2010), this form of soil disturbance appears to confer many advantages for *Iridomyrmex* residing in roadside habitats.

In contrast, *Rhytidoponera metallica*, an Opportunistic species, was recorded in all non-disturbed zones of both wide and narrow roadsides, but was much less abundant in soil disturbed areas. Beaumont et al. (2012) also found that *R. metallica* did not increase in abundance in relation to fire disturbance. However, this result contrasts other studies, where *Rhytidoponera* genus typically shows an increase in its abundance in response to habitat disturbances - but when other dominant species such as *Iridomyrmex* spp. are not present in high numbers (York 2000, Hoffmann and Andersen 2003). *Rhytidoponera* species are subordinate and highly influenced by the presence and interference of dominant *Iridomyrmex* species (e.g. Parr et al. 2007). The abundance of *Iridomyrmex* species in disturbed roadside sites is thought to have strongly influenced the abundance of *Rhytidoponera* species in this study. Indeed, ant species were observed interacting with each other at seed depots during the experiments based on which their competitive status can be inferred (see Chapter 4 for more details). Other studies suggest that such spatial or temporal partitioning of species make the coexistence of dominant and subordinate species possible (Savolainen and Vepsalainen 1988).

Diminished litter cover and other vegetation structure might be another factor limiting the presence of *Rhytidoponera* in disturbed zones, which appear to prefer leaf litter for shelter (Pers. Obs.). Plants, in particular perennial grasses, often rapidly colonize the edges of roadsides providing adequate shelter for *Rhytidoponera*. The lowest occurrence of *I. purpureus* was associated with the
largest occurrence of *R. metallica*, which lends support to a dominant-submissive place in a competitive hierarchy.

### 3.4.3 Functional group approach to understand ant composition

Using the functional group approach, it was hypothesised that soil disturbance and associated habitat simplification should favour Hot Climate Specialists and Dominant Dolichoderines, as both groups are known to typically favour open, well insulated environments (Andersen 1995). This study did find that the abundances of Hot Climate Specialists was significantly greater in the disturbed zones, however results were not as conclusive for Dominant Dolichoderines. This pattern has been previously documented elsewhere in Australia (e.g. Beaumont et al. 2012, Gosper et al. 2015).

Dominant Dolichoderinae generally show an increased response in their abundance to a range of disturbances that result in canopy openness and increased insolation of ground-layer (Hoffman and Andersen 2003). The weak difference in the abundance of Dominant Dolichoderinae between disturbed and non-disturbed zones in this study may be explained by the already open structure and hot conditions typically experienced in most roadsides (both wide and narrow). The abundance of Opportunists decreased with soil disturbance, mainly due to the reduction in the numbers of *R. metallica*. Opportunists tend to increase their abundance to a range of disturbances (King et al., 1998), however they can be
decreasers as well. Nonetheless, when disturbance favours Dominant Dolichoderinae, the abundance of Opportunists actually decreases due to the avoidance of interference with dominant species. Opportunists and Generalized Myrmicinæ showed opposite responses to disturbance (Hoffman and Andersen 2003).

Generalized Myrmicinæ was the third most abundant and widespread group and was the most species-rich group recorded in the study. Generalized Myrmicinæ often show large amongst species variation in their responses to disturbances within its guild (York 2000, Beaumont et al. 2012). For example, their abundance was significantly greater in the disturbed zones, with Monomorium species contributing to this pattern, whereas the abundance of Pheidole was larger in non-disturbed zones. Beaumont et al. (2012) considered that Generalized Myrmicinæ is often limited by the complex structure of non-disturbed environments, which may partly explain individual species preferences for soil disturbances in this study.

The ant functional group scheme provides a useful framework to better understand species responses to disturbances in a range of environments (Gosper et al. 2015), however as discussed, studies have showed varied success in the applicability of this approach (Hoffmann and James 2011, Schooley et al. 2000). Likewise, in this study, although the framework provided a useful basis for investigations, ant composition patterns did not perfectly conform to the functional group model, which may have greater utility in more strongly contrasting sites (e.g.
rarely versus frequently-burnt habitats) (e.g. Andersen et al. 2014, Gosper et al. 2015).

3.4.4 Conclusion

The impacts of soil disturbances from road works largely depend on existing habitat configuration and road verge width. The latter attribute directly influences vegetation and habitat conditions which, in turn, influence nesting and foraging conditions for seed-dispersing ants. As such, soil disturbance from road works represents a frequent and severe disturbance process that confers both beneficial and adverse effects on seed-dispersing ant species. For example, *Rhytidoponera* spp. prefer non-disturbed areas, where their presence may ensure the success of various native seeds to be dispersed into these habitats. In contrast, certain species from the *Melophorus* and *Monomorium* genera prefer disturbed areas, utilising conditions for nesting and greater access to resources. However, the presence of *Iridomyrmex* spp., a competitively dominant species, may strongly influence ant species composition, and in turn, overall seed dispersal patterns in linear road corridors and elsewhere.

Of key interest to managers and researchers is the functional role of ants in dispersing seeds of native plants, particularly in fragmented landscapes (Lomov et al. 2009, Thomson et al. 2016), such as the study area, where remnant ecosystems and restored areas are isolated and/or exist in highly modified environment. This is
because there is growing evidence of a global seed dispersal crisis (Sutherland et al. 2013), which as McConkey et al. (2012) describes, has been largely overlooked due to the long life-span of perennial plants, which are neither being dispersed, nor regenerating, but persist for decades in an apparently healthy state.

The results of this study have identified some of the key ant dispersers in roadside environments that provide refuge for many woodland species, and described the role of soil disturbances in promoting or demoting key dispersing ant mutualists. Further studies of the nature of seed dispersal by ants, e.g. seed removal rates, dispersal distances, in relation to soil (and other) disturbances will greatly enhance efforts to conserve high quality roadside vegetation environments, and more broadly, our understanding of this important ecological process both in Australia and elsewhere.
Chapter 4: The influence of soil disturbance on ant-seed interactions in roadside environments
Chapter 4: The influence of soil disturbance on ant-seed interactions in roadside environments

Abstract

Myrmecochory is an important mutualistic interaction between ants and plants, where ants provide critical seed dispersal services essential for population growth and persistence for various plants. The extent to which anthropogenic soil disturbances may disrupt or benefit ant-seed interactions is largely unknown. I examined the influence of soil disturbance from grading operations on ant-seed interactions, e.g. seed removal, in roadside environments in a typical fragmented woodland landscape in south-eastern Australia. Observational experiments were first performed to ascertain the extent of vertebrate versus invertebrate removal of Acacia seeds – a principal myrmecochorous plant in such habitats. Cafeteria style seed depots were used to determine seed removal rates, and the contribution of different ant species to two main ant-seed interactions: seed removal and elaiosome consumption (cheating). Primarily ants interacted with seeds, where few vertebrate removals were recorded. Overall, soil disturbance led to decreased seed removals and greater cheating events, however individual species responses greatly varied. Twenty-six ant species were recorded interacting with seeds, however only a few species were responsible for 82% of ant-seed interactions. Rhytidoponera metallica and Melophorus bruneus typically removed seeds, while Monomorium and Pheidole almost exclusively carried out cheating. Iridomyrmex showed a mix of the two behaviours. The dominant Iridomyrmex purpureus was observed
performing cheating, however the extent of seed removal activities was similar to the keystone *Rhodiponera metallica*. Soil disturbance resulted in contrasting behaviours between the two main seed removalists: *I. purpureus* removed a higher proportion of seeds in soil disturbed zones, while *R. metallica* carried out more seed removals in the non-disturbed zone. These results highlight the complex nature of ant-plant interactions, and effects of disturbance on a critical seed dispersal service.

**4.1 INTRODUCTION**

Plants have evolved adaptations for dispersing their seeds by various means. Seeds of many plants have external food reward structures that attract animals, to aid in the dispersal of their propagules some distance away from the parental plant (van der Pijl 1982, Bullock and Nathan 2008). The effectiveness of the potential dispersal vector largely depends on the identity of the participant, the nature of the initial encounter with the seed and the habitat circumstances (Beattie and Hughes 2002). Both vertebrates and invertebrates can be potential disperser agents (Howe and Smallwood 1982), and from the latter group, ants provide a disproportionate role as dispersal vectors for many plants (myrmecochores) in various environments (Berg 1975, Beattie and Culver 1981).

Australia is acclaimed as a myrmecochorous ‘hotspot’, where more than 1500 plants are adapted to seed dispersal by ants (Berg 1975, Lengyel et al. 2010).
Such plants have seeds with a specialized food body attached to them, called an elaiosome, which is rich in lipids and proteins (e.g. Brew et al. 1989, Fischer et al. 2008). By collecting the diaspor (seed plus elaiosome), ants gain a nutritious reward, whilst conferring critical dispersal benefits to plants (e.g. Howe and Smallwood 1982, Giladi 2006). Previously it was thought that a positive correlation existed between the number of ants and plants in an ecosystem (Beattie 1985, Berg 1975). However, recent studies have confirmed a more specialized pattern, where few ant species might provide effective services to plants (i.e. greater proportion of seed removal, or faster removal; Schupp et al. 2010). This ‘unevenly diffuse’ mutualism, first described by Gove et al. (2007), highlighted the importance of keystone ant species in plant dispersal.

In the face of large scale habitat modifications, it is important to understand interspecific interactions (e.g. among plants and insects) that ultimately contribute to the structuring of plant populations. These interactions are not always beneficial to the plant. For example, certain ant species are more likely to remove the elaiosome in situ (cheating), without dispersing the seeds (Manzaneda et al. 2007, Jones et al. 2015). Subsequently, these seeds are less attractive for other ant dispersers (Cuautle et al. 2005), thus reducing the chances of seeds being dispersed into safe microsites (i.e. ant nest). Seeds can also be directly predated upon by ants (i.e. so-called seed harvester ants; Morton 1979, Buckley 1982, Andersen 1982, Andersen et al. 2000) or by other predators, such as rodents, birds (Morton 1985). However, this is not always the case as fleshy fruit removal resulted in decreased seed predation in one study (e.g. by deer, Fedriani and Delibes 2013).
Due to prevailing natural and/or anthropogenic disturbance regimes, seed dispersal processes may be disrupted (Crist 2009). Invasive species and edge effects may also lead to the further disruption of this mutualism (i.e. Christian 2001, Ness 2004, Ness and Morin 2008, Christianini and Oliveira 2013). Such changes in myrmecochorous systems have been reported at various spatial and temporal scales (Garrido et al. 2002, Heithaus and Humes 2003, Leal et al. 2013), where different ant assemblages can vary in their behaviour towards seeds (i.e. seed removal vs. cheating). As a result, ant-seed interactions can be species and habitat specific (Pudlo et al. 1980, Mitchell et al. 2002, Alba-Lynn and Henk 2010).

In Australia, to date, variability in ant-seed interactions has been largely investigated in the context of fire or mining disturbances (i.e. Majer 1982, Andersen and Morrison 1998, Parr et al. 2007). However, the effects of anthropogenic soil disturbance regimes, and its influence on important seed-dispersing mutualism, has been poorly studied. In many cleared and fragmented agricultural landscapes, soil disturbance regimes pervade most remnant ecosystems, via the ongoing use and maintenance of tracks and roads (Bognounou et al. 2009). Minor rural road networks dominate such landscapes, are frequently disturbed by soil disturbances from roadwork activities, but provide important refuge for remnant ecosystems and threatened native species (Bennett 1991, Forman and Alexander 1998, Spooner 2015). As such, roadside environments provide an ideal context to study the impact of soil disturbance on mutualistic interactions, such as myrmecochory.

The aim of this study was to investigate the influence of anthropogenic soil disturbances on seed dispersal processes by ants. More specifically, I addressed the
following questions: (1) To what extent do ants remove seeds as compared to other potential seed removers and/or predators (i.e. rodents, small mammals) in roadside environments? (2) How does soil disturbance influence seed removal rates and ant-seed behavioural interactions? and (3) Which are the main seed-dispersing ant species in roadside habitats?

4.2 METHODS

Description of study area is provided in Chapter 2. In brief, the study sites were located in a typical agricultural landscape in southern NSW, in the Lockhart Council local government area, where much of the landscape was cleared and ‘intact’ remnant woodlands persist along ‘road reserves’ (Lunt and Bennett 2000). The research was confined to this area to ensure that a consistent approach to road management was applied across the study area.

4.2.1 Exclusion experiment - seed removal by ants vs. vertebrates

Exclusion experiments were performed to determine the main seed removalist(s), and establish if ants compete with other potential seed removers and/or predators in roadside habitats. Seeds of Acacia pycnantha (a common myrmecochore plant in the selected roadsides) were harvested prior the experiment from the study area
(outside of the experimental study sites, November 2014) and the experiment took place during December 2014, coinciding with high ant activities. Recordings of seed removals were conducted for three treatments:

- **vertebrates excluded**: Ants and other potential invertebrates could access the seeds, but vertebrates were excluded. Seeds were placed in a Petri dish, which was covered by 1 x 1 cm wire mesh, dimensions 20 x 20 x 15 cm, to cover the dish.

- **invertebrates excluded**: A smaller sized Petri-dish was placed in the middle of a larger dish with Tanglefoot® applied between the rims of the two dishes, so only vertebrates could access the seeds. Tanglefoot is an insect trapping adhesive, a non-drying sticky “glue”, which have been commonly used in similar experimental setups (e.g. Hughes and Westoby 1990, Thomson et al. 2016).

- **control**: seeds were placed in a Petri-dish that was not covered with neither a wire mesh nor coated with Tanglefoot®, therefore giving free access to both vertebrates and invertebrates. The bottom of each Petri-dish was also scraped with sandpaper to reduce slipping on the surface.

Each treatment was replicated three times at each site (n = 20), with seed depots placed 5 m apart in random order along a transect placed along the road verge. Seed depots were open and exposed to seed removals for 12 hours, during the day (8:00 am to 8:00 pm) and at night (8:00 pm to 8:00 am). 10 seeds were placed at the start of the experiment at each depot and the remaining number of seeds was counted at the end of each period.
4.2.2 Ant-seed interactions

A cafeteria style experiment (Chapter 2) was carried out to make observations of ants interacting with seeds. Two transects were set up at each site and seed depots (n = 6) were monitored two times a day, in the morning (8:00 – 12:00) and in the afternoon (14:00 – 18:00). Each seed depot initially contained 10 seeds, which were offered to ants at the start of each observational period. During the 4 hour observational period, two people walked along each transect, and randomly selected depots to perform 10min observations to maximise the chances of observing ants interacting with seeds. The following data were recorded: (1) number of seeds removed by the end of the 4 hour session, or if all seeds disappeared sooner, the time when all seeds were removed; and (2) the nature of ant-seed interactions (‘removal’ or ‘cheating’).

A seed removal event was determined when ants removed seeds at least 5 cm away from the edge of the seed depot, and then (usually) carried these seeds to their nests (Chapter 5). When an ant started chewing on the elaiosome, without removing the seed itself, this behaviour was categorised as cheating. The number of seeds ants cheated upon or removed, and the number of cheating individuals was recorded. A seed sometimes experienced both interaction types. If a cheating behaviour was observed first, but it was subsequently removed by the same or a different individual, this was categorised as only seed removal.
Furthermore, two types of cheating occurred: a) individual cheaters and b) those that recruited other nest mates on seeds (to consume the elaiosome in situ). In the former case, the behaviour was classified as cheating if an individual belonging to the same species handled a seed or multiple seeds 10min apart. For the latter category, this often occurred when smaller bodied species interacted on a seed or on multiple seeds at any given seed depot, and their numbers usually increased over the observation period (i.e. max. 4 hours). Recruiting behaviour implies that a number of individuals recruit to a food source where fragments of food (in this case the elaiosome) may be consumed in situ or can be transported back to the nest provided the nest is situated in the vicinity (Planque et al. 2010). In this case, the maximum number of seeds that a particular species was cheated on was included in the statistical analysis (below).

4.2.3 Data analyses

The exclusion experiment

The influence of invertebrates (ants) and vertebrates (i.e. rodents, birds) on the number of seeds left at depots after each session was tested using generalized linear mixed model with repeated measures. Sites were treated as random effects, whereas day and night period was included as repeated measures. Least square
means were generated to graphically present differences in seed removals between the treatments (ant-access, vertebrate-access or both).

**Seed removal**

Seed removal rate was calculated as the number of seeds removed (max = 10) at any given depot during the 4 hours cafeteria experiments and was divided by the time when all seeds disappeared (time units are minutes). If there were seeds left at the depot by the end of the session the number of seeds removed was divided by the end of the observational period (i.e. 240 min). Seed removal rate data was then averaged at the transect (zone) level for each site. The difference in the mean seed removal rates between differing zones and roadside widths was then analysed using a mixed linear model with sites as random effects nested within roadside width, with width (narrow or wide) and zone (disturbed or not disturbed) as fixed factors.

The difference in the percentage of total number of seeds removed (regardless if a seed was seen to be removed or not) between zone, time and width was analysed using generalized linear model with a binomial distribution and a logit link function. Sites were included as random effects and zone and time of sampling as repeated measures within site. Sites were nested within width, and width and zone were included as fixed factors.
Ant-seed interactions

To test for differences in the proportion of observed ant-seed interaction behaviours (seed removal and cheating) between zone, time and width, a further generalised linear model with a binomial distribution and a logit link was fitted. Sites were nested within width, and width and zone (disturbed or not disturbed) were included as fixed factors. For these analyses, the sum of each behavioural type was divided by the sum of the total ant-seed interactions.

The contribution of ant species to the total number of interaction types, in either disturbed or non-disturbed zones, and for different roadside widths (narrow or wide) was calculated from the sum of all interaction behaviours for a given ant species for each site and these site values were then averaged. The percentage contribution that each species contributed to either seed removal or cheating was also calculated and presented graphically.

All analyses were run using SAS/STAT® (SAS 2013) within the SAS Studio (SAS 2015) environment using the SAS university edition license.
4.3 RESULTS

4.3.1 The exclusion experiment

There was a significant difference in the number of seeds left at depots between different treatment types ($F = 197.59, df = 2,38, p < 0.001$), where the vertebrate-only access depots contained significantly more seeds than the other two treatments (Figure 4.1). There was almost no seed removal from seed depots where ants were excluded (vertebrate-access only), while an average of 8 of the 10 seeds disappeared from control and ant-access depots. Most seeds were removed from the control and ant-access treatments by the end of the 12 hour observational sessions, showing that ants are the main dispersal agent of *Acacia* seeds.
4.3.2 Seed removal rates

Most seeds were removed during the cafeteria style experiment (disturbed zone = 83.6%; non-disturbed zone = 85.6%). The rate at which seeds were removed from seed depots (i.e. measured in a 4-hour period) did not significantly differ between disturbed and non-disturbed zones, narrow and wide roadsides or their interaction terms ($p > 0.5$). On average, seeds disappeared within the first two hours of the experiment in both zones (mean $\pm$ SE = $121 \text{min} \pm 4.91$).
There was a significant interaction between disturbance zone and roadside width on the proportion of total seeds removed ($Z = 2.12$, $p = 0.03$). In narrow roadsides, a greater percentage of seeds were removed from disturbed zones, while in wide roadsides non-disturbed zones experienced a larger percentage of seed removals (Figure 4.2).

**Figure 4.2.** Percentage of total seeds removed during the cafeteria style experiment in disturbed (filled bars) and non-disturbed (unfilled bars) zones of narrow and wide roadsides. Total seeds removed are considered, regardless if the ant dispersers were seen or not.
4.3.3 Ant-seed interactions

A total of 5760 seeds were offered to ants using the cafeteria style experiments, where 56% of seeds were observed being taken away from depots, whilst for 13% (n=983) the elaiosomes were consumed *in situ* (cheating). On a few occasions, invertebrates other than ants were also observed interacting with seeds (i.e. cricket), however these events were not included in the analyses. There was a significant difference in the proportion of seed removals ($Z = -3.04, p = 0.002$) and cheating interactions ($Z = 2.43, p = 0.01$) between the disturbed and non-disturbed zones (Figure 4.3).

![Figure 4.3](image_url)  
*Figure 4.3. Mean percentage of observed ant-seed interactions, seed removal (n = 2442) and cheating (n = 983), in disturbed (filled bars) and non-disturbed (unfilled bars) zones (combined for all roadside width). Error bars represent standard error of the mean.*
Twenty-six ant species were observed performing ant-seed interactions, belonging to 12 genera and 5 subfamilies (*Formicinae, Dolichoderinae, Ectatomminae, Myrmicinae* and *Ponerinae*; see Appendix 1 for species list). Six ant species were responsible for 83% of total observed ant-seed interactions (Table 4.1), however their individual contribution to either seed removal or cheating varied according to disturbance zone and roadside width configuration (Figure 4.4).

*Iridomyrmex purpureus* contributed to almost one quarter of the total ant-seed interactions, and was more active in disturbed than in non-disturbed zones (27% and 21% of total ant-seed interactions, respectively (Table 4.1). However, in wide roadsides it was observed removing more seeds in non-disturbed zones (Figure 4.4a). *I. purpureus* frequently displayed both types of behaviour, with a greater overall ratio of seed removals (80%, Table 4.1). *I. rufoniger* also displayed both seed removal and cheating behaviours, but to a smaller extent as compared to *I. purpureus*. *I. rufoniger* appeared to have an affinity to narrow roadsides (Figure 4.4a-b).

Although *Rhytidoponera metallica* was the second most interactive species (21% of all interactions), it almost exclusively participated in seed removal behaviour. The highest frequency of seed removals was carried out by *R. metallica* (29%) with cheating equating to only 1.4% of all its interactions (Table 4.1). *R. metallica* showed a higher overall activity in non-disturbed zones, with seed removals almost twice that in narrow as compared to wide roadsides (Figure 4.4a).
Melophorus bruneus exclusively performed seed removal events (9.13% of all seed removals, Table 4.1), with most activity recorded in soil disturbed zones (Figure 4.4). No cheating events were observed for *M. bruneus*. Almost half of the total cheating activities were performed by two Monomorium species (*M. rothsei* and *M. sydneyense*), while their combined contribution to seed removal was as low as 2%. 95% of the total activity by *M. rothsei* was limited to disturbed zones with much higher affinity shown for wide roadsides (Figure 4.4b). Combined data for all other ant species (‘Others’) showed greater affinity towards non-disturbed zones (Figure 4.4a and b).
Figure 4.4. The percentage contribution of ant species to seed removal (a; n = 2442) and cheating (elaiosome consumption) interactions (b; n = 983) in disturbed (D) and non-disturbed (ND) zones of narrow (n) and wide (w) roadsides.
Table 4.1 The percentage contribution of ant species to ant-seed interactions (R = seed removal, n = 2442; and CH = cheating, n = 983), and frequency (%) of behaviour in disturbed and non-disturbed zones, and for the two zones combined (Total).

<table>
<thead>
<tr>
<th>Ant species</th>
<th>Ant-seed interaction (%)</th>
<th>Behaviour (%)</th>
<th>Ant-seed interaction (%)</th>
<th>Behaviour (%)</th>
<th>Ant-seed interaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DISTURBED</td>
<td></td>
<td>NON-DISTURBED</td>
<td></td>
<td>TOTAL</td>
</tr>
<tr>
<td>Iridomyrmex purpureus</td>
<td>27.8 R = 80.67</td>
<td>Ch = 19.33</td>
<td>21.19 R = 80.72</td>
<td>Ch = 19.28</td>
<td>24.5</td>
</tr>
<tr>
<td>Iridomyrmex rufoniger</td>
<td>14.6 R = 76.40</td>
<td>Ch = 23.60</td>
<td>15.41 R = 64.77</td>
<td>Ch = 35.23</td>
<td>15.01</td>
</tr>
<tr>
<td>Rhytidoponera metallica</td>
<td>14.66 R = 99.60</td>
<td>Ch = 0.40</td>
<td>28.02 R = 97.29</td>
<td>Ch = 2.71</td>
<td>21.34</td>
</tr>
<tr>
<td>Melophorus bruneus</td>
<td>9.29 R = 100</td>
<td>Ch = 0</td>
<td>3.74 R = 100</td>
<td>Ch = 0.00</td>
<td>6.51</td>
</tr>
<tr>
<td>Monomorium sydneyense</td>
<td>9.52 R = 8.59</td>
<td>Ch = 91.41</td>
<td>7.18 R = 8.13</td>
<td>Ch = 91.87</td>
<td>8.35</td>
</tr>
<tr>
<td>Monomorium rothseieni</td>
<td>13.67 R = 9.83</td>
<td>Ch = 90.17</td>
<td>0.7 R = 50</td>
<td>Ch = 50</td>
<td>7.18</td>
</tr>
<tr>
<td>Others</td>
<td>10.46 R = 73.18</td>
<td>Ch = 26.82</td>
<td>23.76 R = 68.55</td>
<td>Ch = 31.45</td>
<td>17.11</td>
</tr>
</tbody>
</table>
4.4 DISCUSSION

4.4.1 Seed removal by ants vs. vertebrates

The exclusion experiments revealed that ants were the main dispersers of Acacia pycnantha seeds in roadside environments. The mean number of seeds removed from control depots versus ant-access depots was very similar, while no removals occurred from seed depots where ants were excluded, which suggests that there is an effective seed removing ant assemblage present in roadsides. This result lends support to the view that seed predation by vertebrates (i.e. rodents) does not pose a threat to seeds in most Australian ecosystems (Morton 1985, Horvitz and Schemske 1986, Smith et al. 1989). However, recent studies have found contrary results in habitats at different altitudes, with vertebrates removing more seeds in higher compared to lower elevations in the Blue Mountains, Australia (Thomson et al. 2016). Here, the authors hypothesized that plants may gain additional benefits by an alternate dispersal (or predator) vector through, for example, increased dispersal distance.

It is possible that the presence of vertebrate seed predators differs between and within different regions in Australia. Birds are thought to be important granivores (seed predators) in arid and semi-arid environments (Morton 1979), however their role in other parts of the continent is not well understood (Hughes and Westoby 1990). Furthermore, the decline and extinction of numerous native mammals in the past 200 years (Short and Smith 1994) might have resulted in the
loss of potential mammalian predators of Australian myrmecochores. Roadside environments provide important habitats for small mammal populations (Bennett 1990, Carthew et al. 2013), although non-native small mammals may outcompete native species (Donaldson and Bennett 2004). Therefore, further studies are required of potential seed predation by either native or non-native vertebrates along roadsides.

Sampling time was not a significant factor in determining the number of seeds left at the depots, which reveals the level of foraging activity that ants perform throughout the day. Such activities encompass a broad range of time periods, both diurnal, and in terms of seasonality, highlighting the robustness of their seed removal abilities (Horvitz and Schemske 1986, Ness and Bressmer 2005). Seed removal experiments by ants have rarely been performed at night time due to difficulties in tracking ants in the dark. Therefore, it is possible that seeds taken during the ‘night’ period, as recorded in this study, might have been removed just before the onset of the dark, or early in the morning.

4.4.2 Ant-seed interactions

Direct observations on ants interacting with seeds revealed an effective assemblage of seed-dispersing ants with occasional mutualism cheaters. Seeds were visited by a number of ant species which employed a variety of behaviours, depending on species identity and habitat configuration. Although there was little segregation in
overall ant-seed interactions between treatments, the examination of the constituent elements (i.e. proportion of observed seed removals and elaiosome consumption) did show varying responses to soil disturbance.

In general, the proportion of observed seed removal events was greater in non-disturbed roadside areas. However, the statistically significant interaction found between disturbance zone and roadsides width reflects a more complex pattern. Seed removal was similar in both disturbed and non-disturbed zones in narrow roadsides, as compared to wide roadsides, where seed removal was greater in non-disturbed areas (Figure 4.2). Individual species results show that these differences are driven by the interplay between the two most commonly recorded ant species. In non-disturbed areas of narrow roadsides, the increased activities of *Rhytidoponera metallica* paralleled the reduced activities of *Iridomyrmex purpureus* in this zone. In wide roadsides, the reduced activities of *Rhytidoponera* in disturbed zones was a strong driver of overall lower levels of seed removal events (Figure 4.4a).

This result can be partly explained by habitat preferences of individual ant species (Chapter 3). For most ants, complex habitat structures make foraging more difficult, especially for larger bodied species (Gibb and Parr 2010). In this study, the verges of narrow roadsides were often dominated by dense swards of tall perennial grasses (Chapter 3), creating a largely composite landscape from an ant’s perspective. Therefore, it is not surprising that *Iridomyrmex*, a large bodied species, carried out more seed removals from the disturbed zones of narrow roadsides. Indeed, *Iridomyrmex* prefer more open and well sunlit conditions (Greaves 1971,
Andrew et al. 2013). In contrast, those habitats unfavoured by *Iridomyrmex* seemed to be more advantageous to *Rhytidoponera*; as shown by observed removal activities. *Rhytidoponera* is a widespread genus, occurs in a wide range of habitats (Shattuck 1999) and is recognised as a disturbance specialist (e.g. Hoffman and Andersen 2003). The pattern found in this study, however, stands in stark contrast with this view, revealing that factors other than habitat conditions may be responsible for the patterns observed.

According to Andersen (1992), ant community composition at any given habitat may be influenced by competitive interactions among species. *Rhytidoponera* prefers to avoid encounters with the dominant *Iridomyrmex* (e.g. Lubertazzi et al. 2010), however its subordinate behaviour allow the co-existence of the two species at the same habitats. Nevertheless, *Rhytidoponera* was responsible for a greater percentage of removal activities in non-disturbed zones, where the dominant species was less abundant. Therefore, the occurrence of both species in the same habitat, and frequently at the same seed depots, could be attributed to differences in their position in the competitive hierarchy (Gibb 2005, Mitchell et al. 2002). Indeed, different ant species were often observed at the same seed depots and such occasions gave insight into their competitive behaviour. For example, when a dominant *I. purpureus* individual encountered another species, which was trying to remove an offered seed from the depot, the former always robbed the seed from the latter. Nevertheless, *R. metallica* was always quick at removing seeds and thus often escaped of being robbed, hence proving its subordinate behaviour.
The complementary pattern of *Iridomyrmex* and *Rhytidoponera* is further evidenced by the high identical seed removal rates between the two zones. Most seeds disappeared at a similar rate, within an average of two hours. Seed removal rates in other studies was also found to be rapid, especially in the first few hours (Beaumont et al. 2012), regardless of habitat disturbance types (Andersen and Morrison 1998). However, Beaumont et al. (2011) found higher removal rates in recently burnt habitats, which mainly occurred due to the presence of another important mutualist partner - *Anonychomyrma* nr. *nitidiceps*, which was facilitated by the prevailing habitat conditions.

Another efficient seed removalist in this study was *Melophorus bruneus*, which exclusively performed seed removal events (no cheating). *Melophorus* is a Hot Climate Specialist (Andersen 1995), and were primarily recorded removing seeds in disturbed zones in the early afternoon period when most other species were inactive. *Melophorus* spp., along with *Rhytidoponera*, are considered beneficial (keystone) mutualists in many Australian ecosystems (Gove et al. 2007, Majer et al. 2011). Ultimately, the large number of seed removal events which occurred within a short period of time, regardless of habitat disturbance, reveals the high dependence of myrmecochores on their mutualistic insect partners in this region and elsewhere (e.g. Berg 1975).

The exploitation of mutualistic interactions, in general, is a risk for plants which may confer no benefits (Bronstein 2001). By consuming the elaiosome, cheating ants remove the food reward from seeds without providing an effective dispersal service, thus reducing the chances of these seeds taking part in further
dispersal events (Auld and Denham 1999, Cuautle et al. 2005) and increasing predation (Christian 2001, Ness and Morin 2008). Reduced advantages of partial or complete removal of the reward have been shown in elaiosome removal experiments (e.g. Garrido et al. 2002), yet small remains of the elaiosome on seeds may still enhance the probability of subsequent seed removal (Bas et al. 2009). In this study, elaiosome consumption by ants (cheating) was greater in soil disturbance zones of roadsides. Smaller bodied Monomorium and Pheidole species, but also two Iridomyrmex species, were mainly responsible.

The reason for cheating behavioural differences between the zones remains unclear, but it is possibly influenced by habitat and commensurate foraging differences of ant species. Other studies have also recorded a high prevalence of cheating behaviour by small bodied ant species (Manzaneda et al. 2007, Harris and Standish 2008, Beaumont et al. 2011). The disruptive effects which can occur on this mutualism include behaviours to actively interfering with seed transport (Leal et al. 2014) or by eliminating important seed dispersers (Christian 2001). In this study, Iridomyrmex and Rhytidoponera were frequently observed robbing seeds from “cheaters” while they were feeding on elaiosomes in situ. Therefore, some seeds involved into cheating (and recorded as such), were actually removed to another site in due course. A rather complex seed dispersal service!
4.4.3 Conclusion

Soil disturbance and roadside verge width influenced ant-seed interactions, the response to which was largely explained by individual species responses to disturbance. The nature of behavioural interactions by commonly observed ant species with seeds explained variations in seed removal rates and elaiosome consumption (cheating behaviour) between disturbed and non-disturbed zones.

The interplay between the benefits (seed removal) and costs (cheating and/or seed predation; Arnan et al. 2012) shape the importance of this mutualism to plants. The extent to which soil disturbance, via mutualistic ants and their seed-dispersing behaviours, influences subsequent patterns of seedling germination and establishment requires further investigation. A greater likelihood of seed removal event confers positive benefits to plants, for example in terms of short- and long-distance dispersal services.
Chapter 5: Seed dispersal mechanism by ants in roadside environments
Chapter 5: Seed dispersal mechanism by ants in roadside environments

Abstract

Ants provide a common dispersal vector for a variety of plants in many environments through a process known as myrmecochory. The efficacy of this dispersal mechanism can largely determine the ability of species to track changes in habitat availability caused by ongoing land-use and associated disturbances, and can be critical for population gene flow and persistence. Field studies were conducted in a typical fragmented agricultural landscape in southern NSW, Australia, to investigate the extent to which dispersal services by ants are influenced by anthropogenic disturbances associated with roadwork activities. Observational experiments were performed in road segments that were divided into disturbed and non-disturbed zones, where *Acacia pycnantha* seeds were offered at multiple seed depots and monitored. For combined species, the mean dispersal distance recorded in the disturbed zone (12.5m) was almost double that recorded in the non-disturbed zone (5.7m) for all roadside sites. The findings show that myrmecochory is an unevenly diffuse mutualism, where few ant species contributed to much of the dispersal of seeds. *Iridomyrmex purpureus* was responsible for all seed dispersal distances > 17 m, where a maximum of 120 m in disturbed versus 69 m in non-disturbed zones, was recorded. *Rhytidoponera metallica* and *Melophorus bruneus* were important seed dispersers in non-disturbed and disturbed zones, respectively. In general, large bodied ants tended to
move more seeds to longer distances in disturbed zones, as opposed to non-disturbed zones, where smaller bodied species carried out a greater percentage of short-distance dispersals (< 1m). I also recorded secondary dispersal events from nests by *I. purpureus*, a phenomenon previously not quantified for this species. Infrequent, long-distance dispersal to suitable sites may be highly important for seedling recruitment in disturbed or modified habitats in otherwise highly fragmented rural environments.

5.1 INTRODUCTION

For many plants, seed dispersal mechanisms following the end of the reproductive cycle are critical for the establishment of future offspring (Wang and Smith 2002, Vander Wall and Longland 2004). Plants use various strategies to disperse their propagules into new habitats, such as by wind, water, vertebrate or ants (van der Pijl 1982, Nathan and Muller-Landau 2000). Dispersal distances can range from 1 m’s (ants) to 100’s km’s (wind) (Corlett 2009, Thomson et al. 2010). However, the limited dispersal distances provided by some dispersal agents (i.e. ballistic and/or ant-mediated) may restrain the extent to which plants may colonize new habitats. As a result, recruitment limitations can occur, particularly in habitats subjected to frequent disturbance activities (i.e. natural and/or anthropogenic). This issue can affect population growth and persistence, unless other forms of infrequent long dispersal events occur (Cain et al. 2000, Nathan et al. 2008).
Seed dispersal by ants (myrmecochory) is considered a globally significant driver of plant diversity and population dynamics (Lengyel et al. 2010). Myrmecochorous plants dominate communities in many habitats, in terms of both species richness and abundance (Handel et al. 1981). There are numerous ant species that remove and transport seeds, however most dispersal events are performed by a few key removers (i.e. Andersen 1988, Gove et al. 2007, Zelikova and Breed 2008, Beaumont et al. 2011). Ants generally disperse seeds only short distances to their nests to feed to their larvae (Culver and Beattie 1978, Hughes and Westoby 1992, Rowles and O’Dowd 2009). However, infrequent long-distance dispersal events can also occur by ants (Andersen 1988). Maximum recorded seed dispersal distances are 77 m (Davidson and Morton 1981) and 180 m (Whitney 2002); both recorded in Australia.

The average dispersal distance and the overall shape of the dispersal curve play a crucial role in the rate of colonization of propagules to new sites (Andersen 1988, Portnoy and Willson 1993). Recent studies have also highlighted that myrmecochory can be a multiphase process, where after an initial transport to a nest, seeds may then be discarded away from the nest via a secondary dispersal process (Beaumont et al. 2012). Ants may remove seeds to nearby rubbish heaps (Berg 1975) or be relocated further distances away from nest entrances (Hughes and Westoby 1992, Lubertazzi et al. 2010, Canner et al. 2012). On most occasions, the elaiosomes of the discarded seeds are removed (Hughes and Westoby 1992, Canner et al. 2012), which assists with breaking seed dormancy and influence

Infrequent, long-distance dispersal events by ants, and secondary seed discarding behaviour, can have various implications for the fate and distribution of seeds. The extent of this dispersal service is strongly tied to the composition (Chapter 3) and behaviour (Chapter 4) of the ant species involved, and is influenced by prevailing disturbance regime(s) (i.e. Beaumont et al. 2012). Disturbances such as fire and soil disturbances can lead to increased dispersal distances, possibly due to the simplification of the foraging landscape for ants (Parr et al. 2007). In many habitats, increased anthropogenic activity poses a threat for many species, where understanding the factors that influence ant-plant interactions and seed dispersal into new environments is critical for conservation management (Thomson et al. 2010, Sorrells and Warren 2011).

I investigated seed dispersal processes and secondary seed relocation in relation to soil disturbances. I undertook investigations in roadside environments in southern NSW, Australia, which provide refuge for endangered woodland communities and associated species (Benson 1991, Schabel and Eldridge 2001). These novel environments are maintained by anthropogenic inputs which can influence seed dispersal, recruitment and overall plant persistence, depending on their life-history traits (Lugo and Gucinki 2000, Gelbard and Belnap 2003, Spooner 2005a). The main objectives of the study were: (i) to investigate the influence of soil disturbance on seed dispersal distances, (ii) identify the relative contribution of
individual ant species to this process, and (iii) determine the extent of secondary seed dispersal performed by ants.

5.2 METHODS

5.2.1 Seed dispersal distances

To assess ant dispersal distances, cafeteria style experiments were carried out in the selected roadside sites (see Chapter 2 for more details). Seed depots of approximately 9 x 9 cm in size were placed at 5 m intervals along two 25 m transects, located parallel to each other in both the disturbed and non-disturbed zones of the selected roadside sites (separated longitudinally by a 10 m gap). At each depot, 10 *Acacia pycnantha* seeds were placed on the ground, which two observers then monitored to record ant-seed interactions at each site. Using this method, the observation of all ant-seed interactions at seed depots was not possible. However, efforts were made to maximise the number of observations in a given time frame (maximum 4 hours or until all seeds were removed).

A seed dispersal event was considered when ants removed seeds at least 5 cm away from the edge of the seed depot, and subsequently, these ants were followed until a destination point was reached. Dispersal distances were measured from the respective seed depot to a given nest (or drop point) using a 50 m tape measure. The fate of dropped seeds (if they were further removed or not) was not
monitored, however the dispersal distance of such seeds was also recorded. As *Iridomyrmex purpureus* were common and dispersed seeds often great distances, I did not directly measure every dispersal event performed by this species to its termination point. Rather, the first *I. purpureus* individual transporting a seed from the seed depot was followed until it reached a particular nest. *I. purpureus* nests are large and conspicuous, where individual nests are normally situated sufficiently far from each other. Following individuals heading towards the same nest site were then only monitored for approximately 5 m from the seed depot, until it could be assumed it would complete the journey to this nest (and this distance recorded). Previous trial experiments proved that a large percent (> 90%) of *I. purpureus* individuals that carried the offered seeds beyond 5 m from the seed depot would reach their nests without dropping them. This method was developed in order to maximise the number of observations of ants at the seed stations during the survey period. All other dispersal events by other ant species were monitored to their final destination, and the distance recorded.

5.2.2 Secondary seed dispersal events - relocation distances from ant nest entrances

To investigate secondary seed dispersal events, I focussed on *Iridomyrmex purpureus* nests, which were abundant in the sampled roadsides. I randomly selected a total of 34 independent *I. purpureus* nests (disturbed n=14; non-
disturbed n=20) at the combined roadside sites. *Acacia pycnantha* seeds were again used to perform these observations, which were collected during the ripening period of 2014 November (prior to the relocation experiment in December 2014). Observations were conducted in the morning period between 9.00 and 12.00 hours when most ant activity occurs. As *I. purpureus* builds large and conspicuous nests which have numerous entrances, I randomly placed 15 individual seeds/nests approximately 5 cm from different nest entrances. Trial experiments showed that seeds placed in the proximity of nest entrances would cause worker ants discard seeds to rubbish heaps or in the surrounding environment instead of taking these seeds inside their nests. The distance ants carried these offered seeds were used to evaluate seed relocation distances from ant nests (see also Beaumont et al. 2013). Elaiosomes were previously detached of seeds to stimulate seed manipulation behaviour of ants that often induces seed discarding behaviour from ant nests (e.g. Martins et al. 2006). Seeds were placed individually to a random entrance and if an ant displaced the seed the distance was recorded.

### 5.2.3 Data analyses

The difference in mean dispersal distances between disturbed and non-disturbed zones and roadside width was assessed using mixed models (factorial ANOVA), where zone and width were entered as fixed factors, and sites as random factor. To ensure equal weights to each station rather than number of seeds, I first calculated the average dispersal distance for each depot then calculated the average for each
zone and site to get the site average distance. Data was log transformed if the assumption of normality was not met. As *I. purpureus* contributed to all dispersal distances > 17m, I used the same statistical configuration to test for differences in mean dispersal distance with data for *I. purpureus* omitted.

Differences in dispersal distances between zones and widths were investigated for species with sufficient dispersal measurements, or more precisely when a species was present in both zones of a site (at least 10 sites). Due to the paired nature of the study design, sites were not included unless dispersal distances were recorded in both zones. Paired T-test or Wilcoxon Signed Rank test was used if the data were normally or not normally distributed, respectively. Descriptive statistics were used to describe differences in mean dispersal distances achieved by respective species in differing zones and roadside widths.

A frequency histogram of dispersal distances was constructed to generate a dispersal curve, by calculating dispersal frequencies for each zone within each site, then a single composite histogram was aggregated by giving equal weight to each site. By doing this I was able to take site variability into account where the number of dispersal events greatly varied due to the patchy nature of ant activities (Nelson 2014).

In order to ascertain differences in secondary dispersal distances by *I. purpureus* between disturbed and non-disturbed zones, I calculated the average relocation distance for each *I. purpureus* nest and used a paired Student’s t-test with Satterthwaite’s correction to compare relocation distances between disturbed
and non-disturbed zones. All statistical tests were carried out using R statistical software (R Development Core team 2013).

5.3 RESULTS

5.3.1 Dispersal distances in relation to anthropogenic soil disturbance

Overall, mean seed dispersal distances were significantly influenced by soil disturbance (zone) \((F = 4.9, \text{ df} = 1, 22, p = 0.03)\) and roadside width \((F = 6.1, \text{ df} = 1, 22, p = 0.02)\) (Figure 5.1). For combined species, the mean dispersal distance recorded in the disturbed zone was almost double to that recorded in the non-disturbed zone \((12.5 \text{ m vs } 5.7 \text{ m}, \text{ respectively})\) for all road widths combined, while wide roadsides had longer dispersal distances than narrow roadsides. Nevertheless, narrow roadsides had both the longest \((14.5 \text{ m})\) and shortest \((2.9 \text{ m})\) mean dispersal distances in the disturbed and non-disturbed zones, respectively, while there was no marked difference in dispersal distances between disturbed and non-disturbed zones in wide roadsides (Figure 5.1).

The main seed-dispersing ant genera observed were *Iridomyrmex* \((43\% \text{ of dispersal events})\), *Rhytidoponera* \((37\%)\), and *Melophorus* \((10\%)\) (Table 5.1). Overall, 19 ant species contributed to the dispersal of seeds in roadside environments, with *I. purpureus* and *R. metallica* dominating the seed dispersal activities \((31\% \text{ and } 28\%)\).
respectively; data combined across sites and zones). When mean dispersal distances for combined species were re-analysed by omitting *I. purpureus*, there were no significant differences in mean distances between road disturbance zone ($F = 0.73$, df = 1,22, $p = 0.4$), road verge width ($F = 2.02$, df = 1,22, $p = 0.16$), or by the interaction effect ($F = 2.33$, df = 1,22, $p = 0.14$).

**Figure 5.1.** Mean dispersal distances (m) carried out by seed-dispersing ant species in disturbed (D) and non-disturbed (ND) zones in narrow (n = 579 in D, n = 376 in ND) and wide roadsides (n = 442 in D, n = 553 in ND) in the Lockhart Shire, southern NSW. Error bars represent standard error of the mean.
Table 5.1. Number of seed dispersal events, number of sites each species occurred at (n=24), mean and maximum dispersal distances, recorded for ant species in disturbed and non-disturbed roadside sites in southern NSW, Lockhart Shire.

<table>
<thead>
<tr>
<th>Ant species</th>
<th>Disturbed zone</th>
<th>Non-disturbed zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>dispersal</td>
<td>distance (m)</td>
</tr>
<tr>
<td>Number of sites</td>
<td>events (No. of sites)</td>
<td></td>
</tr>
<tr>
<td>Iridomyrmex purpureus</td>
<td>352 (19)</td>
<td>20.7</td>
</tr>
<tr>
<td>Iridomyrmex rufoniger</td>
<td>157 (13)</td>
<td>0.4</td>
</tr>
<tr>
<td>Rhytidoponera cristata</td>
<td>57 (13)</td>
<td>5</td>
</tr>
<tr>
<td>Rhytidoponera sp. A (convexa group)</td>
<td>18 (4)</td>
<td>1.7</td>
</tr>
<tr>
<td>Rhytidoponera metallica</td>
<td>266 (18)</td>
<td>1.4</td>
</tr>
<tr>
<td>Melophorus bruneus</td>
<td>132 (15)</td>
<td>2.1</td>
</tr>
<tr>
<td>Camonotus sp. 1</td>
<td>1 (1)</td>
<td>1</td>
</tr>
<tr>
<td>Camponotus sp. A (claripes group)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Camponotus obniger</td>
<td>4 (1)</td>
<td>1.5</td>
</tr>
<tr>
<td>Crematogaster sp. A</td>
<td>12 (1)</td>
<td>0.1</td>
</tr>
<tr>
<td>Melophorus sp. B (aeneovires group)</td>
<td>5 (4)</td>
<td>4</td>
</tr>
<tr>
<td>Meranoplus sp. A (group D)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monomorium sp. B (rothsteini group)</td>
<td>19 (3)</td>
<td>0.5</td>
</tr>
<tr>
<td>Monomorium sp. A (sordidum group)</td>
<td>14 (3)</td>
<td>0.1</td>
</tr>
<tr>
<td>Notoncus ectatomoides</td>
<td>2 (1)</td>
<td>0.2</td>
</tr>
<tr>
<td>Pheidole sp. A</td>
<td>11 (7)</td>
<td>0.2</td>
</tr>
<tr>
<td>Pheidole sp. B</td>
<td>8 (2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Camponotus aeneopilosus</td>
<td>2 (2)</td>
<td>11.5</td>
</tr>
<tr>
<td>Total</td>
<td>1051</td>
<td>12.3</td>
</tr>
</tbody>
</table>
There were no significant differences in the mean dispersal distances of individual species between disturbed and non-disturbed zones, and roads of different width (p > 0.05); nevertheless, there were trends in mean dispersal distances among species according to either disturbance zone and/or roadside width (Table 5.1, Figure 5.2, 5.3). *Iridomyrmex purpureus* dispersed the most seeds over the greatest distances (mean = 18.7 m, maximum = 120 m; Table 5.1). Their average dispersal distances were considerably greater than any other species’ (Figure 5.2 vs. 5.3) and reflect the overall pattern for combined species, where the mean seed dispersal distance was greatest in the disturbed zone of roadsides (Figure 5.3).

The mean dispersal distance for *Rhytidoponera cristata* for combined sites was 4.40 m, and was greater in disturbed zones for both wide and narrow roadsides (Figure 5.2, Table 5.1). In contrast, the mean dispersal distance for *Melophorus bruneus* was longer in the non-disturbed zone of both wide and narrow roadsides (Figure 5.2). *Rhytidoponera metallica* also followed this pattern for wide roadsides. Mean dispersal distances for *Iridomyrmex rufoniger* were much lower than any other species’ (0.60 m). Some ‘other’ species showed slight preferences for disturbance zone and/or roadside width, where mean dispersal distance for *Camponotus* species was much greater in the disturbed zone of narrow roadsides (Figure 5.2).
Figure 5.2 Mean dispersal distances for individual ant species (*I. purpureus* excluded) in disturbed (D, \(n = 669\)) and non-disturbed (ND, \(n = 677\)) zones in narrow and wide roadsides. Others = all other remaining species combined (see Table 5.1). Error bars represent standard error of the mean.
Figure 5.3 Mean dispersal distances (m) for Iridomyrmex purpureus in disturbed (D, n = 352) and non-disturbed (ND, n = 252) zones in narrow and wide roadside environments. Error bars represent standard error of the mean.

5.3.2 Frequency of seed dispersal events

A high frequency (64%) of recorded seed dispersal events was < 3m, where a further 20% of dispersals were carried out at distances ranging 10–40 m (Figure 5.4). The tail end of the dispersal curve shows small frequencies of long-distance dispersal events up to 120 m in the disturbed zone. In contrast, the maximum dispersal distance recorded in the non-disturbed zone was 70 m (Figure 5.5). The
relative high frequency of seed dispersal distances < 3 m was generally similar in both disturbed and non-disturbed zones, however dispersal events > 5 m tended to occur more frequently in the disturbed zone of roadside environments (Figure 5.4). The maximum distances recorded in this study was by *I. purpureus*: 69 m in non-disturbed and 120 m in disturbed zones.

![Dispersal distance and frequency](image.png)

**Figure 5.4.** Overall seed dispersal curve across all seed-dispersing ants. Seed dispersal distances are grouped in distance categories (m) in disturbed (D; n = 1021) and non-disturbed (ND; n = 929) zones of roadside environments in the Lockhart Shire, southern NSW.
Seed dispersal curves markedly differ between different species (Figure 5.6).

The shape of the curve is unique for each species and show a somewhat similar distribution between zones (Figure 5.5 - 5.6).

Figure 5.5. Seed dispersal curve generated by *Iridomyrmex purpureus* in disturbed (D; n = 352) and non-disturbed (ND; n = 252) zones, Lockhart Shire.
Figure 5.6. Seed dispersal curves for different ant species in disturbed (D) and non-disturbed (ND) zones, Lockhart Shire southern NSW. Sample sizes for each species are as follows: *R. metallica*: n = 236 in D, n = 311 in ND; *R. cristata*: n = 57 in D, n = 47 in ND; *M. brunues*: n = 127 in D, n = 62 in ND.
5.3.3 Secondary dispersal distances from nests

There was a significant difference in the secondary dispersal distances \textit{I. purpureus} carried out between disturbed and non-disturbed zones ($t = 4.12$, $df = 20.73$, $p < 0.001$; Figure 5.7).

\textbf{Figure 5.7.} Mean secondary dispersal distances performed by \textit{I. purpureus} colonies between disturbed (D, n=111) and non-disturbed (ND, n=169) zones in the Lockhart Shire. Error bars represent standard error of the mean.
5.4 DISCUSSION

The ant genera responsible for most seed dispersals were *Iridomyrmex*, *Rhytidoponera* and *Melophorus*. Other studies have also found that these genera are the most important dispersers involved in myrmecochory throughout Australia (Berg 1975, Andersen and Morrison 1998, Beaumont et al. 2012). In this study, *Rhytidoponera metallica* and *Iridomyrmex purpureus* were associated with the most seed dispersals (28% and 31% respectively), the latter with maximum dispersal distances. *Melophorus bruneus* carried out a substantial amount of seed dispersal events as well (10%). *Pheidole* is also a well-known seed-taking genus (Beaumont et al. 2012), however it was not a prominent disperser in this study. In general, these findings are consistent with the assertion that myrmecochory is an unevenly diffuse mutualism (Gove et al. 2007), that is few ant species contributed to much of the dispersal of seeds.

In a recent review of myrmecochory, Gomez and Espadaler (2013) found a global mean ant dispersal distance of 1.99 m, and greater mean dispersal distance in the Southern Hemisphere (3.71 m). I recorded dispersal distances much greater than this average (mean 6.26 m in non-disturbed and 12.38 m in disturbed zones) in roadside environments. In contrast, previous myrmecochorous studies (e.g. Andersen and Morrison 1988, Parr et al. 2007, Beaumont et al. 2009) have recorded much shorter mean distances. This finding can be explained by results for *I. purpureus* – which strongly influenced the overall findings, and differences in ant sampling methodology as compared to other studies.
I found that *I. purpureus* was responsible for all seed dispersal distances longer than 17 m (mean = 26.2 m, maximum = 120 m; Table 5.1). Such distances have been recorded in similar studies, but in more arid environments. Whitney (2002) recorded exceptionally long distances (180 m) based on direct observations; and more recently, Pascov et al. (2015) recorded a distance of 417 m using microsatellite markers and parentage assignments for seeds found in ant nest middens. Both studies included measurements on species from the *Iridomyrmex* genus in arid environments. This is the first study providing distances to such a degree outside of the Australian arid zones, which suggests that infrequent long-dispersal distances may not necessarily be restricted to this environment. With data for *I. purpureus* excluded, however, our mean dispersal distances resemble those of other studies of myrmecochory (as reviewed by Gomez and Espadaler 2013).

5.4.1 Benefits of soil disturbance to large bodied ants

Soil disturbance had a significant effect on seed dispersal distances for combined species, where mean distances were greater in disturbed as compared to non-disturbed zones. The mean seed dispersal distances for *I. purpureus* and *R. cristata*, two large-bodied ants (see below), was greater in the soil disturbance zone of roadsides. In contrast, mean dispersal distances for *R. metallic* and *M. bruneus* were greater in non-disturbed roadside environments. The remaining species
showed mixed responses, where few conclusions could be drawn in regard to the effects of soil disturbances.

Parr et al. (2007) found no effects of burning on mean dispersal distances at the species level - only in the case of an *Iridomyrmex* species. Likewise, Andersen and Morrison (1998) found that *Iridomyrmex* contributed to overall longer mean and maximum distances in relation to disturbance from mining activities. It has been suggested that the simplified and obstacle-free habitat conditions which exist post-disturbance, especially from an ant point of view, provide conditions commensurate for effective seed dispersal by larger bodied ants (Davidson and Morton 1981, Parr et al. 2007). Body size has been demonstrated to be a good proxy for assessing likely dispersal distances a species may be able to perform (Ness et al. 2004), and so does their foraging behaviour in search of food resources (e.g. Lubertazzi et al. 2010).

In contrast to results for large bodied ants, I found that a greater percentage of short-distance dispersals (< 1 m) occurred in non-disturbed versus disturbed zones (Figure 5.4), suggesting there is increased activity by smaller bodied species in non-disturbed roadside habitats (i.e. *Monomorium* and *Pheidole*). However, these smaller bodied species showed a relatively large percentage of short-distance dispersals in the disturbed zones as well. Therefore, forming generalisations in terms of soil disturbance effects on small bodied ants is difficult, as dispersal distance is largely a function of disperser identity (Andersen 1988, Gove et al. 2007).
Other authors have hypothesised that moderate levels of disturbances may assist in promoting services by seed-dispersing ants, by providing conditions suitable for the dispersal of seeds beyond the plant canopy. In contrast, however, severely disturbed sites are often coupled with low dispersal distances due to the predominance of small bodied ant species (Pudlo et al. 1980, Andersen and Morrison 1998). Given the predominance of large bodied species I recorded in disturbed zones, and commensurate large distances they dispersed seeds, suggests that the soil disturbance regime imposed in this study is infrequent, or an intensity of which these ants can still effectively nest and forage within.

5.4.2 *I. purpureus* activity in roadside environments

The distribution and density of ant nests can greatly determine the shape of the dispersal curve at any particular site (Andersen 1988). The overall pattern of the seed dispersal curve, with a long tail of infrequent but exceptionally long-dispersal distances, suggests low nest densities (Green 1983). Under such circumstances, the foraging effectiveness of dispersers becomes very important for persistence of plant communities in such environments.

Where *I. purpureus* were present nests were discovered in both the disturbed and non-disturbed zones. Seed dispersal distances by *I. purpureus* were strongly influenced by the spatial location of nests, as individual *I. purpureus* foragers returned to the same nest, or on few occasions to multiple nests (Pers.
This assertion is supported by our seed dispersal distance data (Figure 5.4), where high dispersal frequencies at 30-40m reflect the observed nest distribution patterns.

Field observations revealed that *I. purpureus* used the graded soil surface as a ‘runway’ to access resources at great distances, in both the disturbed and undisturbed areas. Complex vegetation structure is known to reduce the foraging speed and discovery of new resources by ants (Gibb and Parr 2010), therefore this form of soil disturbance appears to confer many advantages for *Iridomyrmex* to access resources in roadside habitats. Undoubtedly, open areas, such as those prevalent in roadsides, may confer advantages to *I. purpureus* in terms of suitable warmth, open conditions for nesting (Greenslade 1976).

The secondary seed dispersal activities I recorded for *I. purpureus* can be associated with increased distance related benefits for plants, providing seeds remain in viable condition after handling by ants. Whitney (2002) analysed the density of discarded seeds from *Iridomyrmex* nests (sometimes up to 25 m), and recorded a high seed viability ratio (40%). Furthermore, middens of *Iridomyrmex* nests were reported to provide conducive environment for seedling growth, which can facilitate range expansion of an invasive plant species in northern Australia (Bebawi and Campbell 2004). Other species were previously reported to discard seeds from their nests (Beaumont et al. 2013), and residual elaiosomes often remained on discarded seeds. These results highlight the nature of a highly complex mutualism, which occurs in Australian woodland environments, and potential seed dispersal services for plants.
5.4.3 Conclusion

As many landscapes have been cleared for agricultural purposes in Australia and elsewhere, roadside environments constitute an important landscape element by providing structural and functional connectivity for many plants and animals (e.g. Bennett 1990, Spooner 2015). Nevertheless, disturbances often occur in such environments, which can be both demoting or promoting, depending on the frequency and intensity of given disturbance regimes (e.g. Forman and Alexander 1998).

Soil disturbance appears to provide ideal foraging habitat for larger bodied ant species (e.g. *Iridomyrmex* spp.) to thrive. As a result, increased range expansion for myrmecochorous plants can occur through greater dispersal distances provided by such ants. The adaptability of residing ant species to soil disturbances, and their competitive interactions, will influence the success of this mutualism. For example, the seed dispersal and nesting activity of *Melophorus* and *Iridomyrmex* in soil disturbed zones show the adaptability of certain ant species to prevailing habitat conditions in roadside environments.

Infrequent, long-distance dispersal to suitable sites, may be highly important for seedling recruitment in disturbed or modified habitats (He et al. 2009, Giladi 2006). The extent to which various seed-dispersing ants contribute to plant population recruitment and structures requires further investigation of spatial analyses of patterns of seedlings establishment in relation to nest sites (e.g. spatial
point pattern analysis, Fedriani et al. 2010). Nonetheless, the potential benefits of multi stages dispersal processes may be particularly important for plants existing in fragmented environments where the maintenance of habitat connectivity may be crucial for overall population persistence.
Chapter 6: Synthesis of the results
Chapter 6: Synthesis of the results

“Now, rather than human development occurring in a matrix of natural landscape, natural areas occur in a matrix of human-dominated landscape.” (Harris and Scheck 1991, p. 189)

Seed dispersal is crucial for the structure, dynamics and persistence of plant populations. Mutualistic ants are important dispersers for many plant species, where this study sought to examine the role they play in the dispersal of Acacia pycnantha seeds (a common myrmecochorous shrub) in roadside environments. Previous research has investigated fire disturbance effects on ant communities, however the effects of soil disturbances has been poorly researched and is not well understood. In Australia and elsewhere, minor rural roads are frequently impacted upon by anthropogenic soil disturbance regimes (Spooner and Lunt 2004, Spooner et al. 2004), where roadside environments often provide refuge for many threatened species and remnant ecosystems. As discussed in Chapters 3-5, knowledge gained from these studies has provided new insights to the ecology of ants in temperate woodland ecosystems in south-eastern Australia, and the extent to which soil disturbances promote or demote short- and long-distance seed dispersal by ants.

As the findings show, the fate of invertebrate-dispersed seeds is largely determined by the identity and behaviour of the disperser, its response to soil disturbance regimes, and the habitat context in which ant-seed interactions occur. Effective seed dispersal by ants, and the dispersal mechanism in general, requires a number of successive steps from seed release to eventual germination. This
research focused on the initial steps of the process; the ant disperser community, seed removal rates, dispersal distances and the nature of ant-seed interactions – which are important parameters of myrmecochory.

The identity of seed-dispersing ants was first determined by carrying out direct observations of ants interacting with seeds, from which compositional and abundance data were constructed (Chapter 3). Based on previous evidence, habitat disturbances were predicted to reduce diversity and overall species richness through promoting disturbance tolerant species (Andersen 1995). Indeed, a greater number of species was recorded in non-disturbed zones. Nevertheless, the composition of the main dispersing species was similar in the two habitats, with individual species showing varied responses to soil disturbance.

Most genera identified in this study are widespread and abundant in other regions of Australia as well, namely *Rhytidoponera*, *Iridomyrmex*, *Melophorus* and *Monomorium*, and known to be involved into myrmecochory. Species from these genera frequently occur in other disturbed habitats and generally show increased responses to disturbances (Hoffmann and Andersen 2003). Although this pattern was also recorded for a number of species, variation in the abundance of commonly recorded *Iridomyrmex* and *Monomorium* genera were related more to site conditions (roadside width and habitat) than soil disturbance. The reduced presence of the subordinate *R. metallica* in disturbed areas was most likely influenced by the dominant *I. purpureus*, which can be inferred from direct observations of such competitive encounters at the same depots. *Iridomyrmex* was
more abundant in the soil disturbed areas of narrow roadsides, where it appears to prefer the warm open conditions that these environments confer.

Soil disturbance confers both beneficial and adverse effects on seed-dispersing ants depending on roadside habitat attributes (Chapter 3). Litter cover was the strongest variable explaining increased species richness and had a more pronounced effect in wide roadsides. On the other hand, the percentage cover of perennial grasses was associated with a reduction in the number of species. This could be because complex vegetation structures can reduce ant foraging speed (Gibb and Parr 2010). *Melophorus* was more prevalent in the soil disturbed areas of roadsides, where data suggests that it uses the advantages of a disturbed area for nesting (warm, open area), but forages in adjacent non-disturbed habitat where resources are plentiful. On the other hand, *Rhytidoponera* was more abundant in non-disturbed zones, and to a greater extent in narrow roadsides. Such spatial and/or temporal partitioning of species make the coexistence of species possible (Savolainen and Vepsalainen 1988).

The results also highlighted the complex nature of ant-plant interactions (Chapter 4) in relation to soil disturbance. Two main interactions were observed – seed removal and elaiosome consumption (or cheating). Occasionally, the combination of both behaviours was also observed. Seed removal behaviour was more frequently performed by ants in the non-disturbed areas, whilst cheating was more common in soil disturbed zone. Twenty-six ant species were recorded interacting with seeds, however only a few species were responsible for most ant-seed interactions. *Rhytidoponera metallica* and *Melophorus bruneus* typically
performed seed removal events, while *Monomorium* and *Pheidole* almost exclusively carried out cheating. *I. purpureus* removed more seeds in disturbed zones and the keystone *R. metallica* was more active in non-disturbed zones. Therefore, a complementary pattern in seed removal activities resulted in a similar percentage of seeds being removed from disturbed and non-disturbed areas.

Seeds disappeared within an average of two hours from both disturbed and non-disturbed areas of roadsides (Chapter 4). This rapid disappearance of seeds from the ground can benefit plants, by minimising predation by other invertebrates and/or vertebrates (Hughes and Westoby 1990). *Iridomyrmex* was responsible for a high proportion of removal activities, where other species did not provide beneficial services (Giladi 2006). Small bodied species showed a particular tendency to feed on the elaiosome in situ (cheating). Species from *Monomorium* and *Pheidole* genera were typical of this behaviour, which is considered to be an exploitative strategy of an otherwise mutualistic relationship (Bronstein 2001). Providing that other mutualist partners are able to outcompete such exploiters, an effective seed removal service is conferred to plants. For example, *I. purpureus* were observed stealing seeds from these “cheaters”, thus providing eventual dispersal success for these seeds.

Generally, seed-dispersing ants are thought to disperse seeds a few metres (Gomez and Espadaler 2013), however occasional long-distance dispersal events have been recorded (e.g. Whitney 2002). My findings (Chapter 5) report mean and maximum dispersal distances which are considerably longer than those previously recorded in most other habitats. For combined species, mean dispersal distances
were greater in disturbed as compared to non-disturbed areas, and this effect was most pronounced in narrow roadsides. Again, this result was largely driven by individual results for *I. purpureus*, a large bodied ant which dominated the examined roadside environments. The maximum dispersal distance of this species was 120 m, recorded in disturbed areas. Other ant species achieved much shorter maximum seed dispersal distances, e.g. 17 m for *Rhytidoponera cristata*.

The simplified and obstacle-free habitat conditions which exist post-disturbance may provide suitable conditions for effective long-distance seed dispersal by larger bodied ants, e.g. *I. purpureus*. In contrast, other smaller bodied ants (e.g. *Monomorium*) performed a high frequency of short-distance dispersal events. Frequent short-distance, and occasional long-distance dispersal events may have important implications for plants relying on ants, through influencing vegetation distribution and structure, especially in areas subjected to various (anthropogenic) disturbance activities. Given the predominance of large bodied species I recorded in disturbed areas, and commensurate long distances they dispersed seeds, suggests that the soil disturbance regime imposed in roadside environments confers benefits for mutualistic ants.
6.1 DIRECTIONS FOR FUTURE STUDIES

The primary focus of this research was to assess the composition and activities of seed-dispersing ants in fragmented roadside habitats, therefore only seed depot (cafeteria style) experiments were carried out. To obtain a better understanding of the effects of soil disturbance on the total ant assemblage, the use of pitfall traps would be useful to obtain a full enumeration of ant species present. Pitfall trapping is a common method to sample ant communities in other contexts (but see Major et al. 1999, Keals and Majer 1991).

A number of research opportunities have been identified to build upon the studies documented in this thesis. Ants proved to be active partners of plants in terms of seed removal and dispersal services, however further studies are required to investigate the beneficial role of ants to seed fitness and germination. Successful seed removal (either from experimental seed depots, or from seeds being on the ground after natural release from the parental plant) does not necessarily connote a successful end of the dispersal process. It, in fact, denotes the start of a myriad of potential pathways a seed may undergo whilst being manipulated by an ant (i.e. Hughes and Westoby 1992).

Consequently, there are numerous ways to complement the findings of this study by investigating further steps in the seed dispersal process, such as: seed fate in ant nests (i.e. burial depths), discarding of seed patterns from the nest, understand the effect of cheaters on seed germination and on seed interactions
with subsequent animal associates (mutualistic and antagonistic ants, granivores, etc.), and seed viability after final ant manipulations. For ‘closing the seed dispersal loop’ (Wang and Smith 2002), further experiments on seedling establishment and survival in relation to ant nests in disturbed versus non-disturbed areas would be warranted. Further experimental research is required to investigate the extent to which anthropogenic soil disturbances may influence final seed deposition patterns, and provide evidence for other, novel, long-distance dispersal events.

Undoubtedly, *Iridomyrmex* plays an important role in the ecology and functioning of various ecosystems, due to their influence on other members of the ant community (e.g. high territoriality and dominant behaviour; Gibb 2005) and also on other members of the flora and fauna (Eastwood and Fraser 1999). Likewise, in this study, it was apparent that *Iridomyrmex* performed an important role in the dispersal of seeds (e.g. by providing enhanced distances), which was influenced by soil disturbance and habitat conditions. Therefore, future studies may direct attention on the autoecology of *Iridomyrmex*, in a similar way to Lubertazzi et al. (2010) and Majer et al. (2011). For example, experiments considering seasonality in foraging, nesting preference, foraging response to seed availability, would reveal the extent to which their activity is well-suited for dispersal of plant species in roadsides in the longer term.

Roadside environments, and their corresponding road maintenance activities, appear to create ideal habitat conditions for *Iridomyrmex*. Different management practices are carried out between gravel and bitumen roads, therefore it would be of interest to compare seed dispersal services and nest
densities of *Iridomyrmex* in these two habitats, as compared to other seed-dispersing ant species. Studies controlling the presence of *Iridomyrmex* may also shed further insights on other important mutualists, such as *Rhytidoponera*. Given their contrasting responses to disturbance, there is a need for studies to assess the comparable role of *Rhytidoponera* and *Iridomyrmex* as dispersal agents, with particular regard to seed survival and future seedling establishment. Such information would be highly beneficial in terms of conservation management, and may help to refine the frequency and extent of road maintenance activities.

The close association of few important seed-dispersing ants to seeds of *Acacia pycnantha* (as used in this study) provides further evidence for the assertion that myrmecochoy is an “unevenly” diffuse mutualism (Gove et al. 2007). The use of seeds from other plants would shed further light on this process, and reveal the extent to which ants may favour certain seeds over others (e.g. different seed sizes). For example, ants were also observed dispersing seeds of non-elaiosome bearing seeds (e.g. Eucalypt seeds; Harris and Standish 2008, Farnese et al. 2011, Barroso et al. 2013). Second, large site- and species-specific variation characterized the dispersal processes recorded in this study, causing difficulties in making generalisations about seed dispersal for conservation practices (following McConkey et al. 2012). To increase potential conservation outcomes, further research should be carried out at larger spatial and temporal scales, taking within site variability into account.
6.2 GENERAL CONCLUSION

This study has provided new insights on the effects of soil disturbances on the processes of seed dispersal by ants, which has been largely overlooked to date. Soil disturbance is another re-occurring disturbance regime which can have important effects on plant species and communities (e.g. Bossard 1991, Milchunas et al. 2000), where humans play a key role in modifying habitats (i.e. tracks, roads and by agricultural activity). Mounting empirical evidence suggests that habitat modification from anthropogenic disturbances may be one of the most significant threats to ecosystems and their components (Miller & Hobbs 2002, Ross et al. 2002).

Understanding what factors influence and shape plant populations is an important first step for further conservation and management. This research was conducted in roadside environments, which are shaped by a rich history of anthropogenic activities (i.e. Spooner and Lunt 2004, Spooner et al. 2004), resulting in roadside vegetation of differing age, structure and condition (Spooner and Smallbone 2009). These residing plant populations are further shaped by the biotic interactions which occur within, as highlighted by this study. Considering both biotic and abiotic factors in conservation management can further our understanding of the dynamics and structure of plant populations in the context of soil- and other habitat disturbances.
Soil disturbance, a prevalent road maintenance activity, did not seem to pose direct adverse effects on the main members of the seed-dispersing ant assemblage. However, this process did influence their abundances. The main species observed interacting with seeds (e.g. *Rhytidoponera, Iridomyrmex*, *Melophorus*) have been identified as dominant seed-dispersing ants in other studies as well (e.g. Andersen and Morrison 1998, Beaumont et al. 2012). *Rhytidoponera*, and to a lesser extent *Melophorus*, are keystone seed dispersers across the continent (Gove et al. 2007, Majer et al. 2011), however their activities in roadsides appeared to be highly influenced by the presence *Iridomyrmex*. Conflicting views prevail in the literature on the role of *Iridomyrmex* as a mutualist partner (Davidson and Morton 1981), however the importance of their activities (e.g. in terms of the ratio of seed removals) in this study is unequivocal, and comparable to the keystone *Rhytidoponera*.

*Iridomyrmex* may be essential in the dispersal mechanism of plants in fragmented roadsides and elsewhere. The occasional long-distance seed dispersal which they perform may have direct consequences for plant communities, by facilitating colonization into new sites, and providing habitat connectivity in an otherwise fragmented and highly modified rural landscape. In summary, the information recorded here, such as seed dispersal rates and distances, all varied according to their individual ant partners, and in turn, response to soil disturbance regimes. This information provides a better understanding on some of the factors which may influence the persistence of remnant plant communities and is critical for future conservation management success of roadside habitats.
Appendices

| Functional groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|-------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Subfamily Dolichoderinae** |   |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Iridomyrmex sp. A (rufoniger gp) | DD | 1 | 7 | 2 | 3 | 5 | 4 | 5 | 5 | 10 | 5 | 6 | 7 | 5 | 8 |   |   |   |   |   |   |   |   |   |   |
| Iridomyrmex purpureus | DD | 3 | 11 | 3 | 4 | 10 | 9 | 8 | 10 | 12 | 6 | 1 | 5 | 11 | 6 | 10 | 3 | 1 | 6 | 5 | 9 |   |   |   |   |
| **Subfamily Ectatomminae** |   |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Rhytidoponera cristata | O | 4 | 2 | 3 | 3 | 1 | 3 | 6 | 3 | 1 | 1 | 4 | 3 | 1 | 1 |   |   |   |   |   |   |   |   |   |   |
| Rhytidoponera metallica | O | 3 | 5 | 1 | 7 | 5 | 1 | 5 | 4 | 3 | 5 | 3 | 1 | 6 | 5 | 4 | 8 | 1 | 4 |   |   |   |   |
| Rhytidoponera sp. A (convexa gp) | O | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Subfamily Formicinae** |   |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Camponotus aeneopilosus | SC | 1 |   | 1 |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Camponotus ephippium | SC | 1 |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Camponotus obniger | SC | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |
| Camponotus sp. 1 | SC |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |
| Melophorus sp. B (aeneovirens gp) | HCS | 1 | 1 | 1 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |
| Melophorus sp. A. (bruneus gp) | HCS | 2 | 1 | 5 | 2 | 2 | 1 | 2 | 4 | 2 | 6 | 2 | 4 |   |   |   |   |   |   |   |   |   |   |   |
| Notoncus ectatommooides | CCS | 1 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Subfamily Myrmicinae** |   |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Crematogaster sp. A | GM | 8 | 11 | 1 | 2 | 2 | 1 | 4 | 2 | 1 | 8 | 4 | 6 | 1 | 1 | 3 |   |   |   |   |   |   |   |   |
| Monomorium sp. A (sordidum) | GM | 3 | 9 | 1 | 4 | 4 | 3 | 11 | 3 | 4 | 1 | 3 | 4 | 6 | 1 | 1 | 3 |   |   |   |   |   |   |   |
| Monomorium sp. B (rothsteini gp) | HCS | 4 | 1 | 1 | 1 | 2 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| Pheidole A | GM |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Subfamily Ponerinae** |   |   |   |   |   |   |   |   |   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Odontomachus sp. A (ruficeps gp) | O |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

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Appendix 1.b. Species list. Ant genera, species and functional groups of NON-DISTURBED zones in 24 sites. Sampling units are presence/absence of ant species at seed depots (n = 12; morning and afternoon data combined/site), during December 2013 - March 2014. Functional groups: DD - Dominant Dolichoderine, OPP - Opportunists, GM - Generalized Myrmicinae, HCS - Hot climate specialists, CCS - Cold climate specialists, SC - Subordinate Camponotini, TCS - Tropical climate specialists.

| Functional groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|-------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| **Subfamily Dolichoderinae** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| *Iridomyrmex* sp. A (rufoniger gp) | DD | 5 | 7 | 4 | 8 | 11 | 1 | 7 | 1 | 10 | 10 | 5 | 3 | 1 | 4 | 5 |   |   |   |   |   |   |   |   |
| *Iridomyrmex* purpureus | DD | 4 | 4 | 9 | 3 | 12 | 9 | 2 | 10 | 6 | 10 |   | 12 | 7 | 3 | 12 | 5 | 10 | 10 |   |   |   |   |   |
| **Subfamily Ectatomminae** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| *Rhytidoponera* cristata | O | 1 | 4 | 1 | 4 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 4 | 8 | 1 |   |   |   |   |   |   |   |   |
| *Rhytidoponera* metallica | O | 1 | 5 | 4 | 6 | 9 | 5 | 3 | 4 | 9 | 4 | 2 | 5 | 6 | 8 | 6 | 4 | 4 | 6 | 10 | 10 | 4 | 9 | 9 | 8 |
| *Rhytidoponera* sp. A (convexa gp) | O | 1 | 1 | 1 | 1 | 3 | 6 | 1 | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Subfamily Formicinae** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| *Camponotus* aeneopilosus | SC | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 7 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Camponotus* ephippium | SC | 1 | 2 | 3 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Camponotus* sp. A (claripes gp) | SC | 1 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Camponotus* sp. 1 | SC |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Melophorus* sp. B (aneovirens gp) | HCS | 1 | 1 | 7 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Melophorus* sp. A. (bruneus gp) | HCS | 1 | 1 | 2 | 1 | 3 | 1 | 1 | 2 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Notoncus* sp. A (enormis gp) | CCS | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Notoncus* ectatommoides | CCS | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| **Subfamily Myrmicinae** |   |   |   |   |   |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| *Crematogaster* sp. A | GM |   |   |   |   |   |   |   |   |    |   | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Monomorium* sp. A (sordidum) | GM | 10 | 5 | 2 | 4 | 3 | 3 | 5 | 3 | 1 | 1 | 3 |   |   |   |   |   |   |   |   |   |   |   |   |
| *Monomorium* sp. A sydneyense) | GM |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Monomorium* sp. C | GM |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Meranoplus* sp. A (Group D) | GM | 1 | 2 | 1 | 1 | 1 | 3 | 1 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Monomorium* sp. B (rothsteini gp) | HCS | 4 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Pheidole* A | GM | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 3 | 1 | 1 | 1 |   |   |   |   |   |   |   |   |   |   |   |
| *Pheidole* B | GM | 1 | 2 | 1 | 2 | 4 | 1 |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| *Podomyrma* adelaide | TCS |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

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