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Ant-mediated seed dispersal in an invaded landscape

Adam John Mears Devenish

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with the requirements for the award of the degree of Doctor of
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ABSTRACT

Human activities are modifying our environments at an unprecedented rate and scale, leading to not only a loss of biodiversity, but also a loss of ecosystem functionality. Arguably, one of the most pervasive of these destructive forces can be seen in the rise of biological invasions, and the spread of non-native invasive ant species. Despite the clear potential of the detrimental ecological and economic pressures posed by these insects, we still lack a comprehensive understanding of their impacts on ecosystem services. One such service is ant-mediated seed dispersal – otherwise known as myrmecochory. In this thesis I address some of these shortcomings by investigating how invasive ants may disrupt the process of myrmecochory in native ecosystems, across a range of different scales. Firstly, I took a broad perspective by looking at evolutionary events of myrmecochory across the ant phylogeny: these analyses revealed that myrmecochory is a diffuse interaction between many ant-plant guilds. Secondly, in-depth analyses of the effects of the non-native invasive Argentine ants (*Linepithema humile*) on both ant and plant communities in South African and Spain suggested that these invaders are ill-equipped to replace the native seed dispersers that they displace. Field observations suggested that invaded ant communities dispersed fewer seeds than non-invaded ant communities, and that this inefficiency is evident at multiple stages in the seed dispersal process. Thirdly, the modifications to the natural ant-plant interactions due to invasive ants may be making these environments more vulnerable to secondary invasions by other invasive myrmecochorous plant species. Finally, I explore the mechanism that may be driving these changes in ant-seed interactions, by exploring how differences in a range of physical and chemical seed traits may explain ant preferences to disperse certain seeds. In conclusion, while myrmecochory may be considered a rather loose ant-plant mutualism, invasive ant species do appear to be altering the nature of the biotic interactions, with impacts permeating through different levels of the ecosystem, and potentially leaving their ecological mark well beyond the mere loss of native ant biodiversity.

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I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: DATE:

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CHAPTER ONE

1. GENERAL INTRODUCTION

“What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions.”

Daniel H. Janzen (1974)

Our ecosystems are structured by a diffuse and dynamic array of ecological interactions between competing species (Schluter 2000). Understanding how and why we find organisms in specific environments and under certain conditions is a key question in ecology today. This requires an understanding of the evolutionary history of species (Webb et al. 2002), as well as an understanding of the nature of the underlying interactions (Chesson 2000). Broadly speaking, most ecological interactions can be viewed as either antagonistic (e.g. predation, competition, host-parasite interactions) or mutualistic (e.g. cooperation, mutualism) (Bascompte 2009). For a long time antagonistic actions related to competition, both between and within species, were viewed as the major driving force in community structure and function (Chesson 2000; Losos et al. 2003). More recent studies, though, have shown that mutualistic interactions are equally, if not more, important (Bulleri et al. 2008; Gross 2008). In this chapter I explore the mechanisms and evolution of one such mutualistic interaction, that of ant-mediated seed dispersal - otherwise known as myrmecochory - a widespread and important ecological mutualism. Although this is a relatively well-studied phenomenon, I identify key limitations in our knowledge of ant-seed mutualisms and argue that these knowledge gaps are of special concern in a global environment of unpredictability, rapid modification and the rise of biological invasions.

1.1 THE EVOLUTION OF MYRMECOCHORY

1.1.1 Dispersal

Dispersal is a fundamental biological process: it describes the movement of an organism away from its natal patch to a region where kin-competition is minimised, making it an important life history trait, encapsulating multiple scales of organisation (Clobert et al. 2012). Beginning at the level of the individual, an organism's ability to disperse within an environment not only influences its growth and survival, but also its reproductive fitness (Bonte et al. 2012; Weiß et al. 2016). The patterns of multiple dispersing individuals, between and within habitats, determines the temporal and spatial structure of communities, which in turn determines population growth (Lehouck et al. 2009; O'Sullivan et al. 2014). The degree of dispersal between populations and potential gene flow can impact both the geographical distribution and adaptive capacity of a species (Kokko and López-Sepulcre

2006). Many organisms can actively disperse through the environment on their own volition; others cannot and have instead evolved a range of passive dispersal mechanisms. Plants are good examples of passive dispersers: they produce propagative dispersal units (disseminules) to move beyond their natal patch and facilitate gene flow. In this case, disseminules are the seeds, spores or fruits (Fenner and Thompson 2005), which rely on a range of abiotic (e.g. wind, water and gravity) and biotic (e.g. invertebrates, birds and mammals) vectors for dispersal.

1.1.2 Myrmecochory – a key ecosystem service

Ants are globally ubiquitous: there are over 13,000 described extant species of ants found on every continent except Antarctica, and they represent a significant portion of the animal biomass in most terrestrial habitats, being functionally dominant in many ecosystems (Hölldobler and Wilson 1990; Bolton 2017). Ants are key drivers of ecosystem functionality and maintenance (Folgarait 1998), and useful bio-indicators of environmental health and change (Andersen and Majer 2004). These facets of ant dominance mean that any change in ant abundance can have serious knock-on effects within an ecosystem.

Ant-seed interactions are an example of an important ecosystem service. Ecosystem services are those provided by the natural world that benefit human society and support wellbeing (Millennium Ecosystem Assessment 2005). Ecosystem services are grouped into four broad categories: (i) *Supporting*: services that maintain ecosystem functionality, for example, nutrient cycling through movement of soil during nest construction; (ii) *Provisioning*: goods obtained from ecosystems, for example, entomophagy (insects as a human food source); (iii) *Regulating*: services that regulate ecosystem processes, such as pollination, predation and seed dispersal; and (iv) *Cultural*: non-material benefits obtained from ecosystems, for example, the use of bullet ants during rituals by indigenous South Americans (reviews in Del Toro et al. 2012). Of these, regulating services are arguably one of the most important, yet perhaps the most difficult to quantify and measure, especially in a traditional economic sense. Regulating services are derived from the interactions between ants and other organisms within the environment and can be either antagonistic (e.g. biological control of pests or animal community regulation) or mutualistic (e.g. seed dispersal and pollination).

Mutualisms between ants and other organisms (particularly plants) have long been recognised as some of the best examples of reciprocity in the natural world (reviews in Bronstein 1998) and most likely have been a driver for the ecological and evolutionary

success of ants generally. Besides benefiting the partners, these mutualistic relationships also shape the composition and function of the wider ecological community (Janzen 1966; Beattie 1985; Christian 2001; O'Dowd et al. 2003). Unfortunately, as our understanding of this complex process grows, so too does our realisation of how vulnerable many of these mutualisms are. This vulnerability stems from increasing rates of ecological instability, linked to climate change, habitat loss, fragmentation and biological invasions. Not only are we losing species at an unprecedented rate (as general extinction rates are estimated to range between 1,000 and 10,000 times that of the natural background rate (Chivian and Bernstein 2008)), but worryingly we are also losing the mutualisms that shape and drive our ecosystems as well. Perhaps one of the best-studied areas of ant mutualism is ant-mediated seed dispersal, better known as myrmecochory.

Sernander first described myrmecochory in 1906: through careful observations he detailed the movement of seeds by ants in the temperate European forests of France and Sweden. He noted that myrmecochorous plants produce seeds with an oily rich appendage, now known as an elaiosome. The elaiosome acts both as a reward and a handle (Beattie 1985; Gómez et al. 2005), luring the ant to remove the diaspore (seed and elaiosome) back to its nest, whereupon the nutrient-rich elaiosome is consumed and the seed is discarded intact, in or around the ant nest (Beattie 1985). In doing so the ant colony benefits from a food source, and the plants benefit from the movement of their seeds away from the parental environment, often out of reach of predators and other destructive forces (Fenner and Thompson 2005).

1.1.3 Phylogenetic distribution of myrmecochory

Little is known about the early evolutionary stages of ant-mediated seed dispersal and other important ant-plant mutualisms (Wilson and Hölldobler 2005; Moreau et al. 2006). This is surprising given the volume of ecological literature on myrmecochory; most likely this paucity of our evolutionary understanding can be attributed to poor fossil representation (Dunn et al. 2007). The available evidence suggests that ant-plant interactions became prominent in the mid-Cretaceous period, when both ants and angiosperms (flowering plants) were undergoing diversification (Wilson and Hölldobler 2005). Many of these interactions can be explained through evolutionary history (Rico-Gray and Oliveira 2007). Evidence for myrmecochory in the plant phylogeny appeared sometime towards the end of the Eocene, around the same time as ants are thought to have undergone rapid radiation, establishing themselves as one of the most dominant terrestrial insect groups (Moreau et al. 2006; Dunn et al. 2007). To date, seed-dispersing ant species

can be found within the five major ant group subfamilies: Dolichoderinae, Ectatomminae, Formicinae, Myrmicinae and Ponerinae (Warren and Giladi 2014). However, we lack a systematic analysis of the phylogenetic distribution of myrmecochory in ants. Such analysis would be valuable as it would help reveal the number of independent origins of the mutualism and may also identify common traits that predispose a lineage to adopt this behaviour.

In contrast, the phylogenetic history of ant-mediated seed dispersal has been relatively well studied among plants. Recent synthesis of extant myrmecochorous flora across the globe revealed that myrmecochory has evolved independently at least 101 times (Lengyel et al. 2010). Today it has been observed in at least 11,000 plant species (4.5% of all plant species), across 334 genera (2.5% of all plant genera), and 77 plant families (17% of all angiosperm plant families) (Lengyel et al. 2010). The phylogenetic distribution is broad: plant genera tend to be geographically distinct and so this phylogenetic distribution suggests that myrmecochory is a prominent form of seed dispersal, found in a wide array of habitats. Indeed, myrmecochory is established in habitats as varied as the deciduous European and North American forests (Beattie and Culver 1981; Gorb and Gorb 1995; Servigne and Detrain 2008; Ness et al. 2009), to the tropics of South America (Horvitz and Beattie 1980; Le Corff and Horvitz 1995; Pizo and Oliveira 2001; Guimaraes and Cogni 2002; Christianini and Oliveira 2010), and the arid dry sclerophyll shrublands of South Africa and Australia (Berg 1975; Hughes and Westoby 1992b; Bond and Slingsby 1983; Gove et al. 2007; Majer et al. 2011) (Fig. 1.1). New records of myrmecochorous interactions appear annually, often from previously understudied systems (e.g. new records of *Odontomachus* and *Paratrechina* ant genera dispersing seeds in China (Zhou et al. 2007; Zhu et al. 2017)).

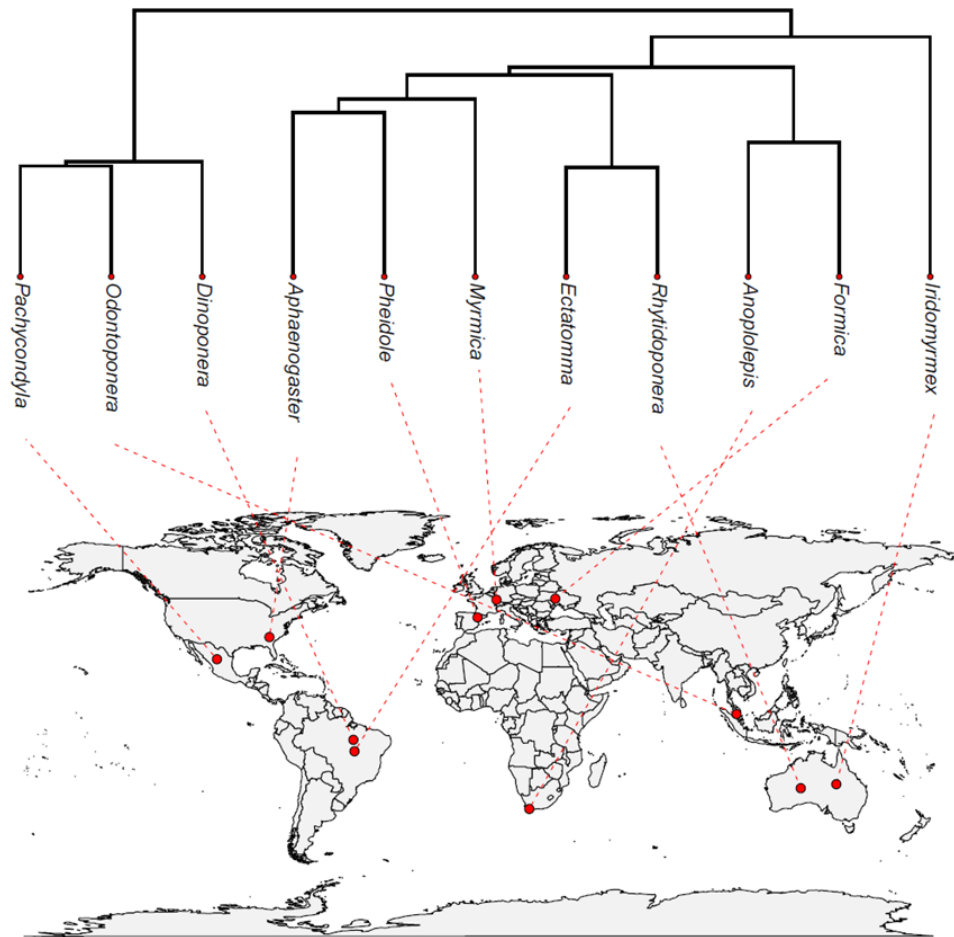


Figure 1.1: Map of the regions where myrmecochory is prevalent, along with a phylogeny of the dominant seed-dispersing ant genera.

Despite the ubiquity of myrmecochory on evolutionary and ecological scales, to date much of our understanding stems from Northern Hemisphere systems; however, the majority of known myrmecochorous plant species (>50%) appear to be concentrated in the arid and nutrient poor habitats of the Southern Hemisphere, in countries such as South Africa and Australia (Rico-Gray and Oliveira 2007; Lengyel et al. 2010). A more diverse approach to studying myrmecochorous processes is required if we are to gain a better understanding of the underlying evolutionary and ecological drivers of this important ecosystem service.

1.2 THE PROCESS OF MYRMECOCHORY

The broad phylogenetic diversity and distribution of myrmecochory and its multiple evolutionary origins, raises the interesting question of what drives the evolution of myrmecochory. This is best explored in terms of the costs and benefits from both the plants' and the ants' perspectives.

1.2.1 Cost and benefits of myrmecochory to plants

While elaiosomes are comparatively cheap to produce relative to other fruits, when compared with other dispersal mechanisms myrmecochory is a short distance event that occurs in close proximity to the parent plant (Goldblatt 1997). A recent survey estimated that the average global ant-mediated seed dispersal distance is no more than 2.24 metres from the parent plant (Gómez and Espadaler 2013); this is significantly shorter than the long-distance seed dispersal achieved by vertebrate dispersers, such as birds. For example, Christianini and Oliveira (2010) found that birds dispersed seeds of the plant *Xylopia aromatica* 40 times further than the dispersal distance achieved by ants. Despite this, studies have shown that ant-dispersed seedlings have a higher post-dispersal survival rate (Gibson 1993) relative to randomly dispersed seeds (Hanzawa et al. 1988). Plants appear to therefore benefit from ant-mediated seed dispersal in more ways than simply avoidance of parent-offspring competition (Howe and Smallwood 1982; Giladi 2006). For example, by removing seeds to ant nests (often below-ground), the seeds escape potential predation by rodents, birds and granivorous ant species (O'Dowd and Hay 1980; Higashi et al. 1989), as seeds that remain above-ground for too long are often quickly found and consumed (Heithaus 1981; Bond and Slingsby 1984; Breytenbach 1986). Transport into an ant nest can also confer other protective benefits, such as escape from bush fires and other destructive forces (Berg 1975; Bond and Slingsby 1984; Hanzawa et al. 1988).

The chemical composition of soil in/near ant nests often differs from surrounding soils; for example, soils in/near nests often have a wide array of elements that are good for seed germination and establishment, with high levels of moisture, organic matter, minerals (such as nitrogen and phosphorous) and micro-organisms content (Beattie and Culver 1983; Gibson 1993; McGinley et al. 1994). The transport of a seed to the ant nest and subsequent placement within their refuse pile results in 'directed dispersal' to nutrient-rich microsites, which improves the chance of seedling establishment (Davidson and Morton 1981; Wagner 1997; Passos and Oliveira 2004). For example, Berg-Binder and Suarez (2012) showed that seeds transported and deposited near *Formica obscuripes* ant mounds (high in both nitrogen and phosphorous) experienced microhabitats that were favourable for seedling establishment. However, several studies have shown that the opposite can also be true, as many of the Southern Hemisphere plants species are directed towards nutrient-poor sites, often low in potassium (Bond and Stock 1989). For example, Bond and Stock (1989) found that the endemic South African seeds of *Leucospermum conocarpodendron* were placed in sites favourable for germination and establishment within ant nests, when those sites

corresponded with a lower phosphorous content compared with the surrounding environment.

Relying on ants for seed dispersal can also be costly for a plant. For example, as seeds may be clumped together within ant refuse piles, plants might experience increased intra- and inter-species competition (Davidson and Morton 1981). Likewise, movement and placement of seeds within the ant nest may not always be optimal for germination (Christian and Stanton 2004; Renard et al. 2010; Canner et al. 2012) as often these seeds are relocated post-dispersal to other sites (above-ground), where they might be predated upon. Therefore, focusing on the nest location as the end-point of a seed's journey might be misleading. In conclusion, there is much compelling evidence in favour of the benefits of myrmecochory for plants, and it is tempting to assume such benefits are equally applicable across different myrmecochorous partners; however, the net costs and benefits to a plant are likely to be highly context dependent on habitat type and ant species composition (Giladi 2006).

1.2.2 Cost and benefits of myrmecochory to ants

Despite a wealth of literature on the benefits of myrmecochory to plants, there are few studies that provide empirical evidence of how seed-dispersal interactions benefit ant mutualists. Elaiosomes are thought to be a reliable source of nutrition for ant colonies, particularly for larvae that require foods both high in protein and fats (Vinson 1968). However, no known ant species relies solely on elaiosomes for survival, although elaiosomes can provide an important stable and predictable supplementary food source, which buffers ant colonies at times when food is scarce (Bono and Heithaus 2002; Clark and King 2012). This is supported by observations that myrmecochorous plant species shed their seed over long seasonal windows (staggered seed release), which often coincides with peaks in ant activity (Ohkawara et al. 1996; Boulay et al. 2005; Giladi 2006). Laboratory studies have shown how ant colonies that are fed elaiosomes benefit from increased gyne (sexual) production (Morales and Heithaus 1998; Bono and Heithaus 2002) and increased larval weight (Gammans et al. 2005; Marussich 2006; Fokuhl et al. 2012), relative to colonies that were not fed elaiosomes. Intriguingly, other studies have found the benefit of elaiosomes to be negligible (Caut et al. 2013) or even detrimental to colony survival (Turner and Frederickson 2013). Generally, therefore, it can be seen that while most studies shed light on the direct benefits linked to elaiosomes, they often fail to also take into account the energetic costs and risks associated with retrieving and/or specialising on elaiosomes as a food sources (Edwards et al. 2006). Also, in some cases plants 'cheat' by

producing the chemical attractant to lure an ant, but then confer no nutritional benefit (Pfeiffer et al. 2010; Turner and Frederickson 2013). Such deceit would incur a significant fitness cost on the ant colony, resulting in selection against myrmecochory. The costs and benefits that ants experience from myrmecochory remain unclear; more field studies are required to assess the long-term impacts of seed dispersal on colony survival and fecundity.

1.2.3 Obligate vs. facultative myrmecochory

It is clear that the presence of an elaiosome can increase the removal rate of seeds by ants (Culver and Beattie 1980; Howe and Smallwood 1982; Leal et al. 2007). However, the importance of this interaction on the survival of both plant and ant partners is less clear. This interaction is usually viewed as facultative, with an exchange of resources (“the elaiosome”) for a service (“seed dispersal”) between a wide array of plant and ant guilds (Bennet and Krebs 1987; Warren and Giladi 2014). Yet, this viewpoint might be biased, as the vast majority of literature on myrmecochory to date is from the Northern Hemisphere (North America and European forests), where many of these plant species are known to be diplochorous (both ballistic and ant-dispersed). In such instances seeds undergo two stages of dispersal: primarily they are ejected away from the parent plant via ballistic ejection, and then ants secondarily move them. This means that while the absence of ants does reduce seed recruitment (Warren et al. 2010; Warren et al. 2012; Warren and Bradford 2014), seed dispersal and plant survival can still occur without any direct ant involvement (Gorb et al. 2000). However, this is far from the norm, as most myrmecochorous plant species occur in the Southern Hemisphere, where seeds must be moved below-ground in order to escape both predation and bush fires (Berg 1975; O’Dowd and Hay 1980). In these circumstances the plant species are obligate mutualists, meaning that without the ant mutualists they achieve no substantial dispersal. This means these plants are restricted to localities where the appropriate seed-dispersing ants occur. It is important to note here that although the plant’s dependency on the ant is obligate, in both instances the ant species remains facultative (it can survive without seeds to disperse). Yet recent evidence from Fiji highlights an obligate relationship for both the ant and the plant: many ant-housing epiphytes, which grow on trees, need their seeds to be dispersed and deposited into cracks within the tree bark. This ensures the seed can germinate and the plant can grow, and in doing so provides the obligate ant species with necessary ‘housing’ in the form of domatia - small chambers within the plant (Chomicki and Renner 2016).

The complex nature and variation in the types of mutualisms found between ant and plant partners means that the evolution of myrmecochory is likely to be driven by selection

for a wide array of ant and plant traits. Where highly dependent and specialised (obligate) interactions have evolved, there are likely to be fewer actors involved, and the associated traits that mediate this interaction are likely to have become more refined and restricted over time.

1.2.4 Multi-stage process

While the mutualistic process of myrmecochory might initially appear to be rather simplistic and linear, there are clear phases to the stages of seed dispersal. The ant must locate and remove the diaspore (Stage 1); it must transport the diaspore back to its nest (Stage 2); it must remove the elaiosome without damaging the seed itself and place the intact seed in a site suitable for germination and seedling establishment (Stage 3) (Fig. 1.2).

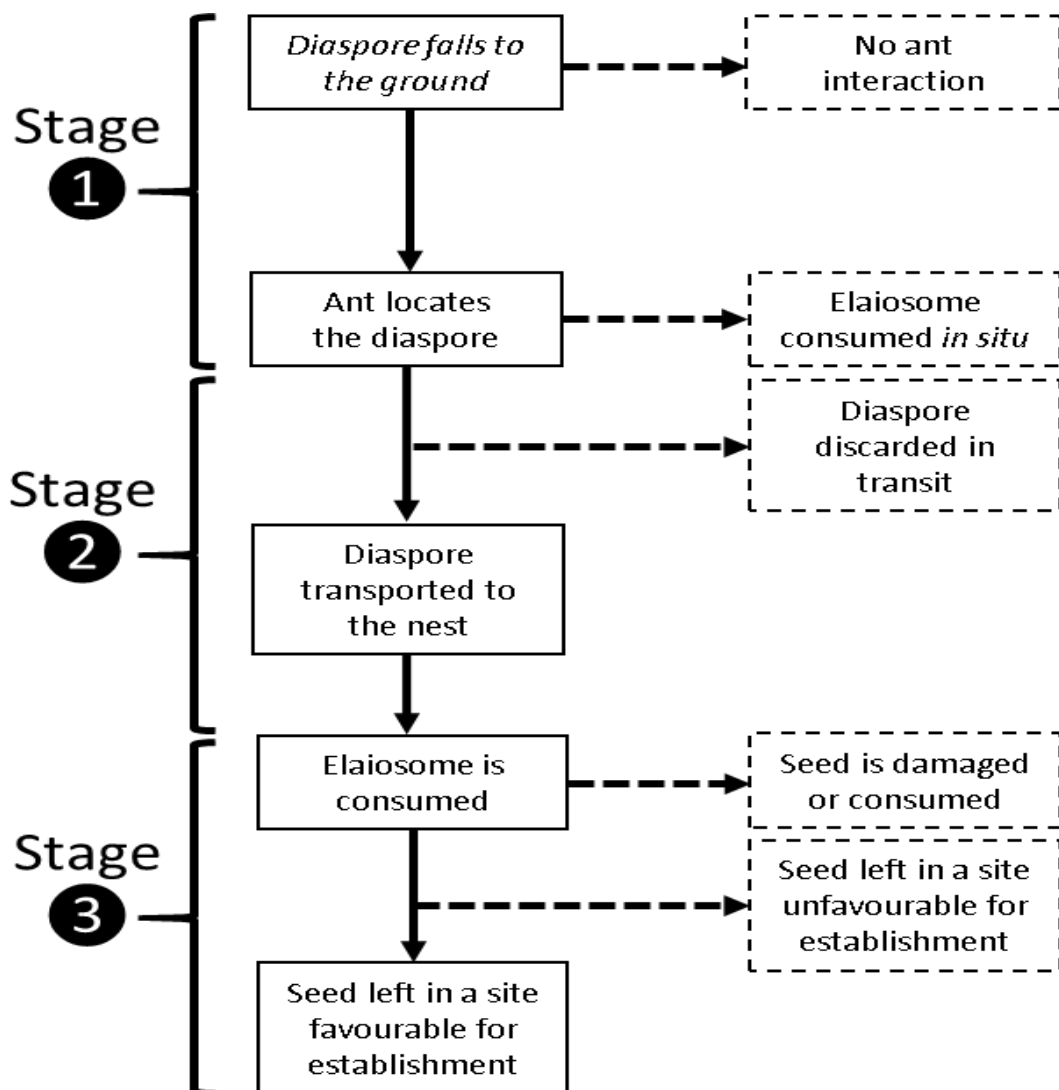


Figure 1.2: Three stages of myrmecochory: dotted boxes indicate behaviours that result in failure points in the seed dispersal process. All behaviours in the solid boxes must be completed in order for a seed to be successfully dispersed.

Understanding ant-seed interactions at each stage of this process is critical for quantifying the efficiency of the dispersal process. Of specific concern is how non-native invasive ant species may influence the efficiency of each phase, and the subsequent impact any changes may have on the ecosystem. For example, many ecosystems require the seeds to be deposited in a specific location (or depth) for seed survival and subsequent seedling establishment. Failure to do so can lead to a modification of the natural plant community structure. Therefore, while many studies may focus on specific aspects of the dispersal process (in particular Stage 1 and 2), we lack a comprehensive understanding of the entire process of myrmecochory (seed removal through to placement for germination). This means that we often lack the information to accurately predict how changes to the seed dispersal process may in turn impact the wider environment, and the ant and plant species within it.

1.2.5 Myrmecochory in the Cape Floristic Region

The Cape Floristic Region (CFR), an area of South Africa spanning 90,000 km², is home to an estimated 9,000 plant taxa, of which 68% are endemic to the region (Cowling et al. 1996; Goldblatt 1997; Goldblatt and Manning 2000). Due to the diversity and rarity of plant species, the CFR is classified as one of the world's five global floral kingdoms, with diversity rivalling even that of the tropical rainforests (Schnitzler et al. 2011). This diversity has puzzled scientists: one explanation is that the unique topography and limited summer rainfall of the area that has favoured short distance seed dispersal mechanisms, such as myrmecochory (Bond and Slingsby 1983; Linder and Hardy 2004).

Flora of the CFR is commonly referred to as fynbos (meaning 'fine-leaved') and it is typically categorised by three key plant families: Proteaceae, Restionaceae and Ericaceae. Much of this diversity is thought to derive from the fact that the CFR is dominated by nutrient poor soils, dry summers and frequent bush fires (Bond and Slingsby 1983; Schnitzler et al. 2011). These conditions are thought to explain why 30% of fynbos plant species are myrmecochorous (approx. 2,500 species) (Bond and Slingsby 1983; Westoby et al. 1991): myrmecochory is thought to provide an ecological advantage over other dispersal mechanisms, as removal of seeds to an underground nest protects the seeds from bush fires, as well as predators (Bond and Slingsby 1983).

Within the CFR, most seed dispersal is achieved by two ant genera: *Anoplolepis* and *Pheidole* (Bond and Slingsby 1983), with the large pugnacious ant (*Anoplolepis custodians*) dispersing the large Proteaceae seeds. Such diversity and specificity between

ant-plant partners comes at considerable cost, as the arrival of the invasive Argentine ant (*Linepithema humile*) has shown (Bond and Slingsby 1984; Christian 2001). Christian (2001) demonstrated that environments invaded by *L. humile* were characterised by the absence of both *Anoplolepis* and *Pheidole* genera. This led to a shift in the composition of plant community structure, in particular a reduction in the density of large-seeded plant species.

1.2.6 Myrmecochory in the Iberian Peninsula

The Iberian Peninsula is an area of Southwestern Europe spanning 582,000 km². It is one of the most species rich areas in the Mediterranean Basin, with an estimated 1,328 endemic plant taxa (Myers et al. 2000; Buira et al. 2017). Of the eight known seed dispersal mechanisms that frequent this region, myrmecochory forms a common element of most woodland communities (approx. 5%) (Gutián and Ma Sánchez 1992). However, while this region of the world has considerably less myrmecochorous flora than the Cape Floristic Region, the two regions share one thing in common: they have been subjected to a number of invasive ant species. Espadaler and Collingwood (2001) identified 12 non-native ant species present in the Iberian Peninsula, including the *L. humile* and the invasive garden ant (*Lasius neglectus*). *L. humile* has been cited as a key disruptor of myrmecochorous seed dispersal processes within the Iberian Peninsula (Gómez and Oliveras 2003; Gomez et al. 2003; Bas et al. 2009).

1.3 PARTNER MATCHING

The pairing of ant with seed (myrmecochorous interactions) is influenced by a wide array of traits, within both the ants and the seeds. Myrmecochorous seeds can vary considerably in size, morphology, chemistry and phenology (Hughes and Westoby 1992a; Garrido et al. 2002; Boulay et al. 2007a). Likewise, ants vary considerably in physical (i.e. mandible gap and worker size) and behavioural (i.e. foraging period and diet) traits that may influence their interactions with seeds (Gómez et al. 2005; Servigne and Detrain 2008). While some factors such as correlation between ant and seed size are easily visually noted, other components of the interaction are less obvious. What is clear, however, is that these differences between ant and plant traits can directly influence the extent and the quality of interactions (Gorb and Gorb 2000; Warren et al. 2012).

1.3.1 Not all ants interact with seeds in the same way

Myrmecochory is typically viewed as a relatively loose association between ant and plant mutualists, with several ant species within a given environment removing seeds

derived from a variety of plant species (Beattie 1985). This is supported by the view that plants within the same species often produce contrasting seed sizes, and/or chemical compositions, and that different ant size classes (i.e. intra-caste variation) prefer different seed sizes (Mark and Olesen 1996; Ness et al. 2004). Such a loose association may appear beneficial as it provides seeds with varied dispersal localities and environmental conditions (i.e. bet hedging).

However, such generalist interactions also come at a cost: studies show that the real fitness benefit of ant-mediated seed dispersal depends heavily on which ant species is interacting with the seeds (Giladi 2006). In fact, depending on how you define the interaction, there are often only a few high-quality seed dispersers in any given environment (Ness et al. 2009; Warren et al. 2014). While several ant species may interact with seeds on the soil surface, most of these interactions result in little, to no dispersal benefit for the seed. For example, many ant species will consume the elaiosome *in situ* (i.e. elaiosome robbing) without transporting it back to the nest (Andersen 1988a; Espadaler and Gómez 1997; Boulay et al. 2007b). Other ant species might transport seeds back to the nest and place them in locations unsuitable for seedling survival. For example, *Aphaenogaster senilis* was initially thought of as a common seed disperser in the Iberian Peninsula, until work by Gomez and Espadaler (1998) revealed that seeds of some plant species were removed and placed at depths not suitable for seedling survival and establishment.

1.3.2 What traits define a high-quality seed disperser?

There are four key traits that are thought to signify a high-quality seed disperser: (i) Large-bodied (often ecologically subordinate) scavenger ant species (Andersen 1992; Boulay et al. 2007b; Ness et al. 2009, Aranda-Rickert and Fracchia 2012) which quickly remove seeds back to their nest, not stopping to consume the elaiosome *in situ*; (ii) Solitary foragers, which forage over large areas in predictable periods of the day, often coinciding with seed release (Boulay et al. 2007b; Warren et al. 2011), thereby increasing their chances of finding a seed on the soil surface; (iii) Ants that handle seeds in a manner that does not damage the seed coat, and that places them at a depth suitable for germination and establishment (Culver and Beattie 1980; Gibson 1993; Bas et al. 2007); and (iv) Species that form relatively small colonies (consisting of a few hundred individuals) that frequently relocate their nests (Hughes 1991), thereby reducing the accumulation of seeds in any one location. These traits are often shared among some of the most widely recognised seed-dispersing ant species (e.g. *Aphaenogaster rudis*). However, the quality of seed dispersal is

likely to depend on the type of seed that is being dispersed: small- and large-seeded myrmecochorous plant species are likely to require different seed dispersers within the same environment, as their optimal conditions for germination and establishment will likely be variable. For example, smaller seeds cannot withstand deep burial (Fenner and Thompson 2005). This pairwise association between ants and plants is indicative of a closely co-evolved mutualism, with seed traits that are ‘designed’ to attract a specific range of ant partners (Giladi 2006).

Predicting high-quality seed dispersal partnerships is important in determining ecosystem stability and trophic balance. Rate of removal is dependent upon both ant and plant seed traits, and so we often make the assumption that a larger elaiosome (reward) will attract potentially larger, and thus higher quality, seed dispersers who might disperse seeds further away from the parent plant (Leal et al. 2007; Aranda-Rickert and Fracchia 2012). Removal rate, however, is likely to depend on additional biological processes, such as the number and quality of offspring (seeds). For example, plant species that produce a large quantity of offspring are likely to have seed traits that favour a broad spectrum of ant dispersers (e.g. Australian *Acacia saligna* (Richardson and Kluge 2008)). In doing so they can buffer a high degree of seed loss that might occur due to a ‘bet-hedging’ strategy that results in placement of seeds in variable habitats (i.e. nests of different ant species). In contrast, plants that produce a small quantity of high-quality offspring are likely to have seed traits that favour a narrow spectrum of ant dispersers (e.g. endemic South African *Leucospermum* (Lamont 1985)), thereby ensuring their seeds are precisely handled and placed in optimal environments for germination and establishment. Such pairwise partner matching and specificity has been suggested as one of the driving forces behind speciation in some myrmecochorous plant lineages (Mucina and Majer 2012), and it is also the reason why we see such high numbers of endemic myrmecochorous plant species in relatively small geographical areas (e.g. CFR in South Africa). Close partner matching comes with a considerable degree of risk, as the more dependent a plant is on a narrow range of dispersers, the more likely a plant species may become extinct, should that specialist disperser disappear. Many large-seeded endemic plant species in the CFR rely solely on dispersal by one ant species. Specialism, therefore, threatens the wider ecosystem stability, as loss of the network between ant and plant mutualists can, in turn, affect other organisms that rely on those plant species for survival.

1.3.3 Black box of myrmecochory

Elaiosomes are specialised plant adaptations that promote the dispersal of their seeds. Bird-dispersed seeds often possess elaiosomes (referred to as arils) that are large and colourful (Murray 2012); in contrast, ant-dispersed seeds produce elaiosomes which are often pale and colourless (Fig. 1.3).



Figure 1.3: Variation in elaiosome types showing both bird- and ant-dispersed seeds. [© Adam Devenish]

This dull appearance is because rather than responding to visual cues, ants detect and respond to olfactory cues through their antennae (Buehlmann et al. 2015). Elaiosomes of ant-dispersed seeds are thought to be reminiscent of dead insects, with fatty acid profiles similar to that of insect haemolymph (Hughes et al. 1994), often containing high concentrations of rare and important amino acids that are vital for larval development (Fischer et al. 2008).

Elaiosome chemistry is not a simple case of mimicry: there is considerable variation in ant responses to elaiosomes of different plant species. Such discrimination has been attributed to elaiosome size (i.e. reward size) (Mark and Olesen 1996; Gorb and Gorb 2001; Leal et al. 2014), morphology (Gómez et al. 2005), and/or variation in fatty acid composition (e.g. oleic acid) (Lanza et al. 1992; Youngsteadt et al. 2011). The latter component has been shown to override the potential ‘reward’ element of the interaction, as some ant species were shown to disperse seeds high in oleic acid, despite no elaiosome (reward) being present (Turner and Frederickson 2013). Leal et al. (2014) showed that high-quality seed dispersers were able to preferentially target seeds with a higher reward (elaiosome mass), whereas low quality seed dispersers showed no discernible preference.

It is therefore plausible that plants have evolved specific traits to target specific dispersers through the modification of their elaiosome traits. Further research is needed to determine whether ants select seeds based on chemical composition alone, as plants within the same species can vary in their elaiosome composition (Boulay et al. 2006).

1.4 IMPACT OF INVASIVE ANTS ON MYRMECOCHORY

Hölldobler and Wilson (1990) famously stated that, “the biggest enemy of ants is other ants”. This is quite apt given that the first organisms to feel the effect of invasive ants (i.e. species become established in regions where they are not native) are the native ant species. Invasive ant species have been widely reported to displace native species (review in Holway et al. 2002) resulting in knock-on effects in a wide array of associated ecosystem services. Of the 19 recognised global invasive ant species (Global Invasive Species Database 2018), most studies to date have focused almost exclusively on two invasive ant species: the Argentine ant (*Linepithema humile*) and the red imported fire ant (*Solenopsis invicta*). Their impacts on myrmecochorous process are broadly considered to be either direct (i.e. their inability to disperse seeds effectively) or indirect (i.e. displacement of native myrmecochorous seed dispersers).

1.4.1 Direct effects

Invasive ant species are widely recognised as inefficient seed dispersers, that may produce a wide array of detrimental effects on the process of myrmecochory (review in Mark and Olesen 1996). The reason for this is that many invasive ant species are seed predators rather than dispersers. For example, both the red imported fire ant (*S. invicta*) and the tropical fire ant (*Solenopsis geminata*) consume seeds, which form up to 30% of their diet (Tennant and Porter 1991; Vogt et al. 2002). In some instances, invasive ants may

be attracted to a seed's elaiosome, but rather than disperse the seed, they feed on the elaiosome *in situ* (termed elaiosome robbing). For example, the little fire ant (*Wasmannia auropunctata*) is too small to provide any discernible seed dispersal service but is attracted to elaiosomes which it then consumes *in situ*. By removing the elaiosome, the seed is not dispersed and moreover will no longer attract an ant species which could have dispersed it (Horvitz and Schemske 1986). Although some mutualistic interactions do occur, the invasive ant species often removes fewer seeds and at a slower rate than the sympatric native seed dispersers. For example, the Asian needle ant (*Brachyponera chinensis*) removed less than 70% of seeds than the native seed-dispersing ant (*Aphaenogaster rudis*) in pine forest ecosystems (Rodriguez-Cabal et al. 2012). Invasive ants may also only transport seeds relatively short distances, resulting in fewer seeds reaching the ant nest. For example, only 7.4% of seeds removed by the invasive Argentine ant (*L. humile*) reach a nest; with 50% of the transported seeds being abandoned on the soil surface (Gómez and Oliveras 2003). As a result, seeds in invaded regions spend more time on the soil surface and are therefore more prone to predation and other destructive forces, compared with seeds in non-invaded regions (Breytenbach 1986; Christian 2001).

The poor dispersal ability of invasive ants has been attributed to the fact that these ants tend to be smaller than the native seed-dispersing ants that they exclude (Ness et al. 2004). Therefore, not only are invasive ants dispersing fewer seeds, with fewer reaching places suitable for germination and establishment, but the invasive ants might also be excluding plant species with seeds within a certain range of traits (e.g. large seed size). This mismatching could be potentially further exacerbated by the spread of myrmecochorous plant species that display traits (e.g. smaller seed size) which are more favourable for dispersal by invasive ant species.

1.4.2 Indirect effects

Failure of invasive ants to disperse seeds directly is further compounded by the fact that native high-quality seed-dispersing ant species are often displaced during ant invasions (Christian 2001; Gómez and Oliveras 2003). Native ant species that coexist with the invasive ant species often show either no interest in seed dispersal or reduced seed-dispersal ability (Christian 2001). In fact, native ants typically show the same seed trait bias exhibited by the invasive ant species (Christian 2001). Changes to the ant community structure, as a product of invasion, can therefore directly alter the dispersal potential of different plant species, which in turn can modify the wider plant community structure. It is, however, difficult to quantify the full effects of an ant invasion without considering the dispersal

potential of seeds in non-invaded ant communities. For example, ant invasions often also result in the displacement of both antagonistic (e.g. seed predators) and mutualistic (e.g. seed dispersers) native ant guilds (Christian 2001; Holway et al. 2002; Gómez and Oliveras 2003; Rodriguez-Cabal et al. 2012). Therefore, while invasion might result in fewer seeds being dispersed, potentially fewer seeds might be predated upon as well. Likewise, invasive ants may disrupt other aspects of the plants' reproductive biology (such as pollination) through the displacement of native mutualists, resulting in fewer seeds available in the first place (Lach 2008; Hansen and Müller 2009).

Invasive ants pose a significant threat to the integrity of natural ecosystems. Taking into consideration both the potential direct and indirect effects of invasive ants on myrmecochorous processes, it becomes clear that it is not always easy to accurately predict their full impact; this is particularly so as we often do not know the extent of the threat, or if indeed how this threat varies across different communities. We therefore require more empirical studies in order to gain a better understanding of the full extent of their impacts on natural ecosystems and supporting ecosystem services, such as myrmecochory.

1.4.3 Argentine ants in Mediterranean systems

The Argentine ant (*L. humile*) is a common invader world-wide. Originating from South America, this inconspicuous species has managed to spread globally to such an extent that today it can be found across six continents and several oceanic islands (Suarez et al. 2001). While this species is generally associated with human-modified and disturbed habitats (Holway et al. 2002), it can also be found in a wide array of natural and pristine habitats (De Kock and Giliomee 1989; Holway 1998; Christian 2001; Gómez and Oliveras 2003). It is commonly discovered in regions with a Mediterranean climate (Tsutsui et al. 2000) which, coincidentally, are areas that often possess high numbers of myrmecochorous species (Lengyel et al. 2009).

The Argentine ant modifies the effectiveness of myrmecochory. A comparative study by Rodriguez-Cabal et al. (2009) showed that the Argentine ants' presence can result in a 92% reduction in the number of native ant seed dispersers. In turn, this can lead to a reduction in both the distance and likelihood of seeds reaching ant nests (Rodriguez-Cabal et al. 2009; Gómez et al. 2003). More often than not, by changing the ant community structure, Argentine ants are able to influence the propensity for certain plant species to be dispersed. For example, Rowles and O'Dowd (2009) showed that interaction between Argentine ants and diaspores was dependent on both seed size and elaiosome reward, with

large seeds eliciting a mass recruitment response, whereby the elaiosome is consumed *in situ*. By contrast, small seeds are dispersed and buried in shallow nests. Ostensibly it appears that the Argentine ant does not stop the dispersal processes, but rather it alters the nature of the ant-plant mutualism; the concern therefore is that over time invasions could lead to a loss of both plant species and seed phenotypes, together with the potential to facilitate the spread of non-native invasive plant species.

Recent records from newly established Argentine ant populations (e.g. Fulham in the UK (Fox and Wang 2016)) along with climate change predictions, means that we are likely to see ongoing and mostly likely increasing reports of this invader. Continuing work is therefore needed to identify vulnerable plant populations that lack the traits suitable for dispersal by this invader.

1.5 CONCLUSION

Myrmecochory is a diffuse and widespread dispersal syndrome, practiced by a large number of ant and plant mutualists. This form of mutualism is mediated by both ant and plant traits. Increased spread of invasive species (ant and/or plant), and displacement of native species is imposing novel selection pressures on ant-plant mutualisms, which means it is likely to lead to shifts in plant community structure over time. This, in turn, could produce knock-on effects in the wider ecosystem, with impacts well beyond just the ants and the plants that were initially affected.

Despite a research history stretching back several decades, our understanding of how and why myrmecochorous species evolved remains nascent. While many ant species participate in this interaction, only a few of these species are documented as providing a beneficial service for plants. Temperate regions have so far been the main focus of myrmecochorous research to date. More empirical studies are required in other regions, with a focus on hotspot areas for biodiversity such as South Africa; as well as regions where myrmecochory is less prevalent such as Central Africa and Asia. In doing so we may be able to gain a broader insight into some of the underlying drivers and evolutionary aspects that shape the process and development of myrmecochory.

1.6 QUESTIONS ADDRESSED IN THIS THESIS

This thesis is comprised of five data chapters and a general discussion which summarises the main findings on the evolutionary history of myrmecochory, along with an

assessment of some of the current impacts *Linepithema humile* has on ant-mediated seed dispersal processes, together with suggestions for future research avenues.

1.6.1 Chapter Two: Myrmecochory from the ants' perspective: a phylogenetic review

Our current understanding of the evolution of myrmecochory as an adaptive trait is limited to work on the biogeographic, taxonomic and phylogenetic distribution of myrmecochory within the angiosperms (Dunn et al. 2007; Lengyel et al. 2010). To understand the evolution of this ant-plant mutualism from the ants' perspective, I constructed a dataset based on the available records of myrmecochory. These data were then used to identify how many possible independent origins of myrmecochory there are within the ant phylogeny. I found that as a behavioural trait, myrmecochory is a widespread phenomenon within the ant phylogeny, with at least ten independent origins. However, the resolution of this study was confounded by a high level of geographic bias in the available literature to temperate regions.

1.6.2 Chapter Three: Effects of an ant invader on native ant community structure

Myrmecochorous seed dispersal processes within a region are ultimately contingent upon the structure of the ant community. Predicting the effect of *L. humile* on native ant community structure is therefore a vital step in understanding the potential knock-on impacts to ecosystem services, such as myrmecochory. Here I explore the impacts of *L. humile* on ant community structure at two geographic regions: South Africa and Spain. The findings of this study suggest that the level of impact can vary considerably across Mediterranean ecosystems; however, a notable observation is that both South Africa and Spain shared one common theme: where *L. humile* were present, the native ant species most commonly associated with myrmecochorous processes were absent.

1.6.3 Chapter Four: Invasive ants alter seed dispersal dynamics

Accurate assessment of the impacts of *L. humile* and other invasive ant species on seed dispersal processes are often based solely on one aspect of the seed dispersal process. In doing so, such studies risk potentially under or overestimating the impact of the invaders. To gain a better understanding of the effects of *L. humile* on the dynamic nature of the seed dispersal process, I conducted a field study in Girona (northern Spain) looking at the impacts of *L. humile* across three stages of the seed dispersal process. Results show that *L. humile* is a poor-quality seed disperser across all three stages of the dispersal process. This suggests that the impact of *L. humile* on Mediterranean ecosystems could be greater than initially reported.

1.6.4 Chapter Five: Interactions between multiple invasive alien species drive cascades in an ant-plant mutualism

Habitats prone to invasion are often subjected to several invasive species at once. Yet, many of the studies contributing to our growing understanding of invasive processes focus on individual impacts, rather than potential invader-invader interactions. In this chapter I explore invader-invader interactions between ant communities invaded by *L. humile* and several invasive myrmecochorous *Acacia* plant species. Results suggest that invaded ant communities have a preference for seeds of invasive *Acacia* plant species over those of the sympatric native plant species; this preference may enhance the invasion potential of these invasive plant species. This therefore suggests that the invasion by one species (the ant) may therefore be creating cascading changes through the plant community by facilitating the spread of invasive plants; this is an example of how one invasive species may alter the conditions of the local environment they invade, such that it is more permeable to other invasive species.

1.6.5 Chapter Six: Variation in myrmecochorous seed traits: evidence from chemical selection and ant community preference

Differences in seed preference are likely to be driven by differences in the traits of both myrmecochorous plant species and ant communities. These traits structure the nature of the biotic interactions. Here I gather data on both the physical and chemical characteristics of seeds and examine whether these traits can explain the ant-seed removal responses seen in Chapter Five. There were no clear consistent differences in the chemical and/or physical traits of the invasive and native plant species; however, the range of seed traits favoured for dispersal in the invaded ant communities were more restricted compared with those in non-invaded communities. This suggests that communities invaded by *L. humile* lose not only plant species, but specific seed traits as well.

1.6.6 Chapter Seven: What have we learned about the impacts of Argentine ants on seed dispersal within Mediterranean ecosystems?

In this final chapter I summarise the main findings and insights of my research and discuss their implications within the wider context of myrmecochorous seed processes, together with considerations for the threats that invasions pose to this important but vulnerable ecosystem service. Furthermore, I identify gaps in our knowledge and highlight future research areas.

CHAPTER TWO

2. MYRMECOCHORY FROM THE ANTS' PERSPECTIVE: A PHYLOGENETIC REVIEW

2.1 ABSTRACT

Ant-plant mutualisms are some of the best examples of reciprocity in the natural world. However, most of our understanding surrounding the origins of these mutualisms comes from either the plants' perspective or from specialised co-evolved mutualisms, such as the fungus-growing ant symbiosis and bull thorn *Acacia* complexes. By considering only one of the protagonists in this interaction we make the biased assumption that the other is passive. This bias in the literature is perhaps best represented by our understanding of the evolution of myrmecochory, which to date has been almost exclusively examined from the plants' perspective. Here I address this shortcoming by also assessing the diversity of myrmecochory from the ants' perspective. I performed an ancestral state reconstruction using records of myrmecochory in ants from the literature to determine how widespread myrmecochory is across ant taxa, and to identify the minimum number of times myrmecochory evolved within the ant phylogeny. This analysis suggests that myrmecochory is a widespread phenomenon, occurring in at least 63 ant genera, with at least 10 independent origins, although this number varies considerably depending on the myrmecochorous classification system used. Overall, the findings provide quantitative evidence to support previous suggestions that myrmecochory is a diffuse interaction between many ant and plant species. However, in order to better understand the mechanisms and processes that drive this important mutualism, a more cosmopolitan approach to data collection is required, focusing especially on understudied regions such as South Africa.

2.2 INTRODUCTION

Global ecosystems are made up of a myriad of biotic interactions, many of which are mutualistic. Mutualisms are broadly defined as the interaction between two different organisms, in which both partners benefit from the activity of the other (Boucher 1988). These mutualistic interactions are a widespread phenomenon, occurring across all levels of biological organisation (Boucher 1988; Szathmáry and Smith 1995; Herre et al. 1999). However, within mutualistic interactions there is considerable variation in their form, ranging from diffuse reciprocal interactions between several species, to highly specialised co-evolved associations between a pair of species. Understanding how these mutualisms arise and are maintained is a major challenge in evolutionary biology and ecology today.

The increasing availability of molecular genetic data generates greater insight into the occurrence of mutualistic interactions. Increasingly researchers are turning to phylogenetic approaches to understand the complex patterns of ecological transmission and evolutionary associations seen in mutualisms (Dunn et al. 2007; Lengyel et al. 2009; Pringle et al. 2012; Chomicki and Renner 2015; Rubin and Moreau 2016; Branstetter et al. 2017; Ward and Branstetter 2017). For example, phylogenetic study of the obligate mutualism between fig trees (*Ficus*) and their fig wasp pollinators has revealed a high degree of congruence and specificity between both groups, indicating pairwise co-evolution at both global and fine scales (Herre et al. 1999). Conversely, the phylogenetic relationship between leaf-cutter ants and their symbiotic fungi show that in some lineages there is no clear concordance or specificity between partners (Hinkle et al. 1994; Mikheyev et al. 2007). This variation within mutualistic interactions is most likely due to differences in the evolutionary forces that shape and maintain them (e.g. differences in the magnitude of the benefit to each partner). Phylogenetic analyses therefore not only allow for a multispecies approach to understanding mutualistic interactions, but also help identify the historical and evolutionary factors that may be driving co-evolution.

Ants and angiosperms are highly speciose and make up a large proportion of global biomass. Of the 13,000 known ant species, many engage in mutualistic interactions with angiosperms. One such mutualism is myrmecochory – ant-mediated seed dispersal. In this interaction, plants produce a specialised appendage (an elaiosome), which attracts the ant to the seed; the ant then removes the seed to its underground nest where the elaiosome is consumed and the seed is discarded, intact, in a place where it can germinate (Beattie 1985). This trait has been extensively explored from the plants' perspective. Dunn et al. (2007) and Lengyel et al. (2009) suggest that this trait has evolved independently at least 101 times

in the angiosperm plant phylogeny and is known to be the primary seed dispersal mechanism for more than 11,000 plant species worldwide (Lengyel et al. 2010). Despite this understanding of the diversity and origins of myrmecochory from the perspective of plants, little is known about patterns of evolution of myrmecochory from the perspective of the ants.

Myrmecochory was first described by Sernander (1906) who based his observations of seed dispersal in temperate European forests. Culver and Beattie (1978) laid the foundation for the understanding of myrmecochory, basing their studies predominantly in Northern Hemisphere environments. However, most myrmecochorous plant species (>50%) are found in the Southern Hemisphere in arid regions of South Africa and Australia (Lengyel et al. 2010). Recent studies on the ecology of seed dispersal in other geographic areas (Le Corff and Horvitz 1995; Christian 2001; Leal et al. 2007; Dominguez-Haydar and Armbrrecht 2011) suggest that ant partners vary across geographic regions, with many associations being typically specialised, whereby plant species rely on only a few specific ant species for dispersal of their seeds (Gove et al. 2007; Ness et al. 2009). For example, displacement of the pugnacious ant (*Anoplolepis custodians*) by the invasive Argentine ant (*Linepithema humile*) within regions of South Africa has resulted in the loss of seed dispersal services for many large-seeded endemic plant species (Christian 2001). This illustrates how some plant species rely on only a single ant species for seed dispersal.

In this study, I explore how widespread seed dispersal as an ant adaptive trait is within the ant phylogeny. The aims were to identify which ant genera disperse seeds and determine the quality of their seed-dispersal service (Aim 1); to determine the phylogenetic relationships between myrmecochorous ant genera and determine the number of evolutionary origins of myrmecochory (Aim 2). To achieve this, I conducted a literature review using the Web of Knowledge to construct a database on how many times ant-mediated seed dispersal has evolved in Formicidae (for Aim 1); I then used ancestral state reconstruction analyses, to take account of shared ancestry, to determine whether this trait is phylogenetically constrained or widespread (for Aim 2). This information is essential for giving us a better understanding into the evolution of myrmecochory.

2.3 METHODS AND MATERIALS

2.3.1 Database construction on occurrence of ant-mediated seed dispersal

2.3.1.1. Literature search

Records of myrmecochory within the ant genera were compiled from a search on the Web of Knowledge database, for all years (1900-2017) using the search string “TS= myrmecoch* OR TS= (ant AND seed AND dispers*)”. I gathered all available information on: (i) seed-dispersing ant genera (subfamily/genus/species/study location); (ii) dispersal quality (number of seeds dispersed/number of seeds interacted with/total number of seeds in the study); and (iii) myrmecochorous plant genera (family/genus) associated with that specific ant.

I imposed several restrictions on the data that was included. Firstly, I restricted the analysis to cases where the ants interact directly with the elaiosome, as this is a trait on which the co-evolved mutualism has evolved. Thus, I excluded studies or dispersal events in which ants disperse fruit pulp (with seed attached) or collected seeds from vertebrate excrement. Secondly, I focused on the first stage in the seed dispersal process – seed removal from a source - irrespective of any data as to whether the seed was placed in a site favourable for germination and establishment. This focus was taken because this level of information was missing in most studies and optimal seed placement is likely to vary between ecosystems. Thirdly, I only included field studies, as laboratory studies have been shown to often modify insect behaviour, creating behavioural artefacts (Jandt et al. 2015) and fail to account for other abiotic or biotic barriers that might influence seed dispersal (e.g. competition), (Warren et al. 2014). Finally, studies vary in their collection methods (e.g. *indirect* cafeteria experiments vs. *direct* seed choice) and the method influences how an ant would interact with seeds in a species-specific manner. I therefore only used studies that used cafeteria experiments to quantify this metric.

2.3.1.2. Presence/absence of myrmecochory

Each ant genus was assigned a discreet binary metric of presence/absence for myrmecochory. To do this I considered any record of an ant removing a seed as evidence for seed dispersal (broad or *sensu lato* definition). This information was limited to genus level because species-level resolution of ant phylogeny is only available for a few genera, and many studies did not report the specific ant species. The data were too sparse to impose a minimum reporting threshold, so a single study on a species was taken as a positive record of myrmecochory in that genus.

2.3.1.3. Quality of seed-dispersal

For positive records of myrmecochory, I generated two continuous metrics – *interaction strength (IS)* and *dispersal score (DS)*. Firstly, *IS* represents the number of seeds removed as a proportion of the number of seeds interacted with. This method was used to quantify the interaction potential between individual ant and plant genera. By contrast, *dispersal score (DS)* represents the number of seeds removed as a proportion of the total number of seeds in that study (e.g. number of seeds removed by species, divided by the total number of seeds used in the study). This method takes account of the fact that although some ant genera seldom interacted with seeds, when they did, they moved all seeds (e.g. *Hypoponera* interacted with one seed, and moved that one seed (Le Corff and Horvitz 1995)). This means that a *dispersal score* based on the number of seeds interacted with, divided by the number of seeds removed, would create false positives. Thus, this measure is expressed as a percentage and gives us a continuous metric measure for the propensity for each ant genus to disperse seeds.

Considering that many ant genera are known to be fully or partially granivorous (i.e. consume seeds), this is likely to influence the quality of the seed dispersal service (i.e. seed survival). I therefore also assigned each ant genus a discreet ternary metric of seed dispersal absence/present-poor/present-good. To do this I inferred *disperser quality (DQ)* based on the propensity for each ant genus to remove and consume seeds. Under this classification system an ant must remove the seed, but not consume it to be considered a seed disperser (narrow or *sensu stricto* definition). Disperser quality was calculated as the sum of the ants' propensity to remove seeds (I) and the ants' propensity to consume seeds (II), with good dispersers scoring 2 or more, poor dispersers scoring 1, and ants that don't disperse seeds scoring 0.

- I. Each ant genus was graded on its propensity to remove seeds where $DS = 0$ [No seeds dispersed], $DS = 1$ [seeds seldom removed; DS between 0.01% and 9.99%], $DS = 2$ [seeds occasionally removed; DS between 10% and 19.99%], and $DS = 3$ [seeds often removed; DS over 20%].
- II. Each ant genus was graded on its known propensity to consume seeds, where 0 = no records of granivory, -1 = opportunistically granivorous (e.g. *Pheidole* and *Tetramorium*) and -2 = primarily granivorous (e.g. *Messor* and *Pogonomyrmex*).

2.3.1.4. Tree construction

The ant phylogeny used was taken from the Moreau and Bell (2013) molecular tree because it provided the best available genus level resolution for this group. The plant

phylogeny was constructed using the Phylomatic tree (R20120829) on the online Phylomatic (version 3) platform (Webb and Donoghue 2005). Tree branch lengths for the plant phylogeny were calculated using the *phylocom* (version 4.2) package (Webb et al. 2008).

2.3.1.5. Ant-plant complexes

I explored the relationship between ant and plant genera using the Interactive Tree of Life (iTOL) platform (Letunic and Bork 2007). A heat map was constructed to visualise the patterns between ant and plant genera using the *IS* metric (see above). Additionally, the number of ant-plant records were compared, based on the number of studies conducted, using a linear regression model in R (version 2.3.2) platform (R Core Team 2017).

2.3.2 Ancestral state reconstruction for the evolution of ant-mediated seed dispersal

I resolved the distribution of myrmecochory within the ant phylogeny using the Maximum Likelihood (ML) method, with continuous-state and discrete-state models, under the Ace function of the *APE* package, version 5.0 (Paradis et al. 2004). The ML method was selected as it estimates the ancestral states based on the statistical likelihood of the known phenotypes, and assumes that not all events are equally likely to occur (Pagel 1999). Calculated *DS* (see section 2.3.1.3) were used to model the continuous trait evolution of seed dispersal in the ant phylogeny. By contrast, in order to identify the number of independent origins, I used the discrete metrics. For the broad definition of myrmecochory, a tree was constructed using the all-rates-equal model (ER), with a binary discrete characteristic (see section 2.3.1.2): (0) absent and (1) present. In this form of analysis, the transition between character states (i.e. from non-seed disperser to seed disperser) is identical. Alternatively, for the narrow definition of myrmecochory, a tree was constructed using the symmetrical model (SYM), with a ternary discrete characteristic (see section 2.3.1.3): (0) myrmecochory absent; (1) myrmecochory present – low-quality; or (2) myrmecochory present – high-quality. In this way the progression from character states is transitional (i.e. non-seed disperser, to low-quality seed disperser, to good quality seed disperser). Because some ant genera are paraphyletic (e.g. *Camponotus*), the discrete and continuous characteristic was mapped onto each node. Discrete-state models were analysed using a Markov chain Monte Carlo (MCMC) method, otherwise referred to as stochastic character mapping (Huelsenbeck et al. 2003) for 100,000 generations. Finally, in all cases the phylogenetic signal (model vs. null model, using a log-likelihood test) was determined using the *phylosig* function in the *phylotools* (version 0.1.2) package (Revell 2012), as well as the *fitDiscrete* function in the *geiger* (version 2.0.6) package (Harmon et al. 2008).

2.4 RESULTS

2.4.1 Database of myrmecochorous ant genera

In total, 160 papers published between 1975 and 2017 showed evidence of myrmecochory and were used in this study (see Appendix 1). I identified evidence of myrmecochory within 63 different genera, which represents 21% of all ant genera, across six subfamilies within the Formicidae (Table 2.1).

Table 2.1: Prevalence of myrmecochory within ant subfamilies. Records of myrmecochorous ant genera within the six ant subfamilies. *Taken from antweb.org

Subfamily	Myrmecochorous genera	Number of all genera*	Myrmecochory (%)
Dolichoderinae	10	28	36
Dorylinae	1	26	4
Ectatomminae	2	4	50
Formicinae	16	51	31
Myrmicinae	24	143	17
Ponerinae	10	47	21
<i>Total</i>	<i>63</i>	<i>299</i>	<i>21</i>

These ant genera were collectively responsible for the dispersal of 109 plant genera, across 52 plant families. Some symmetrical and specialist relationships (one ant, one plant) were observed; however, most observations were asymmetrical with partners being generalists in both directions (one ant to multiple plants, and one plant to multiple ants) (Fig. 2.1).



Figure 2.1: Heatmap based on the records of myrmecochory in the ant phylogeny (y-axis) and their associated plant families (x-axis). Heat map based on the ant – plant *interaction strength (IS)* of 55 ant genera and 52 plant families. Seven ant genera were omitted due to their unresolved position in the phylogeny and/or insufficient data to determine *IS*. Depth of colour indicates the strength of the interaction, whereas no colour indicates no known record.

Notably, plant families such as Euphorbiaceae and Fabaceae, which have a worldwide distribution (Angiosperm Phylogeny Group 2009), showed a loose association with at least 26 different ant genera. Furthermore, the *interaction strength* (i.e. interaction potential between individual ant genera and plant families), varied considerably across taxa, and likely reflects the geographic differences between ant-plant complexes. For example, in North American ecosystems, the genus *Aphaenogaster* is widely cited as the primary (high-quality) seed disperser (Culver and Beattie 1978); however, in both European Mediterranean and Australian ecosystems it is considered a more opportunistic (low-quality) seed disperser (Hughes and Westoby 1992b; Boulay et al. 2006).

While this lack of pattern likely represents the diffuse nature of ant-plant associations in myrmecochory, it could also be because studies are heavily skewed towards specific geographic regions (Fig 2.2; see Appendix 1) or a few well-studied ant genera (Fig. 2.3). For example, out of 160 studies a total of 33 were based on records of *Aphaenogaster rudis* in North America. The number of ant-plant species interactions recorded was strongly correlated with the number of studies sampled (Linear regression: $F = 260.1$, $p < 0.001$) with an R^2 of 0.81.

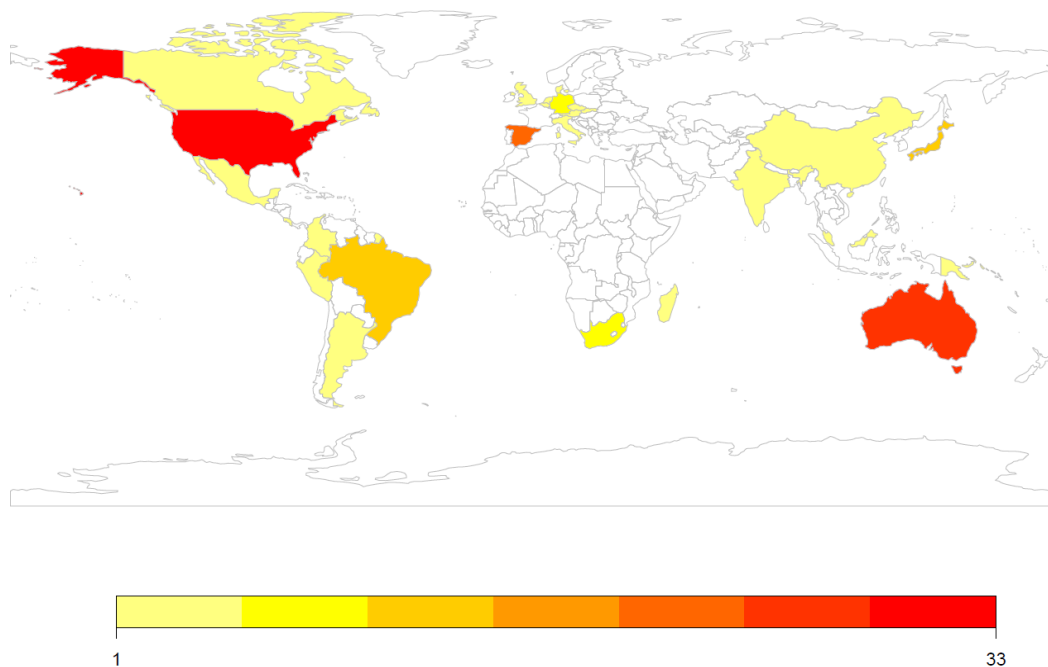


Figure 2.2: Distribution of myrmecochorous records with a heat map showing number of studies in each geographic region.

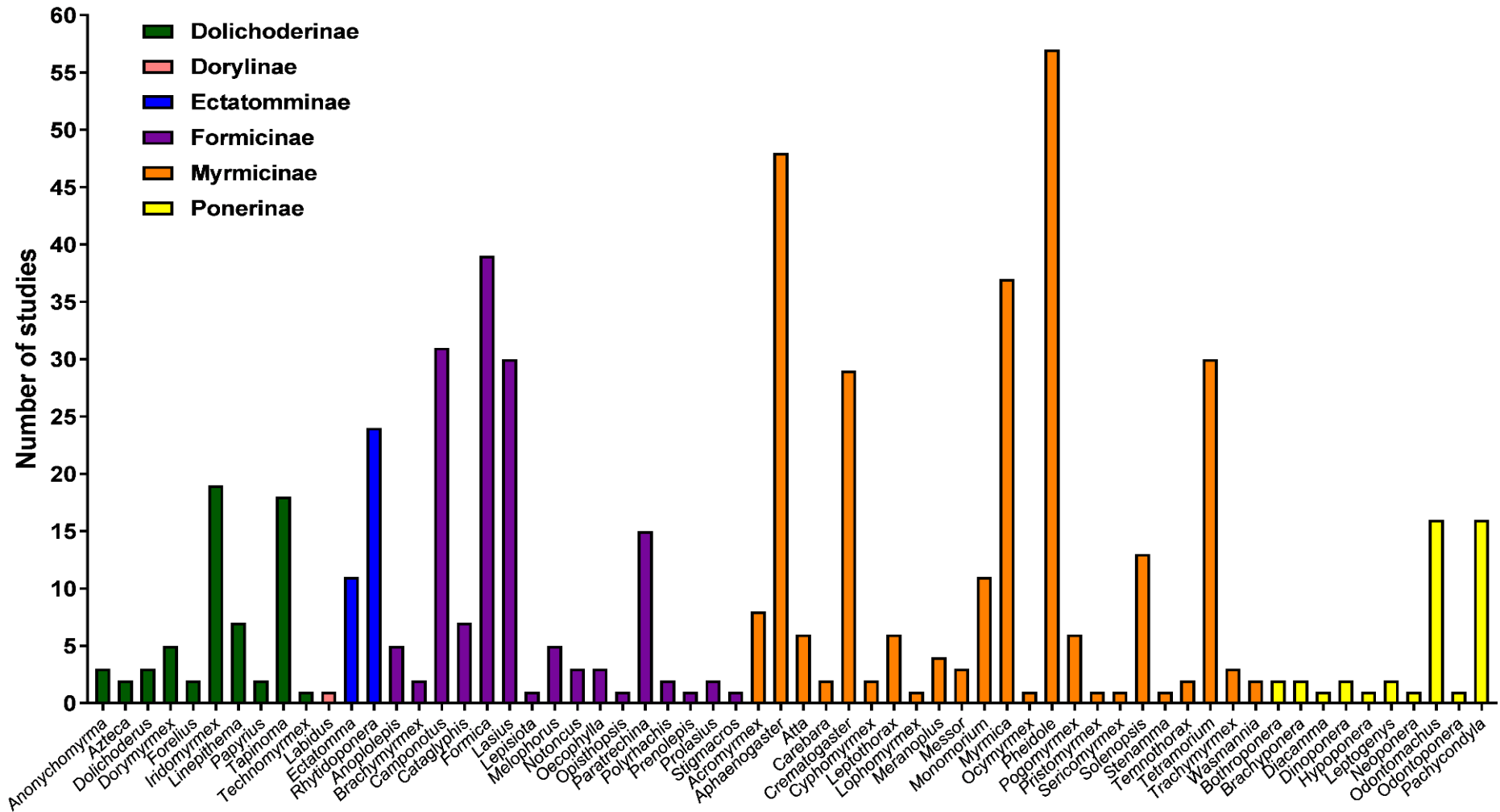


Figure 2.3: Distribution of myrmecochorous records showing the number of studies in which an ant genus is implicated in seed dispersal.

2.4.2 Evolution of myrmecochory

2.4.2.1. Is myrmecochory phylogenetically dispersed or clumped?

Analysis of myrmecochory as a continuous characteristic, using the generated *DS* for each ant genus, indicates that it is dispersed across the ant phylogeny in both old and new world lineages (Fig. 2.4). This distribution, however, did not differ significantly from the null model (Log-likelihood test: $F = 0.353$, $p = 0.164$), indicating that as a continuous trait, there is no phylogenetic signal, (i.e. that *DS* is not influenced by a phylogenetic relationship between ant genera).

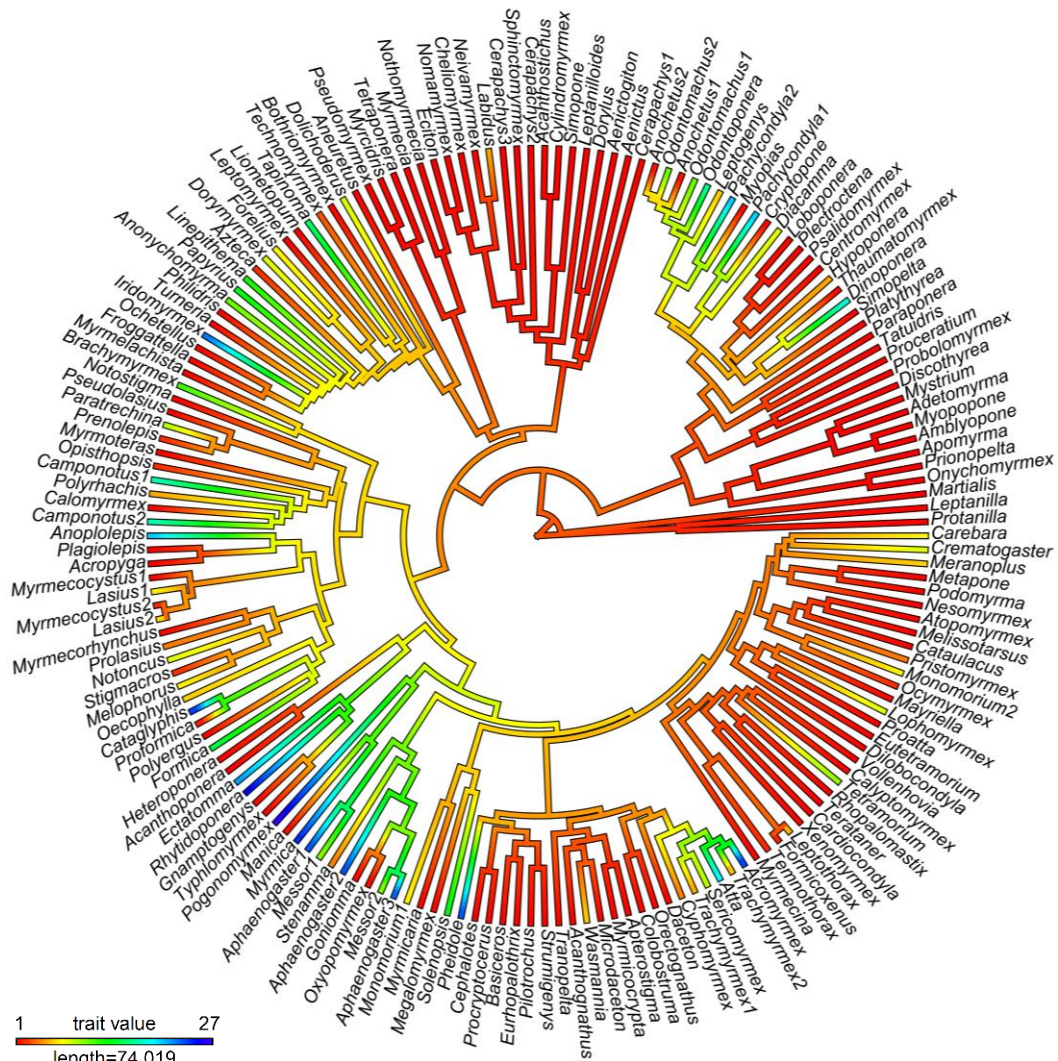


Figure 2.4: Myrmecochory is phylogenetically dispersed, occurring in ant genera from both old and new world lineages. Myrmecochorous ant phylogeny, based on the molecular reconstruction by Moreau and Bell (2014), was re-drawn using R; paraphyletic subgroups are represented with a number next to the genus, in order to distinguish between the subsidiary clades. Trait value, which represents the propensity to disperse seeds (based on a dispersal score), is denoted using the following colour gradient: red indicates the absence of myrmecochory; yellow (low) to blue (high) indicates the presence of myrmecochory. Two ant genera (*Lepisiota* and *Leptogenys*) were omitted due to their unresolved position in the phylogeny.

2.4.2.2. How many times has myrmecochory evolved within the ant phylogeny?

Analysis of myrmecochory as a binary discreet characteristic (presence/absence), suggests, under a broad definition of ant-mediated seed dispersal, that myrmecochory has evolved at least 29 times independently within the ant phylogeny, with the most independent origins appearing within Myrmicinae (n = 18) and Ponerinae (n = 6) subfamilies, respectively (Fig. 2.5). Under this classification there was a strong phylogenetic signal (Log-likelihood test: $F = 10.59$, $p = 0.001$).

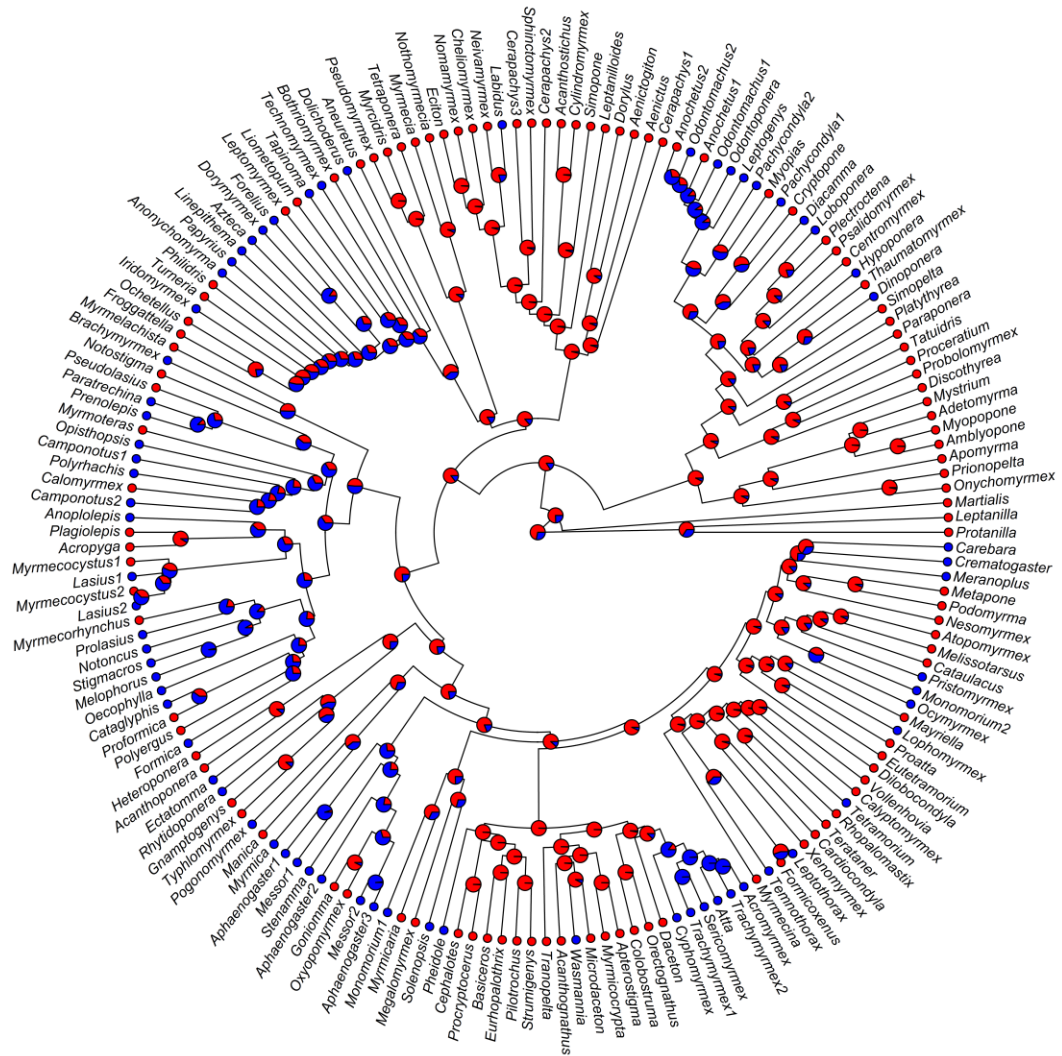


Figure 2.5: Myrmecochory in the broad sense has evolved multiple times within the ant phylogeny. Ancestral state reconstruction of ant-mediated seed dispersal trait using a binary discreet characteristic, under a broad *sensu lato* classification system: red (absent) and blue (present). Two ant genera (*Lepisiota* and *Leptogenys*) were omitted due to their unresolved position in the phylogeny.

Using the narrow definition of ant-mediated seed dispersal - with ternary discreet characteristics (absence/present-low/present-high) - high-quality seed dispersal events (i.e. high propensity to move seeds, low propensity to consume seeds) were found to be restricted to only 12 (4% of all) ant genera (see Appendix 1). After taking the paraphyletic distribution of some of the ant genera into account, myrmecochory has appeared only ten times independently within the ant phylogeny, with the greatest number of independent origins appearing in the Formicinae ($n = 3$), Myrmicinae ($n = 3$) and Ponerinae ($n = 3$) subfamilies respectively (Fig. 2.6). Likewise, under this classification there was also a strong phylogenetic signal (Log-likelihood test: $F = 12.53$, $p = 0.0003$)

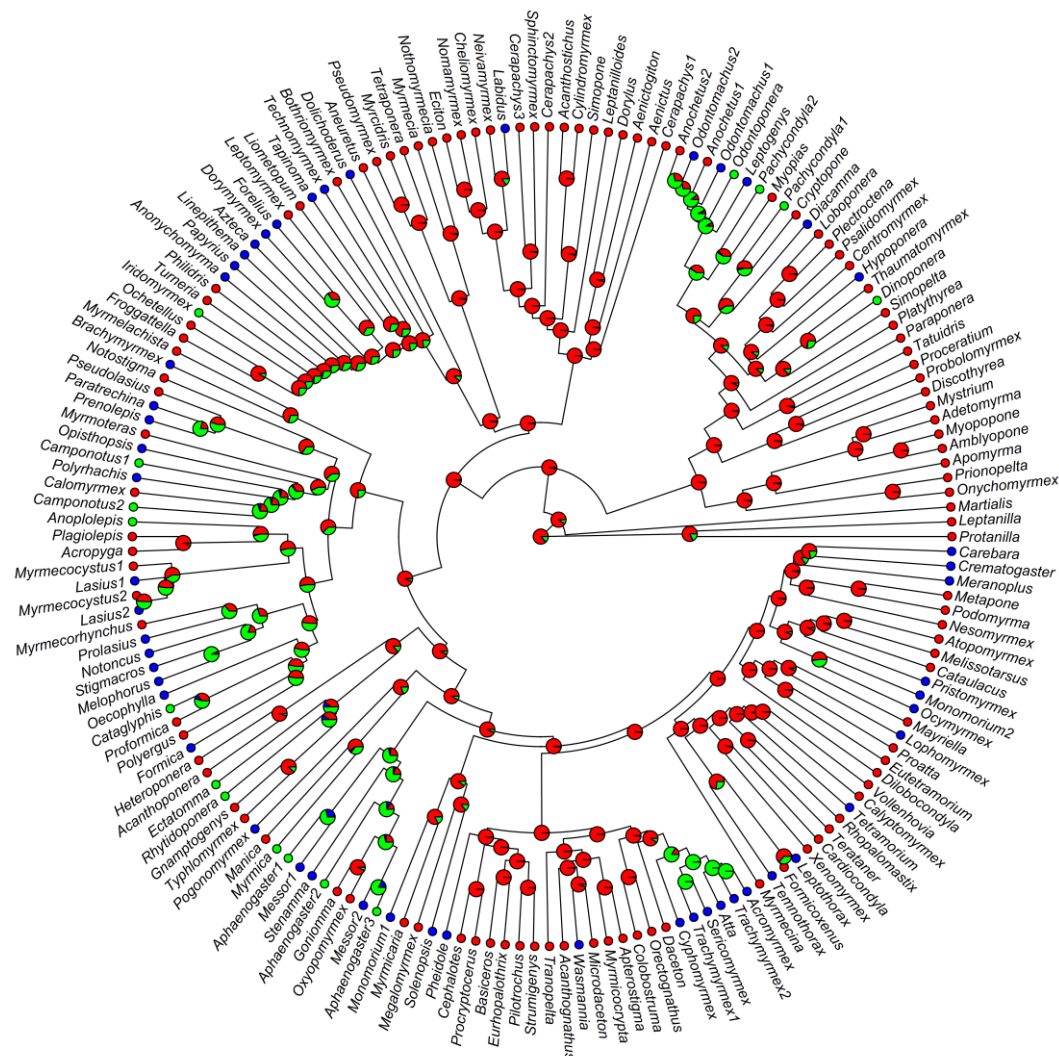


Figure 2.6: Myrmecochory in the narrow sense has evolved multiple times within the ant phylogeny. Ancestral state reconstruction of ant-mediated seed dispersal trait using a ternary discreet characteristic, under a narrow classification system: red (absent), blue (present- low), and green (present- high). Two ant genera (Lepisiota and Leptogenys) were omitted due to their unresolved position in the phylogeny.

2.5 DISCUSSION

Ant-mediated seed dispersal is a key ecosystem service provided by ants around the world. This study attempts to determine how many times myrmecochory has evolved in the ants, and whether there is a strong phylogenetic signal in the evolution of myrmecochory such that it is more likely to evolve in specific clades. The analyses reveal that myrmecochory has evolved independently up to 29 times in the ant phylogeny, and that it is found consistently in at least five of the main ant subfamilies. I found some evidence of phylogenetic signal at the genus level, when using a discreet classification, supporting the suggestion that myrmecochory is a specialist trait of specific types of ants. The patchy geographical distribution of myrmecochorous records and the fact that dispersal score for some ant genera were based solely on one record, highlight some key knowledge gaps on this important ecosystem service.

Using a broad definition of myrmecochory as an adaptive ant trait (whereby the interaction is more commensal (rather than symbiotic) in nature), I found myrmecochory to be widespread throughout the ant phylogeny, being described in over 20% of ant genera. This scattered distribution is likely to reflect the early origins of the interaction, as it has been hypothesised that myrmecochory evolved from antagonistic interactions between ants and seeds (Levey and Byrne 1993). This hypothesis (i.e. that antagonistic interactions with plants were ancestral in ants), could help explain why both seed dispersers (low propensity to consume seeds) and seed consumers (high propensity to consume seeds) were found to be clustered together phylogenetically; for example, within the Myrmicinae ant subfamily, both *Aphaenogaster* (seed dispersers) and *Messor* (seed consumers) are sister genera.

However, under a narrow definition of myrmecochory (whereby interactions are symbiotic (rather than commensal) in nature), it appears to be a specialised ant trait. In this instance, while the trait is still widespread throughout the ant phylogeny, it has considerably fewer independent origins. This pattern may be due to the uneven geographic distribution of ant subfamilies; for example, Myrmicinae and Formicinae are the most ecologically dominant and abundant subfamilies in Northern Hemisphere systems, whereas Dolichoderinae and Ponerinae are more often associated with Southern Hemisphere systems (Andersen 1995; Dunn et al. 2009a). While there is undoubtedly a strong geographic influence as to which ant genus interacts with seeds, certain ant genera (e.g. *Aphaenogaster*) are frequently cited as seed dispersers in both Northern and Southern Hemisphere systems. Notably, *Aphaenogaster* species are cited as high-quality seed dispersers in Northern Hemisphere systems; however, in Southern Hemisphere systems

they are recorded as being low-quality seed dispersers. This discrepancy in part might be due to an elevation bias in studies. For example, Thomson et al. (2016) found that within an Australian site, *Rhytidoponera* was the primary seed dispersing ant genus at low elevations while at high elevations *Aphaenogaster* species were dominant. Elevation and the natural variation in ant community structure (excluding invaded vs. non-invaded) is an aspect seldom studied, in relation to seed dispersal preference, illustrating our lack of in-depth knowledge of this ecological process.

Within the broader framework of mutualistic interactions, myrmecochory fits well within the uni-directional consumer-resource (C-R) model proposed by Holland and De Angelis (2010), alongside other mutualisms such as pollination and ant-lycaenid caterpillar interactions. This framework suggests that resources are produced by the plant (e.g. elaiosomes), and by (the ant) exploiting this resource, the plant benefits from a service (Holland and De Angelis 2010). However, in myrmecochory, most of our understanding of the ant-plant interaction is based largely on the above-ground transport of seeds, rather than on the seed's final placement. This means it is often difficult to confirm any putative positive effects that the ant's behaviour may have on the plant. The loose associations identified in the literature so far are likely because the benefits to ants appear to be rather limited (Oostermeijer 1989; Morales and Heithaus 1998; Bono and Heithaus 2002) when compared with specialised co-evolved ant-plant mutualisms (e.g. fungus-growing ants, bull thorn Acacias); a case in point is that elaiosomes are thought to form a relatively small portion of ant colony diets (Giladi, 2006; Warren and Giladi, 2014). More specifically, with the exception of one study in which the ant species was shown to directly benefit from the plant's survival (Chomicki and Renner 2016), there is very little evidence to support the idea of co-evolution and maintenance of a tight pair-wise two-way obligate mutualism in myrmecochory. In fact, plants might benefit more from such a loose association, as high levels of specialisation on the part of the plant (i.e. dispersal restricted to one ant species) could be detrimental to survival over time, due to nest saturation resulting in increased intra- and inter-seedling competition. Furthermore, this loose association may be easily maintained because elaiosomes are relatively cheap to produce compared with fruits (Goldblatt 1997).

My analyses suggest that myrmecochory evolved at least eight times in the ants. This estimation is likely to be a conservative estimate, and potentially underestimates the true number of independent origins. The reason for this is, firstly, that most of the records of myrmecochorous interactions come from a relatively few well-studied systems. For example, of the three hotspots of myrmecochory reported in the Lengyel et al. (2010) study,

only the temperate forests of North America and Australia have received extensive investigation. In fact, lack of adequate sampling is common for many geographic regions, including Africa, South America and Asia; although South Africa is one of the three hotspots of myrmecochorous flora, only five studies were found in this region. Secondly, by grouping records of species to genus level, possible multiple independent origins within each ant genus will have not been captured. Indeed, several ant genera have a widespread geographic distribution and their role differs according to the environment they are in. For example, in the Iberian Peninsula *Pheidole* are widely reported as the primary seed disperser (Gómez and Oliveras 2003); however, in other systems (e.g. Australia and South America) they are reported as seed predators (Hughes and Westoby 1992b; Aranda-Rickert and Fracchia 2011). This variability in disperser quality is likely to be further confounded because I was unable to include seed placement as a measurement of disperser quality, as this criterion is seldom considered in myrmecochorous studies. Also, what constitutes optimal seed placement is difficult to quantify without in-depth investigation of the seeds' requirements, especially given that it is highly context dependent. For example, seed predation pressure, fire intervals, and/or importance of soil seed banks in plant regeneration may all play an important role in regulating optimal seed placement. Finally, at the analytical resolution currently permitted by the literature, it is likely that certain ant genera listed in Appendix 1 are in fact not seed dispersers at all. This is because some ant genera were classified as seed dispersers from records of only one ant species, this means that there may be insufficient evidence to suggest that seed dispersal is a trait commonly associated with that entire ant genus. Irrespective of these limitations, increased understanding of the mechanisms, or in-site variation between dispersers, may reduce the total number of seed dispersers recorded in this study, whilst perhaps increasing the phylogenetic spread of myrmecochory (and hence the number of independent origins).

This chapter provides a much-needed ants' (as opposed to plants') perspective on the evolutionary patterns and distribution of myrmecochory. The analyses are limited by the quality of the data on ant-seed interactions, and on the geographical and genus-specific bias in the literature. However, irrespective of how myrmecochory is defined (i.e. using a broad or narrow classification), it is clear that there is a strong phylogenetic signal in the evolution of myrmecochory by ants. Future work should focus on encouraging a more cosmopolitan approach to studying myrmecochory, particularly for regions where myrmecochory is less well studied (e.g. South Africa), and in better understanding of the nature of interactions between ants and plants to assess the degree to which the interaction is a true mutualism or not.

CHAPTER THREE

3. EFFECTS OF AN ANT INVADER ON NATIVE ANT COMMUNITY STRUCTURE

3.1 ABSTRACT

Invasive species pose a serious threat to the integrity and function of natural ecosystems. Understanding how these invaders alter natural communities is therefore an important aspect in predicting future outcomes of biological invasions. In this study I investigate the effects of the Argentine ant (*Linepithema humile* Mayr) on native ant community structure within two distinct geographic regions: Spain and South Africa. The main goal of this study was to determine the effect of *L. humile* on the abundance and distribution of native ant species. By comparing invaded and non-invaded ant communities across the two countries, I found that the native ant species more commonly associated with ecosystem services, such as myrmecochory, were absent at invaded sites in both Spain (*Pheidole pallidula*) and South Africa (*Anoplolepis* sp. and *Pheidole capensis*). Overall, the number of native ant species collected in pitfall traps was considerably lower in the presence of *L. humile*. This detected effect was greatest in Spain, where only one native ant species (*Plagiolepis pygmaea*) was found to coexist in invaded sites. By contrast, several native ant species coexisted at sites in South Africa, and the abundance of certain native species appeared to be augmented in the presence of *L. humile*. While these findings, support previous studies that show that *L. humile* generally has a negative effect on the native ant communities, it also clearly highlights how extremely variable the effect can be between geographic regions. As to whether this variability is drive by abiotic (e.g. temperature) or biotic (e.g. resistance of the South African fauna to invasion) conditions clearly warrants further investigation. Future work therefore should expand sampling to more independent invasion zones and explore the idea that the local biotic or abiotic conditions of a region may influence the extent to which native ant communities are affected by the invasion of *L. humile*.

3.2 INTRODUCTION

Non-native invasive species are having a profound negative effect on biodiversity (Bellard et al. 2016) and society as a whole (Essl et al. 2011). For example, invasive insects have been shown to reduce food stocks (Oerke 2006), spread disease (Carpenter et al. 2013; Crowl et al. 2008) and damage infrastructure (Booy et al. 2017). A recent meta-analysis by Bradshaw et al. (2016) estimated that the yearly global cost of invasive insects is at least US\$70 billion. Despite this high financial cost, this value is in fact likely to still fall well below the true costs of invasive insects, as many of their impacts are not easily quantifiable in economic terms; for example, impacts on native biodiversity and disruption to associated ecosystem services.

Impacts resulting from non-native invasive species are often more pervasive than the effects of mere species loss. A recent synthesis on the impacts of invasive plants on community structure revealed that whilst invaded communities were commonly more species poor, they could be at the same time richer in terms of primary production (Vilà et al 2011). For example, displacement of native non-nitrogen fixing plant species in favour of invasive nitrogen fixing plant species, not only alters plant community structure but also alters the underlying ecosystem processes (Vilà et al. 2011; Stinca et al. 2015). Increases in plant productivity as a result of invasion can in turn influence other trophic levels, leading to changes in soil properties and modification of fire regimes through increases in plant biomass (D'Antonio and Vitousek, 1992), as well as reducing fungi and invertebrate species richness (Levine et al. 2003). Likewise, the abundance of native species within sites can also change, as displacement by the invader of one species (often the more dominant native species), can result in community restructuring whereby the number of subordinate native species actually increases (Grime 1998). These changes can lead to positive feedback loops, which may further exacerbate invasion impacts, leading to a long-term and potentially irreversible regime shift (Gaertner et al. 2014). It is therefore not surprising that this innate complexity within invaded communities can make it difficult to accurately predict shifts in species assemblages and ecosystem function.

Invasive ants are among some of the most prominent invasive species. Five invasive ant species (*Anoplolepis gracilipes*, *Linepithema humile*, *Pheidole megacephala*, *Solenopsis invicta*, *Wasmannia auropunctata*) are recognised among the world's 100 worst invaders (Global Invasive Species Database 2018). As with other invasive species, these ants have benefited from the breakdown of geographical barriers and increase in globalisation, such that today they can be found on every continent except Antarctica

(Bertelsmeier et al. 2017). A combination of their social life and small body size means that many ant invasions go unnoticed until already well established (Rabitsch 2011). Once present, these invaders displace native species and cause a rapid shift in the community structure (Sanders et al. 2003; Wittman 2014). For example, areas in which they are found can show up to a 90% reduction in the native ant community species richness (Holway et al. 2002). To date much of our understanding surrounding the impact of invasive ants are derived from ‘snapshot’ short-term post-invasion studies comparing invaded and non-invaded regions. The fact that impact of these invasions can vary through time (Morrison 2002) has led some researchers to question whether these invasive ant species truly are the ‘drivers’ of ecosystem change, or merely the ‘passengers’ (Didham et al. 2005; MacDougall and Turkington 2005; King and Tschinkel 2006). We therefore require more empirical studies on the effects of invasive species on native ant community structure, especially across sites with long invasion histories.

The Argentine ant (*Linepithema humile* Mayr), within the subfamily Dolichoderinae, is a frequently encountered invader, with a world-wide distribution. Originating from South America, this inconspicuous species can now be found across six continents and several oceanic islands (Suarez et al. 2001). Whilst this species is generally associated with human-modified and disturbed habitats (Holway et al. 2002), it can also be found in a wide array of natural and pristine habitats (De Kock and Giliomee 1989; Christian 2001; Holway et al. 2002; Gómez and Oliveras 2003) and is commonly established in regions with a Mediterranean climate (Tsutsui et al. 2000). It is often difficult to compare the extent of the impact of this invader, because studies use very different methods or sampling periods in which to measure the ant community structure. This inconsistency means it can be difficult to accurately predict the potential effects of an invasive ant species on native ant community structure, especially as many of our studies may come from one specific geographic region. For example, of the regions that *L. humile* has invaded, the South African Cape Floristic Region is one of the least studied areas, despite its high levels of plant endemism and long invasion history (Mothapo and Wossler 2011).

Here I investigate the effects of *L. humile* presence on native ant community structure within two distinct geographic regions, South African and Spain, using a comparable sampling method. I predict that the impacts of *L. humile* on native ant fauna to be comparable across both regions; if so, this would suggest that the differences previously identified in the literature were down to biases in the different types of sampling methods deployed, rather than any real biological difference in effects.

3.3 METHODS AND MATERIALS

3.3.1 Study Area

3.3.1.1. Spain

Linepithema humile was first recorded in the Iberian Peninsula at the beginning of the nineteenth century (Espadaler and Gómez 2003). Since then its population has expanded considerably in Northern Spain, particularly in coastal regions at a rate of 7.94 (\pm 2.99) metres per year (Roura-Pascual et al. 2010). The study in Spain was conducted at eight sites in and around Girona (North-eastern Spain). Four of the selected sites were previously known to be invaded with *L. humile*; two ($>$ 500m apart) of which were near University of Girona Montilivi Campus (41°58'59.20"N, 02°49'29.75"E) and two ($>$ 500m apart) near Castell d'Aro (30 km away) (41°49'04.61"N, 03°04'00.68"E). These sites were selected on the basis that they had been previously sampled for studies on the impact of *L. humile* on myrmecochorous seed dispersal processes (Espadaler and Gómez 1997; Gómez and Oliveras 2003; Bas et al. 2009) and therefore had a potentially long invasion history ($>$ 10 years). To contrast this, the other four sites were not invaded by *L. humile*; two ($>$ 500m apart) of these were near the University of Girona Montilivi Campus (41°58'59.20"N, 02°49'29.75"E) (providing a paired comparison with two of the invaded sites) and two ($>$ 500m apart) near Santuari dels Angels (15 km away) (41°58'31.18"N, 02°54'34.02"E). The vegetation at all sites was a combination of open cork-oak secondary forest, dominated by *Quercus* and *Pinus* tree species, with herbaceous myrmecochorous plant species in the clearings. Sampling was carried out during the summer months of June-July 2014, when both native and invasive ant species were active.

3.3.1.2. South Africa

Linepithema humile has been found in a number of otherwise pristine and undisturbed nature reserves within South Africa (Bond and Slingsby 1984; De Kock and Giliomee 1989) and was first detected in Jonkershoek in the 1980s, within the Swartboskloof region (De Kock and Giliomee 1989). Jonkershoek Nature Reserve forms part of the Boland Mountain Complex and contains several endemic vegetation types, including Kogelberg Sandstone Fynbos, Cape Winelands Shale Fynbos, and Boland Granite Fynbos. The study in South Africa was conducted at 11 sites within the Jonkershoek Nature Reserve (33°55'51''S, 18°51'16'' E). Five sites were within the *L. humile* invasion zone first identified by De Kock and Giliomee (1989). These invaded sites were paired with six sites that were outside the invasion zone, but between 0.5-1.5km away from the invasion front. This meant that the invaded and non-invaded sites were of a similar local biome and plant

community, providing as good as possible an attempt at a paired comparison of ant community structure in the presence or absence of *L. humile*, short of performing exclusion experiments (i.e. extermination of the invasive ant, which was not permitted in the National Park). All sites were near ($\pm 50\text{m}$) the road running through Jonkershoek Nature Reserve. The vegetation at all sites was endemic mountain fynbos, dominated by *Protea repens* and *P. netifolia*, as well as various endemic Restionaceae and Ericaceae. Sampling was carried out during the summer months of November-December 2014, when both native and invasive ant species were active.

3.3.2 Sampling design

Within each site a 100 m transect was set up. Two commonly used methods (Agosti et al. 2000) were then employed to determine the spatial and temporal distribution of invasive and native ant species. Pitfall traps, which are an effective and often used method (e.g. Rodriguez-Cabal et al. 2012) for sampling active epigaetic (ground foraging) ant species, were used to determine abundance and community composition of both invaded and non-invaded ant communities. Additionally, baiting traps (e.g. Albrecht and Gotelli 2001) were used to detect temporal (diurnal) partitioning of ant activity within each ant community.

3.3.2.1. Pitfall sampling

Twenty pitfall traps were placed at five-metre intervals along each 100m transect in each of the sites to sample the ant community structure. Each pitfall trap consisted of a 150 ml beaker, which was buried into the ground, flush with the surface of the soil, into which 50 ml of propylene glycol was added. The pitfall traps were left out for 72 hours (duration found to be sufficient for appraising ant assemblages in other Mediterranean habitats, such as Australia (Agosti et al. 2000; Borgelt and New 2005)), after which time all ants were stored in 70% alcohol for species identification.

3.3.2.2. Bait sampling

Ten baiting traps were placed along each 100m transect at 10 m intervals, in order to monitor diurnal rhythms of species and consequently detect temporal variation in ant community structure during the day. A distance of 10m is typical for such sampling methods, as it ensures each baiting station is effectively independent (Holway 1998; Andersen et al. 2002; Parr et al. 2004; King and Porter 2005). Each baiting trap consisted of 5 g of ant bait (five to one mix of tuna and honey) placed on a 10 cm² white laminated card. Baiting traps were set at 8:00 hours (morning), 12:00 hours (afternoon) and 16:00 hours (evening). An additional survey at 20:00 hours was done in Spain. While widely

used, this method favours detection of dominant ant species; therefore, the temporal activity of more subordinate ant species may not be accurately reflected. After one-hour, species (rather than individual) occurrence was recorded by collecting ants from each trap and storing them in 70% alcohol for species identification. Additionally, soil temperature was recorded (at each baiting trap) at the time of collection using an electronic infrared soil probe to distinguish between diurnal periods. Soil temperature is widely cited as a good predictor for foraging patterns in ants and other invertebrates (Porter and Tschinkel 1987).

3.3.3 Data analysis

For each country, ants were identified to species level (where possible), using keys from Fisher and Bolton (2016) or Gomez and Espadaler (2007). Only ant workers were recorded, as alates collected in the pitfall traps do not accurately indicate the presence of an established colony (Fisher 1998). Localities within each country were then grouped and compared according the presence/absence of *L. humile* and classified as either invaded or non-invaded. Subsequent analyses were conducted on invaded and non-invaded sites within each geographic region.

3.3.3.1. Ant diversity and abundance

Species accumulation curves were calculated using the *specaccum* function in *vegan* (version 2.4.4) package in R (Oksanen et al. 2012) for each site, to explore the relationship between species richness and number of pitfall traps sampled. Ant diversity, evenness and abundance was calculated (where appropriate) for each site, using a range of widely used ecological metrics (Morris et al. 2014), including: Species richness [N]; Simpson's index [$D = \sum (n/N)^2$]; Shannon-Weiner index [$H = -\sum_{i=1}^S P_i \ln P_i$]; Pielou's evenness index [$J = \frac{H}{H_{max}}$]; Fisher's alpha index [$\alpha = N(1 - x/x)$]. Furthermore, I tested for spatial autocorrelation between sites using the Moran's I statistic (Moran 1950). No evidence for spatial autocorrelation was found for the Spanish dataset ($I = 0.08$, $p = 0.21$), but a significant effect of spatial autocorrelation was detected for the South African dataset ($I = 0.20$, $p < 0.01$). This spatial autocorrelation was therefore controlled for using a Generalised Least Square (GLS) regression model (Dormann et al. 2007) to analyse the effects of invasion status (invaded vs. non-invaded) on each diversity metrics, using the *gls* function in *nlme* (version 3.1.1) package (Pinheiro et al. 2018).

3.3.3.2. Composition of ant communities

Native ant community structure of invaded and non-invaded sites were compared using a Bray-Curtis dissimilarity index (Bray and Curtis 1957). Records of *L. humile* were excluded from this analysis. Furthermore, to achieve data normality and reduce homoscedasticity, all data were log-transformed prior to analysis. Non-metric multidimensional scaling (NMDS) ordination (on all samples) and hierarchical clustering analysis (pooled samples) methods were used to explore the effects of *L. humile* invasion on native ant community structure. The significance of this difference (i.e. ant species abundance) was determined by using an Analysis of Similarities (ANOSIM) and Similarity Percentage (SIMPER) test. Additionally, a Mantel test was used to check for potential effects of spatial autocorrelation on native ant community structure between sites (Mantel and Valand 1970). Due to the innate contrasting features of ant community structures and their biology in Spain and South Africa, results for these analyses are presented at a country-specific level.

3.3.3.3. Diurnal ant activity

Diurnal temporal differences between, and within, invaded and non-invaded ant communities were compared using a Sorensen-Dice coefficient (Dice 1945). Hierarchical clustering analysis (samples pooled by sampling period) was performed on presence/absence data collected from baits, with traps where no ant activity recorded being excluded (following methods described in Magurran 2004). The significance of this difference was determined by using an ANOSIM test.

3.4 RESULTS

3.4.1 Spain

A total of 16,406 individual ants were collected in the 160 pitfall traps across 8 sites, belonging to 31 species, from 15 genera (Table 3.1).

Table 3.1: Ant species collected from pitfall traps (n = 160) in *L. humile* invaded and non-invaded regions during June 2014. Localities coded according to geographic location (UG = University of Girona, Montilivi Campus; CdA = Castell d'Aro; SdA = Santuari dels Angels) and transect number.

Ant Species	Status	Invaded				Non-Invaded			
		UG1*	UG2*	CdA1*	CdA2*	SdA1*	SdA2*	UG3*	UG4*
Dolichoderinae									
<i>Linepithema humile</i> Mayr [‡]	Invasive	2105	3166	3490	2377	0	0	0	0
<i>Tapinoma madeirense</i> Forel [‡]	Native	0	0	0	0	6	13	7	0
Formicinae									
<i>Camponotus aethiops</i> Latreille [‡]	Native	0	0	0	0	0	0	1	6
<i>Camponotus cruentatus</i> Latreille [‡]	Native	0	0	0	0	0	0	1	182
<i>Camponotus lateralis</i> Olivier [‡]	Native	0	0	0	0	1	0	0	0
<i>Camponotus pilicornis</i> Roger	Native	0	0	0	0	0	0	0	6
<i>Camponotus piceus</i> Leach	Native	0	0	0	0	0	4	1	0
<i>Camponotus truncatus</i> Spinola	Native	0	0	0	0	1	0	0	0
<i>Cataglyphis piliscapa</i> Forel [‡]	Native	0	0	0	0	0	28	33	4
<i>Formica cunicularia</i> Latreille [‡]	Native	0	0	0	0	0	3	0	0
<i>Formica gagates</i> Latreille	Native	0	0	0	0	1	5	5	2
<i>Formica gerardi</i> Bondroit [‡]	Native	0	0	0	0	6	0	1	2
<i>Lasius emarginatus</i> Olivier [‡]	Native	0	0	0	0	18	0	1	0
<i>Lasius grandis</i> Forel [‡]	Native	0	0	0	0	20	0	86	1
<i>Plagiolepis pygmaea</i> Latreille [‡]	Native	23	27	30	34	0	45	85	74
Myrmicinae									
<i>Aphaenogaster subterranea</i> Latreille [‡]	Native	0	0	0	0	40	0	29	31
<i>Crematogaster auberti</i> Emery	Native	0	0	0	0	3	0	0	1
<i>Crematogaster scutellaris</i> Olivier [‡]	Native	0	0	0	0	12	1	8	1
<i>Diplorhoptrum</i> sp.	Native	0	0	0	0	0	5	4	23
<i>Messor barbarus</i> Linnaeus [‡]	Native	0	0	0	0	0	3	0	2
<i>Messor capitatus</i> Latreille	Native	0	0	0	0	0	150	0	0
<i>Messor structor</i> Latreille [‡]	Native	0	0	0	0	2	24	0	0
<i>Myrmica</i> sp. [‡]	Native	0	0	0	0	2	7	74	10
<i>Pheidole pallidula</i> Nylander [‡]	Native	0	0	0	0	97	511	597	2081
<i>Solenopsis</i> sp.	Native	0	0	0	0	0	0	0	3
<i>Temnothorax racovitzai</i> Bondroit	Native	0	0	0	0	2	0	0	0
<i>Temnothorax unifasciatus</i> Latreille	Native	0	0	0	0	2	0	0	0
<i>Tetramorium caespitum</i> Linnaeus [‡]	Native	0	0	0	0	2	391	224	26
<i>Tetramorium semilaeve</i> Andre [‡]	Native	0	0	0	0	2	0	9	22
<i>Tetramorium</i> sp. [‡]	Native	0	0	0	0	0	106	46	0
Ponerinae									
<i>Hypoponera eduardi</i> Forel	Native	0	0	0	0	1	0	1	0
Total number of ants		2128	3193	3520	2411	218	1296	1213	2477
Species richness		2	2	2	2	18	15	19	18
Simpson's Index		0.02	0.02	0.02	0.03	0.75	0.73	0.71	0.29
Shannon-Weiner Diversity index		0.06	0.05	0.05	0.07	1.87	1.62	1.69	0.73
Pielou's evenness index		0.09	0.07	0.07	0.11	0.65	0.60	0.57	0.25

[‡]Identified in both pitfall and baiting traps

3.4.1.1. Ant diversity and abundance

Of the 31-ant species recorded, 30 were found exclusively in non-invaded ($n = 4$) sites. By contrast, only two ant species were recorded within invaded ($n = 4$) sites (Table 3.1). This difference is clearly reflected in the species accumulation curves (Fig 3.1), as pitfall traps sampled in invaded sites approached an asymptote far more rapidly than those placed in non-invaded sites (Fig. 3.1b). Irrespective of this difference, all sites ($n = 8$) reached an asymptote (Fig 3.1a), thereby indicating that 20 pitfalls traps per site were sufficient to capture most of the local epigaeic ant species richness.

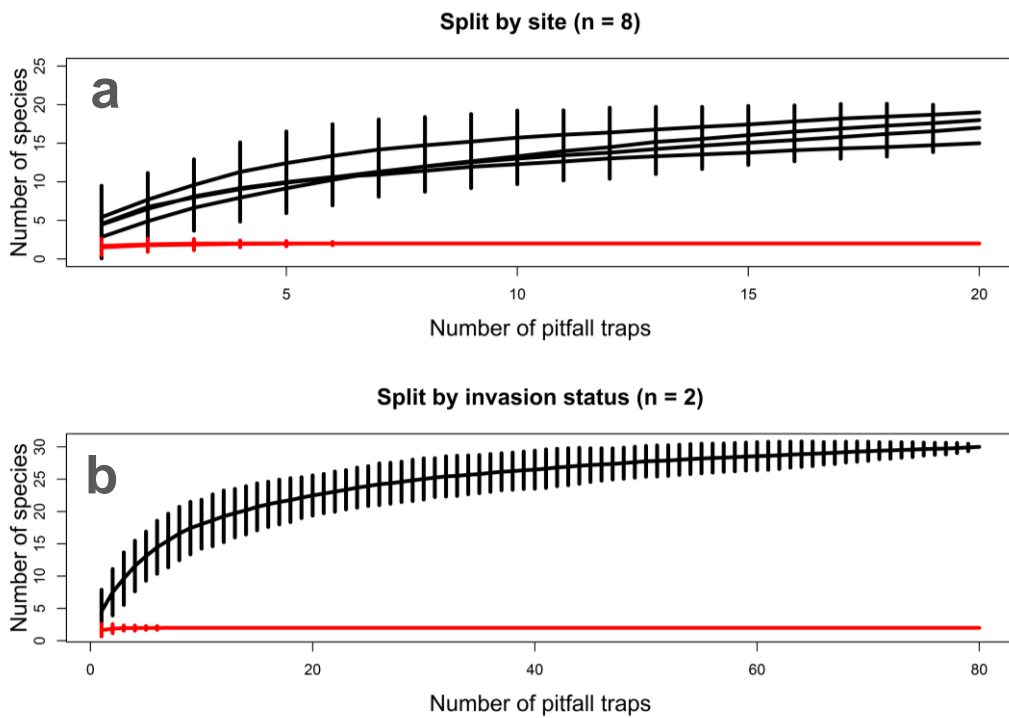


Figure 3.1: Species-based accumulation curves of epigaeic ant species collected in Spain. Cumulative number of species collected from pitfall traps: split according to site (a) and invasion status (b). Overall species richness is estimated across randomly sampled pitfall traps for 999 permutations (mean \pm SD). Line corresponds to invasion status: red (invaded) and black (non-invaded).

Even though more ants were collected in pitfall traps placed in invaded sites than uninvaded sites (Table 3.1), the invaded sites scored significantly lower in terms of species richness, diversity, and evenness (Table 3.2; Fig. 3.2) when compared with non-invaded sites. Of the 30-native species found in non-invaded sites, *Pheidole pallidula* was found to be the most abundant (accounting for 63% of all individuals collected in pitfall traps).

Table 3.2: Effects of invasion status on species richness, diversity (Simpson's and Shannon-Weiner Index) and evenness (Pileou's Index) using a Generalised Least Square regression model. Mean scores shown for invaded and non-invaded sites in Spain.

<i>Invasion status</i>	Species richness	Simpson's Index	Shannon-Weiner Index	Pileou's Index
Invaded ($n = 4$)	2.00	0.02	0.06	0.09
Non-invaded ($n = 4$)	17.50	0.62	1.48	0.52
<i>b</i>	15.5	0.60	1.42	0.43
(Standard Error)	(0.50)	(0.00)	(0.03)	(0.00)
Sig. ^a	***	***	***	***

^aOne-tailed significant levels for directional hypothesis.

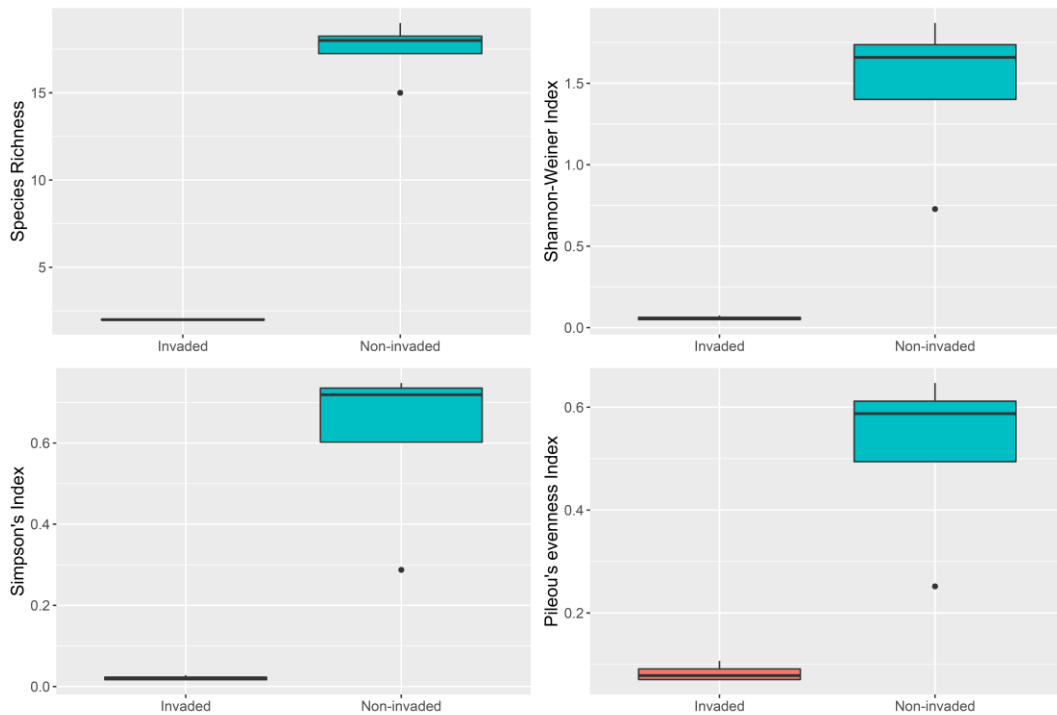


Figure 3.2: Comparison of species richness, diversity (Simpson's and Shannon-Weiner Index) and evenness (Pileou's Index) of ants collected in pitfall traps placed in invaded and non-invaded regions of Spain. Box plot (with 25-75% quartiles) values of pooled pitfall traps ($n = 20$ per site) placed within invaded ($n=4$) and non-invaded ($n=4$) sites.

3.4.1.2. Composition of ant communities

The presence of *L. humile* was associated with a significant shift in native ant community structure (Fig 3.3). Native ant community composition differed significantly (ANOSIM: permutations 999, $r = 1.00$, $p < 0.04$) between invaded and non-invaded sites (Fig. 3.3b), which is reflected in the NMDS analysis (Stress = 0.08; Fig. 3.3a). Checks for potential spatial autocorrelation revealed that geographical position was not a sufficient predictor of differences in native ant community structure (Mantel test: $r = -0.12$, $p = 0.71$).

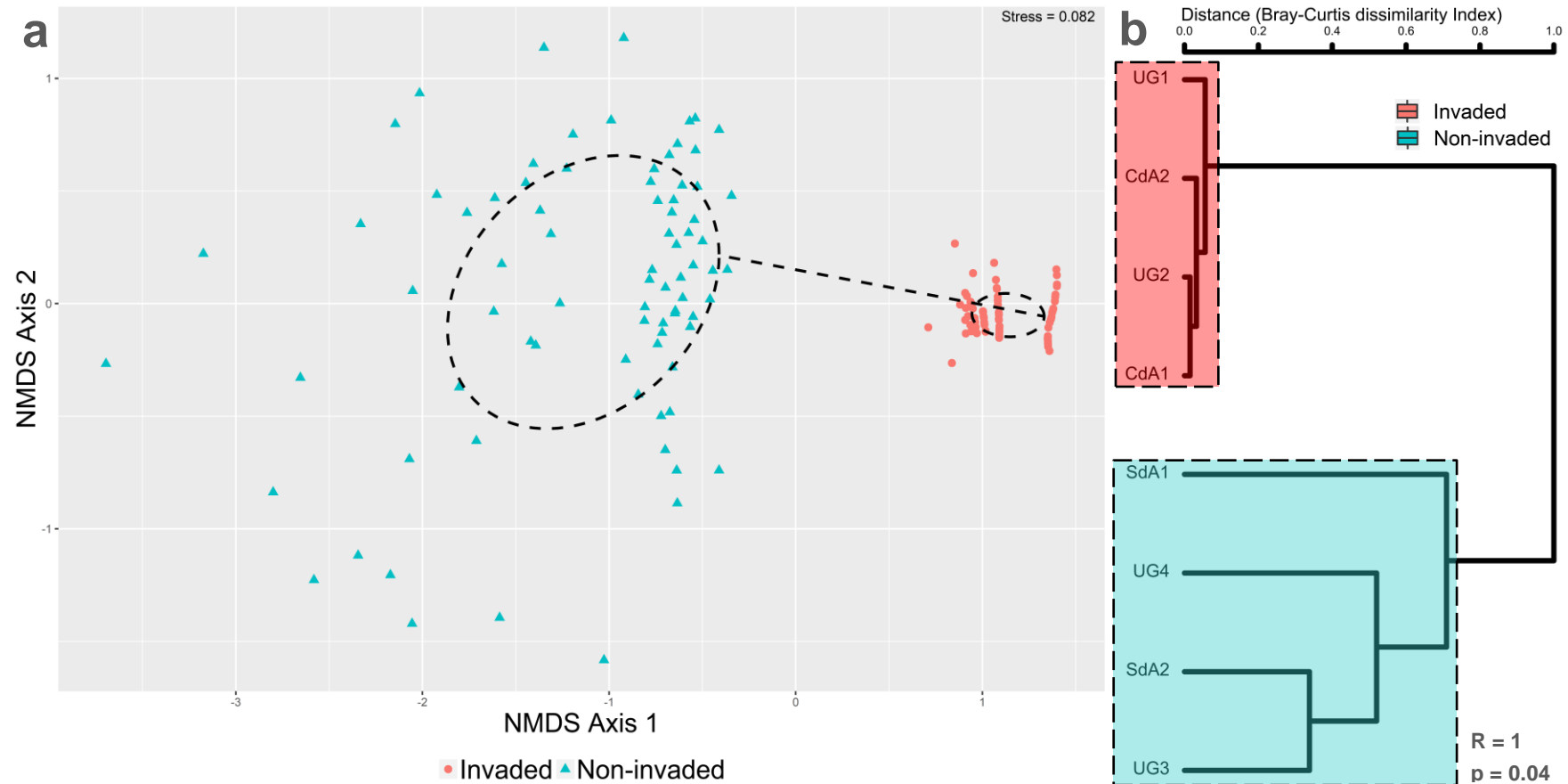


Figure 3.3: Comparison of native ant community structure between invaded (red) and non-invaded (blue) sites in Spain. (a) Non-metric multidimensional scaling (NMDS) ordination plot based on the composition of ants collected in pitfall traps ($n = 160$) within eight sites spread across invaded (circle) and non-invaded (triangle) sites (Stress = 0.08). (b) Hierarchical cluster analysis of native ant community structure from eight sites (20 pitfalls per site). Distances for both (a) and (b) were calculated using Bray-Curtis dissimilarity index using log-transformed pitfall trap data.

SIMPER analysis revealed that five ant species were the main contributors (>50%) to this difference (overall dissimilarity = 90%; Table 3.3). Only one ant species (*Plagiolepis pygmaea*) was found to coexist with *L. humile* in invaded regions; its abundance did not differ significantly between invaded and non-invaded sites (Table 3.3).

Table 3.3: Results of similarity percentage (SIMPER) analysis for 30 ant species present within both invaded and non-invaded sites in Spain.

Species	Invaded mean abundance	Non-invaded mean abundance	% contribution to dissimilarity	Cumulative %	Sig.
<i>Pheidole pallidula</i>	0.00	6.21	0.15	0.15	*
<i>Tetramorium caespitum</i>	0.00	3.95	0.09	0.24	*
<i>Aphaenogaster subterranea</i>	0.00	2.65	0.07	0.31	*
<i>Myrmica</i> sp.	0.00	2.47	0.06	0.36	*
<i>Lasius grandis</i>	0.00	2.05	0.05	0.41	*
<i>Cataglyphis piliscapa</i>	0.00	2.13	0.05	0.46	*
<i>Tetramorium</i> sp.	0.00	2.13	0.05	0.51	*
<i>Tapinoma madeirense</i>	0.00	1.67	0.04	0.55	*
<i>Plagiolepis pygmaea</i>	3.38	3.15	0.04	0.59	0.08 ^{NS}
<i>Crematogaster scutellaris</i>	0.00	1.54	0.04	0.63	*
<i>Tetramorium semilaeve</i>	0.00	1.63	0.04	0.67	*
<i>Diplorhoptrum</i> sp.	0.00	1.64	0.04	0.71	*
<i>Camponotus cruentatus</i>	0.00	1.48	0.03	0.74	*
<i>Formica gagates</i>	0.00	1.34	0.03	0.77	*
<i>Messor capitatus</i>	0.00	1.25	0.03	0.80	*
<i>Lasius emarginatus</i>	0.00	0.91	0.03	0.83	*
<i>Messor structor</i>	0.00	1.08	0.03	0.85	*
<i>Formica gerardi</i>	0.00	0.93	0.03	0.88	*
<i>Crematogaster auberti</i>	0.00	0.52	0.02	0.89	*
<i>Camponotus aethiops</i>	0.00	0.66	0.01	0.91	*
<i>Messor barbarus</i>	0.00	0.62	0.01	0.92	*
<i>Camponotus piceus</i>	0.00	0.58	0.01	0.94	*
<i>Camponotus pilicornis</i>	0.00	0.49	0.01	0.95	*
<i>Hypoponera eduardi</i>	0.00	0.35	0.01	0.96	*
<i>Temnothorax racovitzai</i>	0.00	0.27	0.01	0.96	*
<i>Temnothorax unifasciatus</i>	0.00	0.27	0.01	0.97	*
<i>Solenopsis</i> sp.	0.00	0.35	0.01	0.98	*
<i>Formica cunicularia</i>	0.00	0.35	0.01	0.99	*
<i>Camponotus lateralis</i>	0.00	0.17	0.01	0.99	*
<i>Camponotus truncatus</i>	0.00	0.17	0.01	1.00	*

3.4.1.3. Diurnal ant activity

Comparison of ant activity across invaded and non-invaded sites found that the presence of ant species at baits was not significantly influenced by sampling period (ANOSIM: permutations 999, $r = -0.09$, $p = 0.99$) (Fig. 3.4).

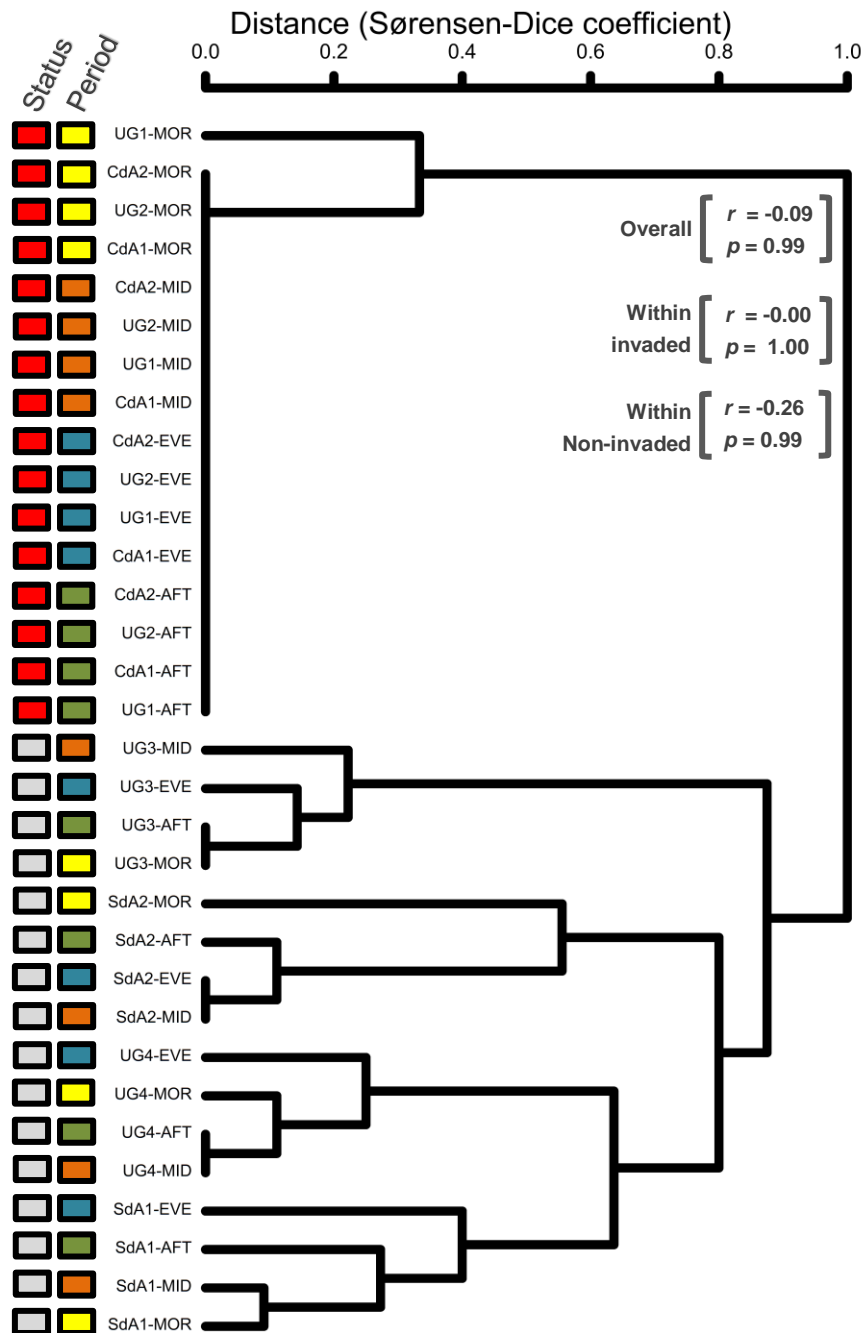


Figure 3.4: Diurnal ant activity is not influenced by sampling period. Hierarchical clustering of ant presence at baits sampled within invaded (red) and non-invaded (grey) sites in Spain. Baits were sampled across four-time periods: morning (yellow), midday (orange), afternoon (green), and evening (blue). Distances calculated using Sorensen-dice coefficient using presence/absence data collected at tuna and honey baits.

3.4.2 South Africa

A total of 9,166 individual ants were collected in the 220 pitfall traps across 11 sites, belonging to 41 species, from 22 genera (Table 3.4).

Table 3.4: Ant species collected from pitfall traps (n = 220) in *L. humile* invaded and non-invaded sites during November 2014 in Jonkershoek Nature Reserve, South Africa.

Ant Species	Status	Invaded				Non-Invaded						
		A	Ax	Bx	E	B	C	D	Dx	Ex	F	G
Dolichoderinae												
<i>Axinidris lignicola</i> Snelling	Native	0	0	1	0	0	0	0	0	0	0	0
<i>Linepithema humile</i> Mayr [‡]	Invasive	89	128	47	88	0	0	0	0	0	0	0
Dorylinae												
<i>Dorylus</i> sp.	Native	0	0	0	0	1	0	0	0	0	0	0
Formicinae												
<i>Anoplolepis custodiens</i> Smith [‡]	Native	0	0	0	0	0	4	11	0	238	138	802
<i>Anoplolepis steingroeveri</i> Forel [‡]	Native	0	0	0	0	0	0	461	43	88	197	42
<i>Anoplolepis</i> sp.	Native	0	0	0	0	0	2	2	0	7	10	22
<i>Acropyga arnoldi</i> Santschi	Native	0	0	0	0	0	0	0	0	0	2	0
<i>Camponotus baynei</i> Arnold	Native	0	0	0	0	3	0	0	0	0	0	2
<i>Camponotus cuneiscapus</i> Forel	Native	0	5	0	0	0	2	3	0	1	6	1
<i>Camponotus havilandi</i> Arnold	Native	0	2	0	1	0	3	2	0	4	0	0
<i>Camponotus maculatus</i> Fabricius	Native	33	1	4	4	10	4	4	4	8	0	2
<i>Camponotus niveosetosus</i> Mayr [‡]	Native	3	0	1	0	3	4	0	1	2	2	5
<i>Camponotus vestitus</i> Smith [‡]	Native	2	1	1	0	2	4	2	0	4	0	4
<i>Camponotus werthi</i> Forel [‡]	Native	0	1	10	0	8	2	2	3	11	0	0
<i>Lepisiota capensis</i> Mayr [‡]	Native	0	1	1	0	41	462	1207	12	109	63	146
<i>Lepisiota</i> sp.	Native	0	0	0	0	2	17	9	0	2	3	10
<i>Plagiolepis capensis</i> Mayr	Native	0	0	4	0	0	0	2	1	1	0	0
<i>Plagiolepis deweti</i> Forel [‡]	Native	3	1	1	1	2	8	0	1	1	0	1
<i>Tapinolepis trimenii</i> Arnold	Native	0	0	0	0	0	0	1	0	1	0	0
Myrmicinae												
<i>Cardiocondyla</i> sp.	Native	0	0	0	4	9	2	5	1	2	2	3
<i>Crematogaster peringueyi</i> Emery [‡]	Native	0	0	6	0	0	0	1	10	0	0	1
<i>Meranoplus peringueyi</i> Emery [‡]	Native	42	5	35	11	22	0	0	8	0	0	3
<i>Messor capensis</i> Mayr	Native	0	0	0	0	1	8	12	46	2	14	8
<i>Monomorium macrops</i> Arnold [‡]	Native	27	16	26	31	243	3	0	33	1	11	3
<i>Monomorium</i> sp.1	Native	9	8	4	4	2	1	2	1	2	5	5
<i>Monomorium</i> sp.2	Native	0	0	0	1	0	0	0	2	0	0	1
<i>Nesomyrmex denticulatus</i> Mayr	Native	0	1	0	0	0	0	0	0	2	0	2
<i>Ocymyrmex barbiger</i> Emery [‡]	Native	92	49	21	25	50	55	82	16	113	17	106
<i>Pheidole capensis</i> Mayr [‡]	Native	0	1	0	0	295	339	612	208	95	149	110
<i>Pheidole</i> sp.	Native	0	0	0	0	16	6	7	40	0	5	4
<i>Strumigenys</i> sp.	Native	0	1	0	0	0	0	0	0	3	0	0
<i>Sylophopsis modesta</i> Santschi	Native	0	3	2	1	0	0	3	3	3	6	1
<i>Tetramorium arnoldi</i> Forel	Native	0	0	0	0	0	0	2	0	0	2	0
<i>Tetramorium sericeiventre</i> Emery [‡]	Native	114	46	64	132	87	143	9	4	0	40	16
<i>Tetramorium</i> sp.1 [‡]	Native	25	14	6	30	8	3	1	10	1	11	1
<i>Tetramorium</i> sp.2	Native	25	24	3	23	15	4	10	7	9	29	51
<i>Tetramorium</i> sp.3	Native	5	3	0	1	8	0	0	8	0	66	10
<i>Tetramorium</i> sp.4	Native	1	0	0	11	0	0	14	6	10	27	4
<i>Tetramorium</i> sp.5	Native	0	0	0	10	0	0	8	2	5	13	11
Ponerinae												
<i>Hypoponera</i> sp.	Native	1	0	0	0	0	1	0	0	0	0	0
<i>Leptogenys intermedia</i> Emery	Native	0	0	0	0	0	0	0	0	0	0	1
Total number of ants		471	311	237	378	828	1077	2474	470	725	818	1377
Species richness		15	17	20	18	21	22	26	23	30	24	27
Simpson's Index		2.09	1.99	1.93	2.15	1.88	1.53	1.39	2.33	1.63	2.09	2.02
Shannon-Weiner Diversity index		0.85	0.80	0.77	0.84	0.77	0.70	0.66	0.86	0.63	0.77	0.81
Pielou's evenness index		0.77	0.70	0.64	0.74	0.62	0.49	0.43	0.74	0.48	0.66	0.61
Fisher's alpha index		2.95	3.66	4.77	4.53	3.92	3.91	4.05	4.40	5.41	5.35	5.53

‡Identified in both pitfall and baiting traps

3.4.2.1. Ant diversity and abundance

In total, 39 ant species were recorded within non-invaded ($n = 7$) sites. By contrast, only 29 ant species were recorded within invaded ($n = 4$) sites (Table 3.4). Pitfall traps sampled in invaded regions approached asymptote more rapidly, than those placed in non-invaded regions (Fig. 3.5b). Irrespective of this difference, all sites ($n = 11$) reached an asymptote (Fig 3.5a), thereby indicating that 20 pitfalls traps were sufficient to capture most of the local epigaeic ant species richness.

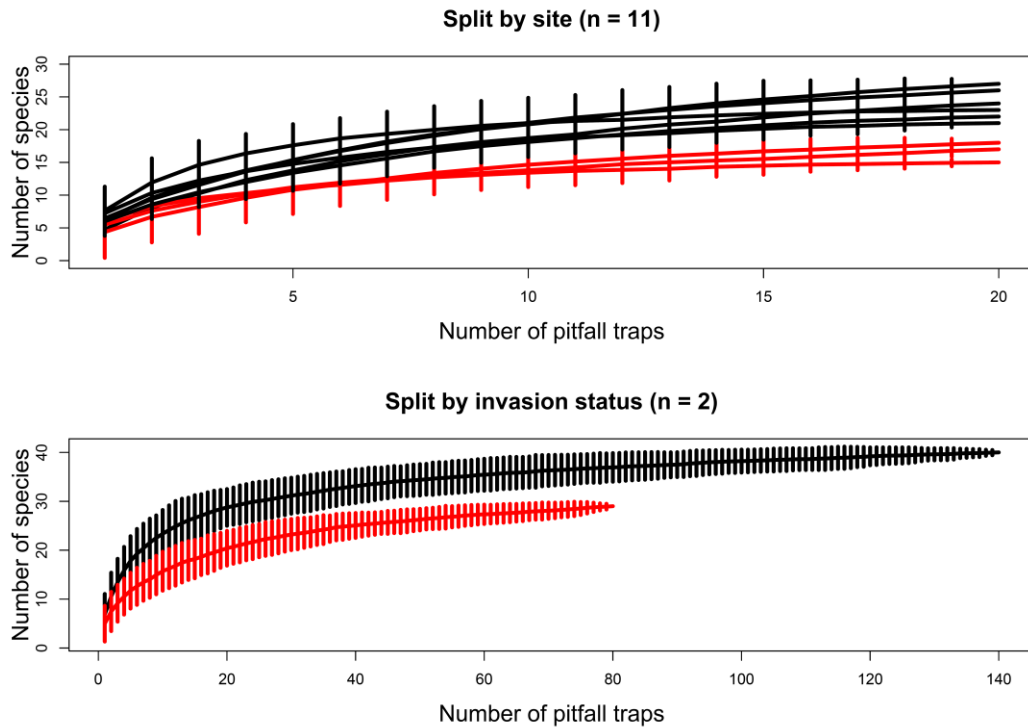


Figure 3.5: Species-based accumulation curves of epigaeic ant species collected in South Africa. Cumulative number of species collected from pitfall traps: split according to site (a) and invasion status (b). Overall species richness is estimated across randomly sampled pitfall traps for 999 permutations (mean \pm SD). Line corresponds to invasion status: red (invaded) and black (non-invaded).

Non-invaded sites were found to be significantly more species rich (Table 3.5; Fig. 3.6) than invaded sites. However, despite having more native ant species present, no significant effects on species diversity (Table 3.5; Fig 3.6) were detected. This may be because invaded sites scored higher in terms of both species abundance and evenness than non-invaded sites (Table 3.5; Fig. 3.6).

Table 3.5: Effects of invasion status on species richness, diversity (Simpson's and Shannon-Weiner Index), evenness (Pileou's Index) and abundance (Fisher's Index) using a Generalised Least Square regression model. Mean scores shown for invaded and non-invaded sites in South Africa.

<i>Invasion status</i>	Species richness	Simpson's Index	Shannon-Weiner Index	Pileou's Index	Fisher's Index
Invaded ($n = 4$)	17.50	0.81	2.04	0.72	3.98
Non-invaded ($n = 7$)	24.71	0.74	1.84	0.58	4.65
<i>b</i>	7.21	-0.07	-0.20	-0.14	0.68
(Standard Error)	(1.67)	(0.04)	(0.15)	(0.05)	(0.29)
Sig. ^a	**	0.15 ^{NS}	0.39 ^{NS}	*	*

^aOne-tailed significant levels for directional hypothesis.

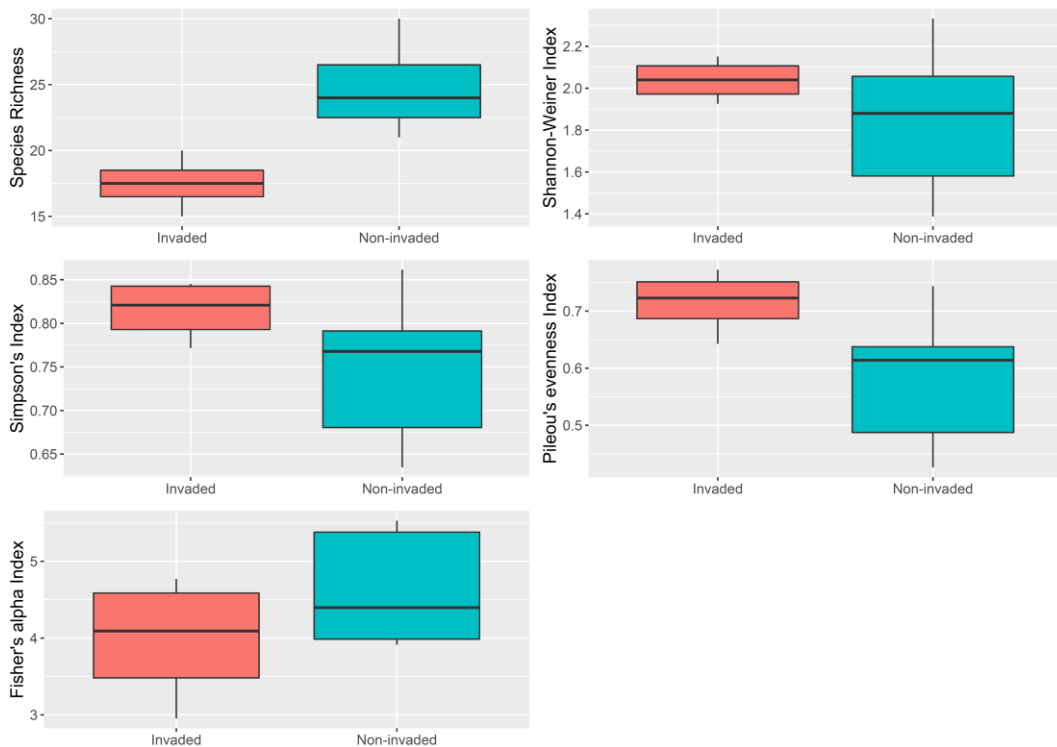


Figure 3.6: Comparison of species richness, diversity (Simpson's and Shannon-Weiner Index), evenness (Pileou's Index) and abundance (Fisher's Index) of native ants collected in pitfall traps placed in invaded and non-invaded regions of South Africa. Box plot show median (with 25-75% quartiles) values of pooled pitfall traps ($n = 20$ per site) placed across invaded ($n = 4$) and non-invaded ($n = 7$) sites.

3.4.2.2. Composition of ant communities

Native ant community structure differed significantly (ANOSIM: permutations 999, $r = 0.84$, $p < 0.001$) between invaded and non-invaded sites (Fig. 3.7b), which is reflected in the NMDS analysis (Stress = 0.22; Fig. 3.7a). The spatial arrangement of invaded and non-invaded sites was found to be a strong predictor of native ant community structure (Mantel test: $r = 0.57$, $p < 0.02$).

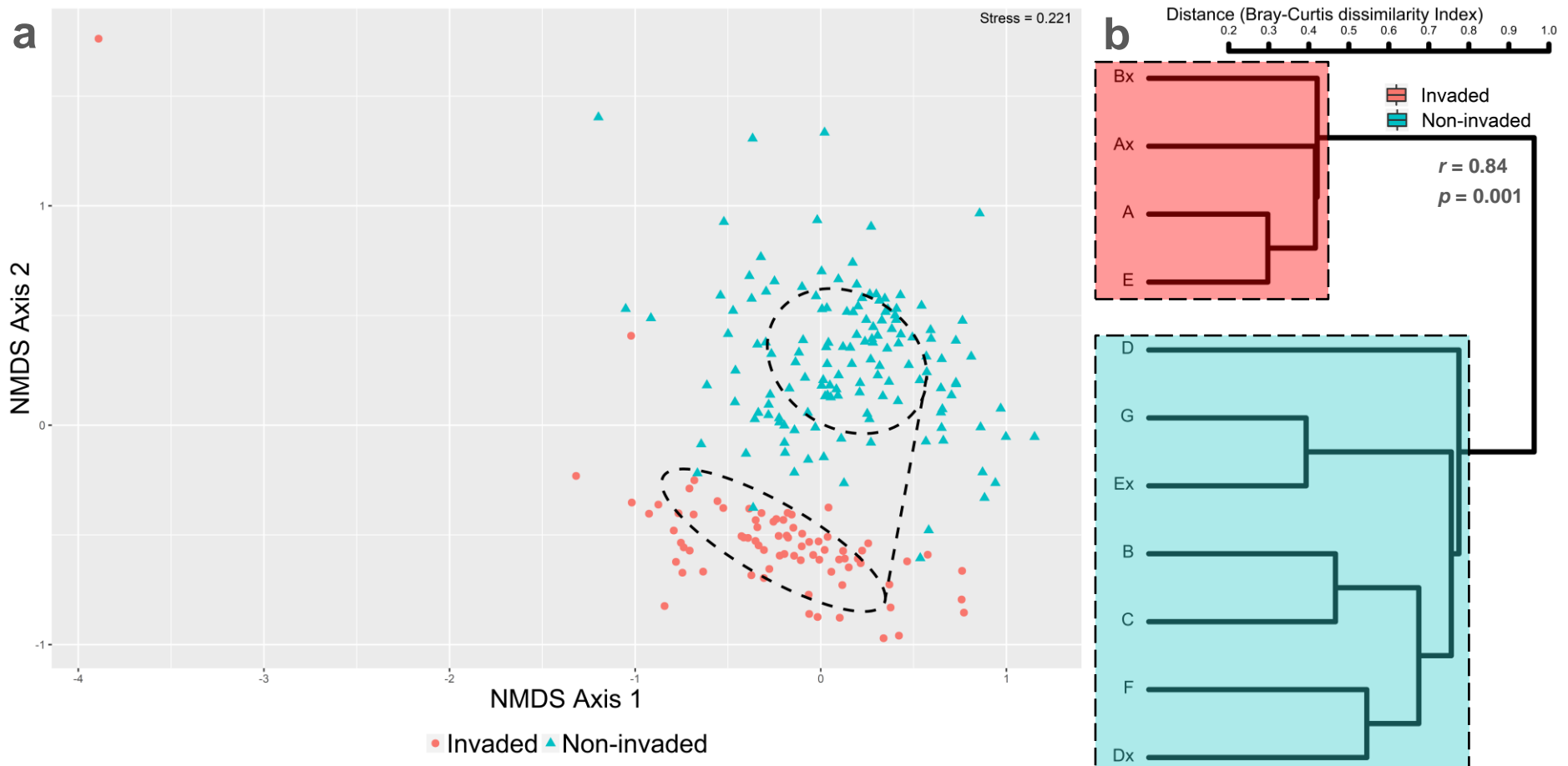


Figure 3.7: Comparison of native ant community structure between invaded (red) and non-invaded (blue) sites in South Africa. (a) Non-metric multidimensional scaling (NMDS) ordination plot based on the composition of ants collected in pitfall traps ($n = 220$) across 11 sites spread across invaded (circle) and non-invaded (triangle) sites. index (Stress = 0.22). (b) Hierarchical cluster analysis of native ant community structure from eight sites (20 pitfalls per site). Distances for both (a) and (b) were calculated using Bray-Curtis dissimilarity index using log-transformed pitfall trap data.

SIMPER analysis revealed that nine ant species were the main contributors (>50%) to this difference (overall dissimilarity = 55%; Table 3.6) between invaded and non-invaded sites. Importantly, all the native ant species more commonly associated with myrmecochory (e.g. *Anoplolepis custodiens*, *A. steingroeveri*, and *Pheidole capensis*) and granivory (e.g. *Messor capensis*) were absent from invaded sites (Table 3.6), while other native ant species were more abundant (e.g. *Meranoplus peringueyi* and *Tetramorium sericeiventre*) in invaded sites, than non-invaded sites (Table 3.4).

Table 3.6: Results of similarity percentage (*SIMPER*) analysis for 40 ant species found within both invaded and non-invaded sites in South Africa.

Species	Invaded mean abundance	Non-invaded mean abundance	% contribution to dissimilarity	Cumulative %	Sig.
<i>Pheidole capensis</i>	0.17	5.37	0.15	0.11	**
<i>Lepisiota capensis</i>	0.35	4.77	0.09	0.20	**
<i>Anoplolepis steingroeveri</i>	0.00	3.35	0.07	0.27	*
<i>Anoplolepis custodiens</i>	0.00	3.03	0.06	0.33	*
<i>Messor capensis</i>	0.00	2.19	0.05	0.37	**
<i>Meranoplus peringueyi</i>	2.91	0.96	0.04	0.41	*
<i>Pheidole</i> sp.	0.00	2.00	0.04	0.46	**
<i>Monomorium macrops</i>	3.23	2.14	0.04	0.49	0.12 ^{NS}
<i>Tetramorium sericeiventre</i>	4.42	2.34	0.04	0.53	*
<i>Lepisiota</i> sp.	0.00	1.60	0.03	0.56	**
<i>Tetramorium</i> sp4	0.79	1.71	0.03	0.59	0.28 ^{NS}
<i>Tetramorium</i> sp3	0.97	1.57	0.03	0.62	0.52 ^{NS}
<i>Tetramorium</i> sp1	2.84	1.51	0.03	0.65	*
<i>Anoplolepis</i> sp.	0.00	1.40	0.03	0.68	*
<i>Tetramorium</i> sp5	0.60	1.46	0.03	0.71	0.21 ^{NS}
<i>Cardiocondyla</i> sp.	0.40	1.35	0.02	0.73	*
<i>Camponotus werthi</i>	0.77	1.18	0.02	0.75	0.41 ^{NS}
<i>Camponotus maculatus</i>	1.86	1.50	0.02	0.77	0.40 ^{NS}
<i>Tetramorium</i> sp2	2.76	2.64	0.02	0.79	0.26 ^{NS}
<i>Camponotus cuneiscapus</i>	0.45	0.83	0.02	0.81	0.25 ^{NS}
<i>Monomorium</i> sp1	1.93	1.18	0.02	0.83	*
<i>Camponotus niveosetosus</i>	0.52	1.10	0.02	0.85	0.11 ^{NS}
<i>Crematogaster peringueyi</i>	0.49	0.54	0.02	0.86	0.53 ^{NS}
<i>Ocymymex barbiger</i>	3.70	3.93	0.02	0.88	0.47 ^{NS}
<i>Camponotus vestitus</i>	0.62	1.00	0.02	0.89	0.20 ^{NS}
<i>Sylophopsis modesta</i>	0.79	0.97	0.01	0.91	0.68 ^{NS}
<i>Camponotus havilandi</i>	0.45	0.58	0.01	0.92	0.54 ^{NS}
<i>Plagiolepis capensis</i>	0.40	0.35	0.01	0.93	0.49 ^{NS}
<i>Plagiolepis deweti</i>	0.87	0.77	0.01	0.95	0.54 ^{NS}
<i>Nesomyrmex denticulatus</i>	0.17	0.31	0.01	0.95	0.38 ^{NS}
<i>Camponotus baynei</i>	0.00	0.35	0.01	0.96	0.38 ^{NS}
<i>Monomorium</i> sp2	0.17	0.26	0.01	0.97	0.56 ^{NS}
<i>Strumigenys</i> sp.	0.17	0.20	0.01	0.97	0.38 ^{NS}
<i>Tetramorium arnoldi</i>	0.00	0.31	0.01	0.98	0.63 ^{NS}
<i>Hypoponera</i> sp.	0.17	0.10	0.00	0.99	0.35 ^{NS}
<i>Tapinolepis trimenii</i>	0.00	0.20	0.00	0.99	0.38 ^{NS}
<i>Axinidris lignicola</i>	0.17	0.00	0.00	0.99	0.38 ^{NS}
<i>Acropyga arnoldi</i>	0.00	0.16	0.00	1.00	0.37 ^{NS}
<i>Dorylus</i> sp.	0.00	0.10	0.00	1.00	0.38 ^{NS}
<i>Leptogenys intermedia</i>	0.00	0.10	0.00	1.00	0.33 ^{NS}

3.4.2.3. Diurnal ant activity

Comparison of ant activity within invaded and non-invaded sites showed that the presence of ant species at baits was significantly influenced by sampling period (ANOSIM: permutations 999, $r = 0.40$, $p = 0.001$) (Fig. 3.7).

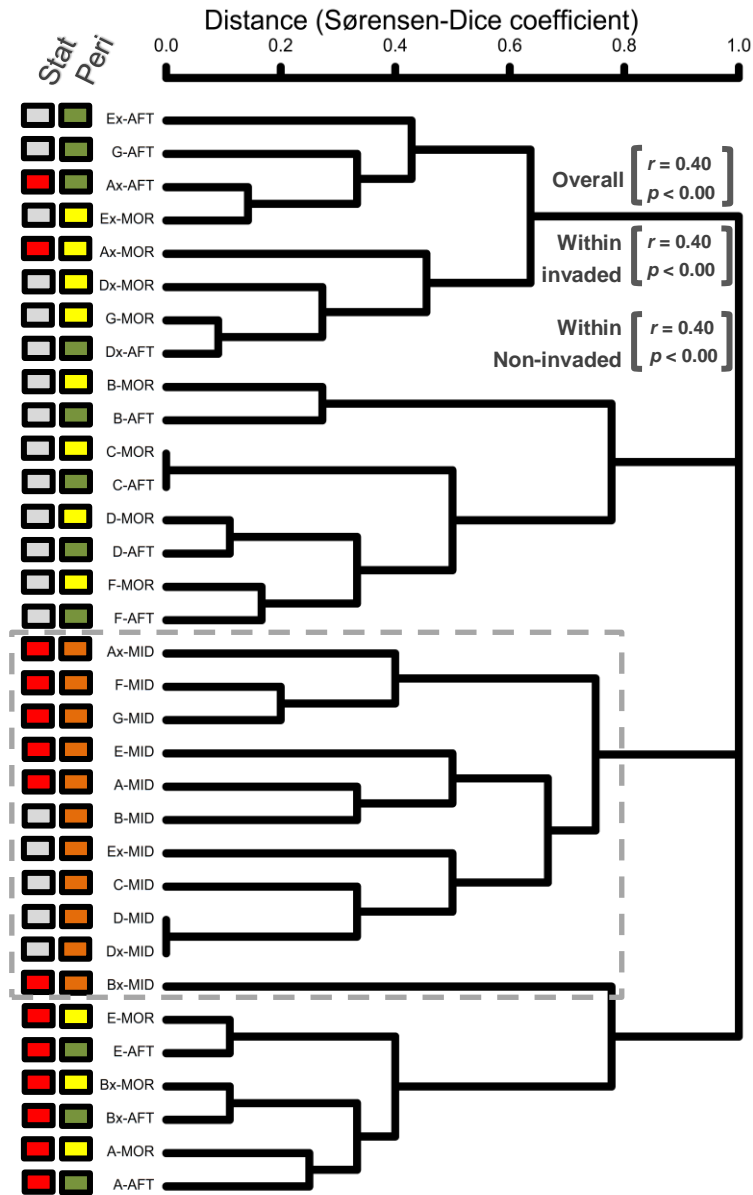


Figure 3.7: Diurnal ant activity is influenced by sampling period. Hierarchical clustering of ant presence at baits sampled within invaded (red) and non-invaded (grey) sites in South Africa. Baits were sampled across four-time periods: morning (yellow; MOR), midday (orange; MID) and afternoon (green; AFT). Distances calculated using Sorensen-dice coefficient using presence/absence data collected at tuna and honey baits. Sites denoted by letters A to G.

Notably, midday sampling periods (12-1pm) clustered, irrespective of invasion status (Fig. 3.7). These periods were associated with a significant rise in ground temperatures (ANOVA: $F=101.6$ $DF=2$, $p<0.0001$) and presence of the thermophilic ant species, *Ocymyrmex barbiger* (GLM: $SE=0.03$, $z=6.07$, $p<0.0001$). By contrast, the presence of *L. humile* in invaded sites decreased with rising ground temperatures (GLM: $SE=0.03$, $z=-4.22$, $p<0.0001$). This trend indicates that there appears to be a degree of temporal partitioning in ant activity within both invaded and non-invaded sites.

3.5 DISCUSSION

Overall, this study found evidence to suggest that the presence of the invasive *L. humile* may be having a negative effect on the native ant community structure within invaded regions of Spain and South Africa. These findings are consistent with similar studies conducted, both within Spain and South Africa, as well as other parts of the world (Ward 1987; Suarez et al. 1998; Holway 1998; Christian 2001; Espadaler and Gómez 2003; Gómez and Oliveras 2003; Sanders et al. 2003; Holway and Suarez 2006; Rowles and O'Dowd 2009). However, given the scarcity of research on this subject in South Africa, this study provides greater insight into the potential effects of *L. humile* invasion on native ant community structure and reveals how region-specific effects of *L. humile* may be significant.

The results of this study suggest that the invasive ant *L. humile* alters native ant species communities in the regions that it invades. However, the degree of displacement and the specific effects on the diversity and abundance of native ant species appears to vary considerably across countries or biomes. In Spain I found that *L. humile* displaced all but one native ant species, whereas in South Africa a considerable number of native species were able to coexist (e.g. Spain: *P. pygmaea*; South Africa: *M. peringueyi*, *Monomorium* sp., and *O. barbiger*). Most notably, at least two *Tetramorium* and one *Meranoplus* species were found to be more abundant in invaded sites within South Africa. Whether this increase in species abundance is due to presence of *L. humile*, rather than differences in site topography and vegetation remains to be resolved. My findings are similar to those of Christian (2001) on native ant community structure in the neighbouring Kogelberg Nature Reserve, where, for example, *M. peringueyi* and *T. sericeiventre* were found to coexist with *L. humile* in invaded sites. This ability to co-exist could in part be due to a range of factors, including temporal separation, enhanced competitive ability, and avoidance mechanisms (Andersen et al. 1991; Witt and Giliomee 1991; Human and Gordon 1996; Rowles and O'Dowd 2009). Species that are displaced are often aggressive ant species (e.g. *Anoplolepis* sp., *Lepisiota* and *Pheidole*); species that co-exist exhibit a range of passive resistance methods, such as feigning death or nest entrance sealing to repel ant invaders (e.g. *Tetramorium*) (Löhr 1992; Witt and Giliomee 1991; Addison and Samways 2000). Likewise, *Monomorium* species in other regions of the world exhibit active resistance to *L. humile* through expulsion of chemical repellents (Andersen et al. 1991; Holway 1999). Interestingly, the species found to be displaced in my study (e.g. *Anoplolepis*, *Lepisiota*, and *Pheidole*), tended to be those that were most abundant (in pitfall traps) in the non-invaded sites. This displacement may be attributable to interference competition, the

mechanism most commonly associated with *L. humile* invasions in other regions of the world. For example, in Australia *L. humile* displaced the most dominant native species through direct exclusion of resources (food or nesting sites) (Rowles and O'Dowd 2009).

This study clearly demonstrates how the impact of *L. humile* can vary, depending on the geographic region that it is found in. For example, the abundance of *L. humile* was considerably higher in Spain compared with that of South Africa, despite the fact that natural background level of native ant species appeared fairly similar in both geographic regions. This effect has been previously detected in a study by Oliveras et al. (2005), in which they found that *L. humile* invasion increased the number of ant workers detected. This numerical dominance may, in part, be linked to the access of carbohydrate-rich resources (e.g. plant exudates, hemipteran honeydew). Several invasive ant species (incl. *L. humile*) have shown increased aggression, colony size and activity when they have access to sugar rich resources (Grover et al. 2007; Wittman et al. 2018). Unsurprisingly, honeydew collected from hemipterans such as aphids forms a significant portion of the diet of *L. humile* in invaded regions of Spain (Abril et al. 2007). However, access to these resources are considerably more limited in South African fynbos, as aphid abundances are found globally to decline with increasing plant species richness (Dixon et al. 1987).

Alternatively, another factor that might explain this difference in the magnitude of the impact could be that of time-since-invasion. While these sites were selected on the basis that they have a documented invasion history, the nondescript nature of *L. humile* means that the actual first appearance of these invaders in these sites is not known. For example, previous studies of invasion history have shown that impacts of invasive species can either decrease (Simberloff and Gibbon 2004; Strayer 2012) or increase (Strayer et al. 2006) as a function of time. This means that magnitude of impact may be a product of the variable invasion history of the sites. However, a recent study of the long-term impacts of *L. humile* on native ant species richness in Northern California found the impact to be unaffected by time, instead the magnitude of the effect was thought to be driven by site-specific factors (Menke et al. 2018). It is therefore likely that these observed differences in this study is due to the innate differences in either biotic or abiotic conditions in the Cape Floristic Region (Hamish Robertson pers. comm.). In fact, the impact of invasive ants can in part be explained by the intrinsic character traits of the recipient native ant community (Andersen et al. 1991; Witt and Giliomee 1991; Human and Gordon 1996; Rowles and O'Dowd 2009). For example, many of the native ant species found to coexist with *L. humile* in invaded sites exhibit a range of passive resistance methods, such as feigning death or nest entrance sealing to repel ant invaders (e.g. *Tetramorium*) (Löhr 1992; Witt and Giliomee 1991;

Addison and Samways 2000). Likewise, *Monomorium* species in other regions of the world exhibit active resistance to *L. humile* through expulsion of chemical repellents (Andersen et al. 1991; Holway 1999). This means one might expect that South African ant fauna are more resistant to invasion of *L. humile* as they possess avoidance mechanisms that the native ant species in Spain do not possess. Alternatively, this difference might not be driven by biotic factors, but instead by abiotic factors, such as temperature. Interestingly, *L. humile* was not observed at baits sampled during midday in South Africa, whereas in Spain, *L. humile* occupied all baits irrespective of sampling period. Abiotic factors, such as ground temperature, may therefore be limiting when *L. humile* can forage and could help explain why some ant species can co-exist in South Africa. For example, *Ocymyrmex barbiger* is recognised as a thermophilic ant species (Bolton and March 1989) and by foraging at period when *L. humile* is absent is able to avoid direct contact. Further work would be required to determine whether these temporal patterns are consistent throughout the year, as sampling in my study was limited to the summer, the hottest time of year when rainfall is relatively scarce in the Cape Floristic Region. An interesting (and potentially important) finding of this study (and one that is of particular importance to the subject of this thesis) is that in both geographic regions the presence of *L. humile* is associated with the displacement of ant genera most commonly associated with seed dispersal (e.g. *Anoplolepis* and *Pheidole*) and seed predation (e.g. *Messor*). This is an example of character displacement theory (Brown and Wilson 1956), in which *L. humile* are only displacing functional groups and/or species that occupy similar niches to themselves.

There are several limitations to this study that preclude me making any firm conclusions from, or detailed interpretations of, the findings. The first is replication across countries/biomes. One strength of this study is that it employed similar sampling methods in two different geographic regions; this means that the differences observed between countries is likely to reflect differences in local abiotic and biotic factors, and not the sampling method itself. However, with only two countries sampled, and with such contrasting patterns of effects in the two, it may be premature to draw any firm conclusions about specific effects of *L. humile*, other than that its presence is likely to cause changes in the native ant community. Replicating the same sampling regime across many invaded regions in different countries is required to ascertain the specific effects of *L. humile*. Ultimately, two key aims for future work should be to: (1) identify the *general* effects of *L. humile*, irrespective of local conditions (this requires replication of the same sampling regime across several countries with different biomes); and (2) identify *specific* effects common to a biome/geographic region/environment. Such information would be useful in advising management of the invasive species, and in prioritising conservation action; e.g.

further confirmation of the results presented here may lead to recommendations to prioritise conservation management of *L. humile* invasions in a Spanish-like biome (where the invasive ant appears to almost completely deplete the native ant community) over one like South Africa (where some native ants appear to be able to co-exist with the invader).

The second shortcoming of my study is one that most studies of invasion suffer from (Porter and Savignano 1990; Suarez et al. 1998; Holway 1998; Vanderwoude et al. 2000; Carpintero et al. 2005; Guenard and Dunn 2010): the observations here represent only a snapshot approach to exploring the effects of *L. humile* on native ant community structure, lack experimental manipulations (e.g. exclusion experiments), and so do not allow a direct comparison of ant community in the presence/absence of the invasive ant *within* the same site to be made. It is therefore difficult to say with any certainty that the study sites and therefore the native ant community structure did not differ intrinsically in some way prior to invasion, e.g. in terms of the levels of disturbance or resource availability. Previous research has shown that variability in habitat quality is a good predictor of invader abundance; for example, invasive *Solenopsis invicta* ant populations within North America are more often associated with disturbed than undisturbed habitats (King and Tschinkel 2013a, b) and so ant diversity prior to invasion may have been already depleted, irrespective to presence of the invasive species. This means that the presence of an invasive species alone may be insufficient evidence to infer native species displacement.

A third limitation of this study is that sampling of ant communities was limited to one season. The reason for this was that the wider context of the study (subsequent chapters in this thesis) was to examine the effects of *L. humile* on the provision of seed dispersing services through myrmecochory; thus, the sampling period was focused on the time of year when most seed dispersal takes place. Additional sampling across other times of year (e.g. in the spring and autumn) would therefore be required in order to establish whether this pattern is representative of the wider native ant community structure (both within invaded and non-invaded regions) all year round.

A fourth limitation of this study is the relatively low level of replication across sites, within countries. Sites were necessarily selected based on the known presence of the invasive ant and in areas which were known to have a long invasion history (>10 years). Given that invasions often have an acute and chronic phase (Strayer et al. 2006), it is considered to be more appropriate to sample sites with a long (and well documented) invasion history to achieve a more accurate picture of the longer term impacts of an ant invader on native ant community structure. Replication across multiple sites is challenging.

If multiple invasion zones are known, then there are additional problems with comparing across sites: e.g. the effects of the invasive species may vary depending on the length of time since invasion (Morrison 2002; Walker 2006; Strayer et al. 2006; Menke et al. 2018); thus it would be best to select sites with comparable invasion histories.

In conclusion, despite the stated limitations of this study, my analyses suggest that the presence of *L. humile* in both Spain and South Africa appears to be having a discernible impact on the structure of native ant communities. My study finds some tentative evidence of associations between species declines and the presence of *L. humile*; however, further research is required with higher levels of replication across sites and across different countries/biomes in order to corroborate these patterns. Future work should also aim to identify the mechanisms that allow some native ants to co-exist with invaders, whilst others are excluded. However, in the context of this thesis, these analyses provide a necessary bedrock of knowledge on the ant community structure (and species lists) in invaded and non-invaded sites, within which I study the dynamic process of the ant-seed interaction (Chapter 4), and the potential for cascading, knock-on effects to the wider ecosystem via the preferential dispersal of seeds from alien plants (Chapter 5) and the potential mechanisms underpinning this (Chapter 6).

CHAPTER FOUR

4. INVASIVE ANTS ALTER SEED DISPERSAL DYNAMICS

Parts of this chapter are adapted from the publication Devenish et al. (2018) Invasive ants take and squander native seeds: implications for native plant communities, *Biological Invasions*. <https://doi.org/10.1007/s10530-018-1829-6>

4.1 ABSTRACT

Seed dispersal is a fundamental process in the lifecycle of all flowering plants. Many plant species have evolved specialist associations with biotic vectors to facilitate dispersal. Such specialised interactions mean that these associations are potentially highly sensitive to disruption, e.g. from invasive species. However, despite this threat we still understand remarkably little about how such perturbations affect the dynamics and efficiency of the seed-dispersal process. In this study I quantify the impacts of an invasive ant across three key phases of the seed dispersal process - seed removal, distribution and placement - in order to determine the stages of seed dispersal most vulnerable to disruption by invaders. Using the Argentine ant (*Linepithema humile*) as a model, I show that invaded sites exhibited a significant decrease in seed dispersal services across all three phases of the dispersal process, relative to non-invaded sites. Seeds dispersed in invaded sites were: (a) less likely to be moved, (b) potentially distributed over a smaller spatial area, and (c) less likely to be placed at soil depths favourable for germination and establishment compared with those dispersed in non-invaded sites. These results reveal that ant-mediated seed dispersal services are significantly reduced by an invasive species at multiple stages in the dispersal process. Reductions in the efficacy of seed dispersal, combined with shifts in the ecological and geographical patterns of dispersal, may lead to cascading impacts on plant species composition and community structure. This study shows how an invasive ant can affect seed dispersal at several stages in the dispersal process.

4.2 INTRODUCTION

Seed dispersal is a vital process in the life cycle of all flowering plants (O'Dowd and Hay 1980; Howe and Smallwood 1982; Hanzawa et al. 1988). Because plants are sessile, they rely on abiotic (e.g. wind and water) and biotic (e.g. insect, bird, and mammal) vectors to disperse their seeds. Invasions by non-native species threaten the efficacy of biotic seed-dispersers, with potentially serious knock-on effects to the natural plant community structure (Ricklefs and Renner 1994; Webb and Peart 2001; Christian 2001; Ozinga et al. 2009; Ruxton and Schaefer 2012). Despite this threat, we often lack comprehensive data on how specific biological invasions alter important ecosystem processes like seed dispersal. This is because assessments are often based on studies that focus on only one aspect of the seed dispersal process (e.g. seed choice), without consideration of the entire process (e.g. whether seeds ultimately end up in favourable germination sites). This latter example can be used as a proxy for understanding the ultimate impact on plant community composition.

Ants are one of the major seed dispersal agents for angiosperms in Mediterranean climates (Lengyel et al. 2010). Ant-mediated seed dispersal (myrmecochory) is geographically widespread, and observed in at least 11 000 (4.5% of all) angiosperm plant species, across 77 (12% of all) families (Bronstein et al. 2006; Lengyel et al. 2010). Plant species that rely on this mode of dispersal use an oily seed appendage (called an elaiosome) to attract ants which then remove the seed back to their nest (Beattie 1985). In doing so, ants place the seed out of reach from seed predators and of destruction by fire and waterlogging (Bond and Stock 1989; Cuautle et al. 2005; Fenner and Thompson 2005). Ants in turn benefit from the nutritious elaiosome (Gammans et al. 2005), which they feed to their larvae (Beattie 1985).

Ant behavioural and biological traits influence the efficacy of myrmecochory and thus the seed-dispersal process. These traits include: Seed removal - an ant must locate a seed and remove it from where it fell; Nest distribution - an ant must deliver the seed to its nest in an area away from the parent plant; Seed placement - an ant must remove the elaiosome on arrival at the nest, and discard the intact seed in a suitable place for germination and establishment (e.g. on refuse piles in or around the ant nest) (Culver and Beattie 1980; Oliveras et al. 2005b). A mismatch between ants and their plant partners at any point in the seed dispersal process will likely modify the nature of this mutualism, reducing both seed dispersal and the survival of myrmecochorous flora. For example, not all ant species handle

seeds in a way that will result in successful germination and seedling establishment (Gómez and Espadaler 1998a, c). The need for successful processing of seeds to occur at all steps makes many ant-plant interactions far more specialised than they might initially appear to be, and therefore this process is much more vulnerable to disruption than might be expected. Even small changes to ant communities may alter the composition of plant communities (Andersen and Morrison 1998; Christian 2001; Ruxton and Schaefer 2012; Warren and Bradford 2014), which can result in shorter seed dispersal distances, reduced transportation rates of seeds to ant nests, and reduced seedling germination and establishment (Bond and Slingsby 1984; Christian 2001; Gómez and Oliveras 2003; Rodriguez-Cabal et al. 2009). In addition, the effects will not necessarily be distributed equally across the native flora, which means there can be significant shifts in plant community structure, together with shifts in functional and taxonomic diversity (Bond and Slingsby 1984; Christian 2001).

Invasions by non-native ant species can significantly alter the ecological distribution and phylogenetic structure of native ant communities (Tsutsui et al. 2000; Lessard et al. 2009). These invaded ant communities show reduced genetic and ecological diversity (Holway and Suarez 2006), resulting in a loss of both keystone ant species (Christian 2001) and ecosystem function (Andersen 1997). For example, Lessard *et al.* (2009) showed that across several studies, ant invaders act as a form of environmental filter, resulting in a loss of native species in a non-random manner. This disassembly of the native ant community structure often results in the loss of specialist ant groups, such as seed dispersers (Suarez et al. 1998; Christian 2001).

Linepithema humile Mayr, the Argentine ant (Hymenoptera: Formicidae) is one of the most invasive ant species in the world (Holway et al. 2002). Human activities have caused its rapid global spread since the 1800s, such that established populations have been found across six continents, in at least 55 countries, and on several oceanic islands (Suarez et al. 2001; Tsutsui et al. 2001; Wetterer et al. 2009). In regions with a Mediterranean climate and/or mild winters, the first recorded introductions were in 1858 (Holway 1998; Wetterer et al. 2009). *Linepithema humile* invades both disturbed and undisturbed habitats, resulting in changes to the native ant community structure (Bond and Slingsby 1984; Holway 1998; Christian 2001; Holway et al. 2002).

Observations of invasive *L. humile* populations in native ecosystems have shown that they can alter the patterns of myrmecochory. For example, a quantitative analysis on the effects of *L. humile* on native ant community structure by Rodriguez-Cabal *et al.* (2009)

found an average 92% reduction in the number of native ant seed disperser species within invaded regions. This loss in native seed dispersers has been demonstrated to have a detrimental impact on seed dispersal processes, with a reduction in both the distance seeds are transported and their likelihood of reaching ant nests (Gómez and Oliveras 2003; Gómez et al. 2003). While this effect has been detected in a wide variety of habitats (Christian 2001; Gómez and Oliveras 2003; Rowles and O’Dowd 2009), the degree of effect can vary tremendously between studies, either because of differences in habitat types or sampling method used.

Much of the work on the impacts of *L. humile* over the last few decades has focused on assessing their effects on seed dispersal distance, often using a single plant species (Bond and Slingsby 1984; Quilichini and Debussche 2000; Carney et al. 2003; Oliveras et al. 2005a). However, other aspects of ant behaviour and ecology, such as seed preference (variation in removal efficiency relative to different plant species), nesting ecology (distribution and location of nests across a landscape), and seed placement (post-dispersal burial depth) are often omitted or overlooked, even though they are likely to affect seed dispersal and survival (Bas et al. 2007; Renard et al. 2010). We therefore lack a single study that examines the impact of *L. humile* on seed dispersal across the full process, from seed removal, to seed placement in the nest. Such information is essential if we are to make accurate assessments of the impacts of an invasive ant species on this important ecosystem service.

In this study I evaluate how seed dispersal efficiency in invaded and non-invaded areas is affected by differences in the ants’ seed-handling behaviour at three key phases in the seed dispersal process. Specifically, across four sites in Spain, I compared the seed-handling behaviour of both *L. humile* and a sympatric native seed-dispersing ant, *Pheidole pallidula*. I test the hypotheses that compared with seeds placed in non-invaded sites, seeds in sites invaded by *L. humile* are: (1) less likely to be removed (seed removal: hypothesis 1); (2) distributed over a smaller area (nest distribution: hypothesis 2); and (3) placed at depths less favourable for germination and establishment (seed placement: hypothesis 3). Identifying how *L. humile* ants differ from sympatric native seed-dispersers, with regard to how they handle seeds at different phases of the seed dispersal process, will help achieve more accurate predictions as to the detrimental impact of ant invasions on this important ecosystem service. This research may then help inform any future efforts aimed at mitigating the consequences of invasion by ants.

4.3 METHODS AND MATERIALS

The Argentine ant was first recorded in the Iberian Peninsula at the beginning of the nineteenth century (Espadaler and Gómez 2003). Since then its population has expanded considerably in Northern Spain, particularly in coastal regions at a rate of 7.94 (\pm 2.99) metres per year (Roura-Pascual et al. 2010). The study was conducted at four sites in June–July 2014 and July–September 2015, when myrmecochorous seeds were naturally dehiscing. Two of the selected sites were known to be invaded with *L. humile* (Montilivi Campus [Site 1]: 41°58'59.20"N, 02°49'29.75"E and Castell d'Aro [Site 2]: 41°49'04.612"N, 03°04'00.68"E); the other two sites were areas not invaded by *L. humile* (Montilivi Campus [Site 3]: 41°58'59.20"N, 02°49'29.75"E and Santuari dels Angels [Site 4]: 41°58'31.18"N, 02°54'34.02"E).

Invasion status was confirmed, and ant community composition assessed for each site using both baiting and pitfall traps (see Chapter Three). At invaded sites, only two ant species were present; the non-native invasive *L. humile* and native non-seed dispersing *Plagiolepis pygmaea* (see Chapter Three). By contrast, at the non-invaded sites, 30 species of ants were present. The sympatric native seed-dispersing species was *P. pallidula*, which represented 62% ($n = 3286$) of ant individuals collected (Table 3.1). *Pheidole pallidula* is a socially polymorphic ant species with dimorphic castes (worker size: 2.2 – 4.5 mm) (Gómez and Espadaler 1994; Fournier et al. 2016). This species has an omnivorous diet and is characterised as a short distance seed disperser, with a mean seed transport distance of 0.46 m (Gómez and Espadaler 1998a, b, c). According to the Giladi (2006) seed collecting ant guild behavioural classification system, both the invasive *L. humile* and native *P. pallidula* would loosely fall within the second guild (“high quality dispersers” or “removalists”) of seed dispersers.

The vegetation at all sites was a combination of open cork-oak secondary forest, dominated by *Quercus* and *Pinus* tree species, with herbaceous myrmecochorous plant species in the clearings.

4.3.1 Seed collection

Ten locally abundant myrmecochorous plant species (Castroviejo 2012) were used in this study (Table 4.1). These species were selected because they exhibited a range of seed shapes and weights (Table 4.1). For each species, fruits near maturation were collected from at least 25 plants in and around the study plots and allowed to dehisce naturally in ambient conditions in the laboratory. Seeds were stored at room temperature and handled

with forceps and gloves throughout the study. All trials were conducted within two to three weeks of fruit collection.

Table 4.1: Plant species used in experiments and their respective seed traits, collected in Girona, Northern Spain, between June 2014 and July 2015. Mean seed weights based on 250 seeds [± 1 SD].

Plant Family	Plant Species	Collection Date	Seed shape	Mean Seed Weight (mg)	Experiment
Asteraceae	<i>Cirsium vulgare</i> (Savi) Ten.	June 2014	elliptic	2.9 (± 0.26)	1 (Removal)
Euphorbiaceae	<i>Euphorbia characias</i> L.	June 2014	cylindrical	5.8 (± 0.39)	1 (Removal)
Euphorbiaceae	<i>Euphorbia nicaeensis</i> All.	June 2014	cylindrical	6.1 (± 0.20)	1 (Removal)
Euphorbiaceae	<i>Euphorbia serrata</i> L.	June 2014	cylindrical	6.2 (± 0.15)	1 (Removal)
Asteraceae	<i>Galactites tomentosa</i> Moench	June 2014	elliptic	11.2 (± 1.54)	1 (Removal)
Fabaceae	<i>Genista linifolia</i> L.	June 2014	ovoid	6.5 (± 0.24)	1 (Removal)
Fabaceae	<i>Genista monspessulana</i> (L.) L.A.S. Johnson	July 2015	globular	5.8 (± 0.14)	2 (Placement)
Fabaceae	<i>Sarothamnus arboreus</i> Boiss.	July 2015	ovoid	6.9 (± 0.31)	2 (Placement)
Asteraceae	<i>Sylibum marianum</i> (L.) Gaertn.	June 2014	elliptic	13.2 (± 1.29)	1 (Removal)
Fabaceae	<i>Ulex parviflorus</i> Pourr.	June 2014	ovoid	4.1 (± 0.25)	1 (Removal)

4.3.2 Field experiments

4.3.2.1. Hypothesis 1: Seeds in sites invaded by *L. humile* are less likely to be removed and transported than seeds in non-invaded sites.

The dispersal rate of seeds in invaded and non-invaded sites was investigated using seed choice experiments. Within each site, ten seed hubs were set up at 10 m intervals, along a transect that was previously used in the ant community surveys (see Chapter three). Each seed hub consisted of a 10 cm² white card with a dome wire mesh placed on top. Ants were able to access the seeds, but larger arthropods and vertebrates were not.

In total 40 seed hubs (10 per site) were set up within the four sites. Out of the eight selected plant species, six seeds were placed on each hub (three seeds taken from two randomly chosen plant species). The seeds were placed on the seed hubs at 08:00 hours and surveyed at 0.5, 1, 2, 3, 6, 12 and 24 hours thereafter. At each of these time points, the different ant species on or within 5 cm of the hub, and the total number of seeds from each species remaining on the hub, were recorded. The seed choice experiments were run for six consecutive days, with seeds from each plant species being placed once on each hub. In total, 870 seeds were used in this study, with 431 seeds placed in invaded ($n = 2$) and 439 seeds in non-invaded ($n = 2$) ant communities.

All statistical analyses were carried out in R (version 2.3.2) program (R Core Team 2017). I compared seed removal rates between and within invaded and non-invaded sites using a Cox Proportional Hazard-model (Cox 1972) and Log-rank test (Bland and Altman 2004) in the *survival* (version 2.42-3) and *coxme* (version 2.2-10) packages (Therneau and Grambsch 2000; Therneau 2018). Generalised linear mixed models (GLMM) using a Poisson error distribution in the *lme4* (version 1.1-17) package (Bates et al. 2015) were

then used to analyse seed removal frequencies (portion of seeds removed from seed hubs after 12 hours) in invaded and non-invaded sites (Crawley 2012). In both the survival and GLMM analyses, the fixed effects were invasion status (invaded vs. non-invaded) and plant species ID ($n = 8$; Experiment 1, Table 4.1); seed hub ID (nested within transect) was included as a random factor, which controls for the effects of site and repeated sampling of seed hubs. All generated p values were subjected to the Benjamini-Hochberg correction method (Benjamini and Hochberg 1995) to control for false discovery rates.

4.3.2.2. Hypothesis 2: *L. humile* nests in invaded sites are less evenly distributed than *P. pallidula* nests in non-invaded sites.

Since seeds are likely to be transported back to the ants' nest, seed distribution can be heavily influenced by the spatial distribution of nests. Therefore, the spatial patterns of ant nests within a site can potentially determine both the structure and dynamics of plant populations (Nathan and Muller-Landau 2000; Berg-Binder and Suarez 2012). If nests are clumped, seeds are less likely to be widely distributed than if nests are evenly dispersed.

The spatial distribution of nests of the primary seed dispersing ant species in invaded (*L. humile*) and non-invaded (*P. pallidula*) sites was compared between the four sites. Within each site, 5 randomly positioned grids (30.25 m²) were set up at least 20 metres apart, each consisting of 144 white 5 cm² cards, separated from each other by 50 cm (Fig. 4.1a). Each card was supplied with 5 g of ant bait consisting of a mix of tuna and honey (5:1 ratio). Each card was observed for 4 hours, between 8:00 am and 12.00 noon (peak foraging activity for both species (Adam Devenish pers. obs.)), for 10 consecutive days. The numbers of ant trails were recorded on each card, and trails were followed back to their nests. Each quartet of cards (Fig. 4.1b) was scored according to whether there was a nest present (≥ 1) or absent (0) (Fig. 4.1c), together with the number of ant trails leading into the nest (Fig. 4.1d). The number of ant trails leading to a nest entrance within a quartet was taken as an estimate for nest size; a potential limitation of this method is that I am assuming that these foraging numbers stay consistent over time. From these snapshot data, nest density for each grid ($n = 20$) was calculated based on the total number of quartets occupied by ant nests and the relative nest size. Ant trails that either originated from, or extended beyond the grids, were not included.

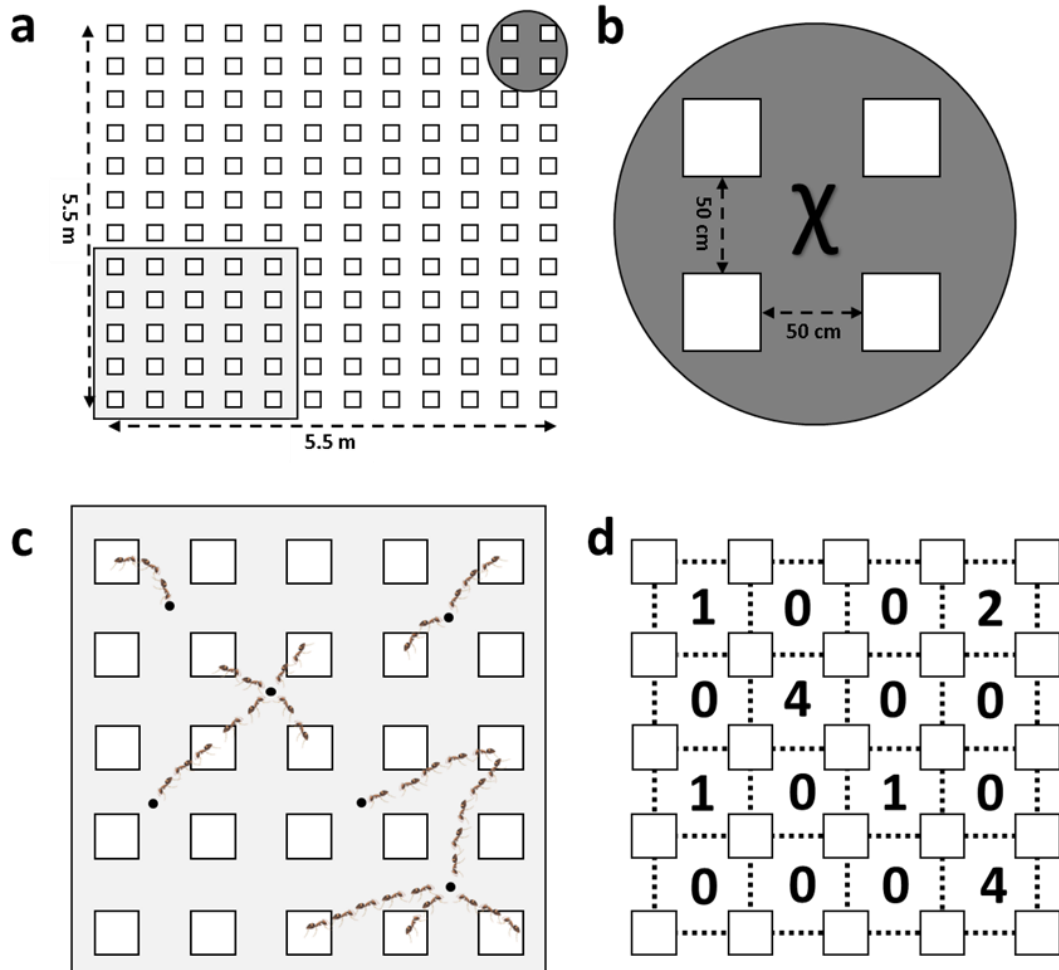


Figure 4.1: Trails from ants attracted to tuna and honey bait cards (placed 50 cm apart in a grid of 30.25 m²) were used to assess nest distribution (size and density) in invaded and non-invaded sites (Hypothesis 2). (a) Grid layout; (b) a “quartet” (cluster of four cards) in a grid and the assigned score (χ = number of trails leading into the grid); (c) ant trails leading back to nests in each quartet were recorded daily over a four-hour period for 10 days; (d) the number of ant trails within each quartet was used as a measure of ant density and hence nest size.

Grid scores were analysed using the PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis Tool (version 2) program (Rosenberg and Anderson 2011). This generates a dispersion index value (D), based on variance (σ^2) to mean (μ) ratio (VMR; $D = \sigma^2/\mu$), which represents how clustered or dispersed the sample is. Mean grid scores were compared between sites using a GLMM model, where the fixed effects were ant species (*L. humile* vs. *P. pallidula*), with site ID included as a random factor.

4.3.2.3. Hypothesis 3: Seeds in sites with *L. humile* invasions are less likely to be placed in locations favourable for germination and establishment compared with non-invaded sites.

There are a number of components (e.g. number of seeds transported) that need to be considered when assessing the quality of a seed disperser (Schupp et al. 2010). Within

sclerophyllous vegetation, final seed placement is often considered to be one of the key aspects associated with high-quality seed dispersers. In particular post-dispersal seed placement within an ant nest heavily influences both seed germination and seedling survival (Christian and Stanton 2004; Gómez et al. 2005; Cumberland and Kirkman 2013). However, transport of a seed into the nest alone should not be considered sufficient evidence for optimal placement, as ant species may place seeds at depths that could be detrimental to seedling survival (Gómez and Espadaler 1998). Seed placement depth was therefore assessed in 20 nests of *L. humile* in an invaded locality (Castell d'Aro), and 20 nests of *P. pallidula* ants in a non-invaded site (Montilivi Campus). Only nests that were at least 5 m apart from each other were selected. Each nest was presented with 40 seeds, placed within 5 cm of the nest entrance: 20 of *Genista monspessulana* (French broom) and 20 of *Sarothamnus arboreus* (Black broom). These native plant species were chosen as they were not present in either locality but were naturally dispersing at the time of the trial. This means that all seeds of these species found in the ant nests would be from the experiment, rather than having been naturally dispersed.

To ensure only each target ant nest retrieved seeds, I observed them for 30 minutes until all the seeds were taken into the nest. If any seeds remained on the surface after this time, a Petri dish was used to cover the seeds and the nest entrance overnight. The following morning any seeds remaining on the surface were collected. Seeds that were not collected are assumed to have been retrieved by the ants into their nests. After 72 hours, a radius of 20 cm around each nest entrance was inspected and any discarded seeds collected. This distance was selected as the capacity for an ant to transport a seed after removal of the elaiosome is limited by the morphology of the ant species (body length and mandible gap size; Gómez et al. 2005). Moreover, these seeds when discarded are often placed on refuse piles above-ground, in close proximity (< 20cm) to the nest (Narbona et al. 2014). I am unable to rule out the potential of any post-dispersal predation from refuse piles in non-invaded regions; however, in invaded regions, no clear above-ground refuse piles for *L. humile* were reported (Adam Devenish pers. obs.). This difference in post-dispersal behaviour is likely to be due to the fact that *L. humile* (gap size: 0.6mm; worker body length: 2.6 mm) have a relatively smaller mandible gap size and body size than *P. pallidula* (gap size: 0.64 mm; worker body length: 2.2 - 4.5 mm) and is therefore unable to eject seeds from their nests (Crisanto Gomez, pers. obs.).

Following the above-ground inspection of the nests, each nest was excavated to a depth of 10 cm, and soil was panned using a graduated sieve (minimum wire mesh aperture: 0.5 mm) to collect any seeds. Depths below 10 cm were not excavated, as seeds deposited

below this depth are unlikely to emerge (Bas et al. 2007). I thus make the explicit assumption that any seed not found within a 20 cm radius of the nest entrance, nor within 10 cm depth in the nest classifies as an unsuccessful dispersal event.

All seeds collected were inspected for the presence of an elaiosome and for signs of seed coat damage. Seeds were classified as either: *ejected* (collected from refuse piles above-ground); *buried* (collected from within the nest up to a depth of 10 cm); or *fate unknown* (not retrieved). I make the explicit assumption that these ‘not retrieved’ seeds were either buried deeper than > 10 cm and thus unlikely to survive; or in the instance of non-invaded regions, these seeds may also have been subject to post-dispersal predation by granivorous ants from refuse piles. The numbers of seeds ejected or buried in *L. humile* and *P. pallidula* nests were compared using a binomial GLMM model, where the fixed effects were ant species (*L. humile* vs. *P. pallidula*), with nest ID included as a random factor. A Mann-Whitney U test was used to compare post-dispersal placement of seeds within and outside of *P. pallidula* nests.

4.4 RESULTS

4.4.1 Hypothesis 1: Seeds in sites invaded by *L. humile* are less likely to be removed and transported than in non-invaded sites

Seeds from all plant species were more likely to be dispersed by ants in non-invaded sites compared with invaded sites (Fig. 4.2a). After 12 h, 95% (\pm SEM 3.6%, $n = 80$) of seeds had been removed from hubs in the non-invaded sites, compared with 49% (\pm SEM 1.9%, $n = 80$) in the invaded sites (GLMM: $F = 65.722$, $DF = 1$, $p < 0.0001$). Comparison of seed removal rates revealed that seeds were ten times more likely to be removed from seed hubs in non-invaded sites compared with invaded sites (Hazard ratio, $n = 870$, 95% CI [3.8, 28.7]; Table 4.2).

Table 4.2: Effects of invasion status (Model 1) and plant species (Model 2) on the removal rate of seeds from hubs using a Cox's regression analysis. Hazard Ratio (HR) is determined by the difference between the slopes of the corresponding treatments and represents the likelihood of a seed dispersal event occurring. Hubs within transects are included as random factors, to control for the non-independence of replicating hubs within sites.

Fixed effects	Coefficient	SE	Hazard Ratio (HR)	95% Confidence intervals for HR	<i>p</i>	<i>q</i>
Invasion status ¹	2.33	0.52	10.66	3.76 - 28.71	***	***
Within invaded sites ²						
<i>Cirsium vulgare</i>	0.77	0.18	2.16	1.52 - 3.09	***	***
<i>Euphorbia characias</i>	0.01	0.15	1.01	0.75 - 1.37	0.94 ^{NS}	0.94 ^{NS}
<i>Euphorbia nicaeensis</i>	0.26	0.15	1.30	0.97 - 1.75	0.08 ^{NS}	0.13 ^{NS}
<i>Euphorbia serrata</i>	0.71	0.15	2.03	1.51 - 2.71	***	***
<i>Galactites tomentosa</i>	-0.82	0.21	0.44	0.29 - 0.66	***	***
<i>Genista linifolia</i>	-0.61	0.18	0.54	0.39 - 0.77	***	***
<i>Sylibum marianum</i>	0.07	0.15	1.07	0.79 - 1.45	0.64 ^{NS}	0.85 ^{NS}
<i>Ulex parviflorus</i>	-0.03	0.18	0.97	0.68 - 1.38	0.86 ^{NS}	0.94 ^{NS}
Within non-invaded sites ²						
<i>Cirsium vulgare</i>	0.28	0.18	1.33	0.94 - 1.87	0.11 ^{NS}	0.22 ^{NS}
<i>Euphorbia characias</i>	0.30	0.14	1.35	1.02 - 1.77	*	0.14 ^{NS}
<i>Euphorbia nicaeensis</i>	-0.09	0.15	0.92	0.69 - 1.22	0.55 ^{NS}	0.73 ^{NS}
<i>Euphorbia serrata</i>	0.26	0.14	1.30	0.99 - 1.71	0.06 ^{NS}	0.17 ^{NS}
<i>Galactites tomentosa</i>	-0.16	0.15	0.85	0.64 - 1.14	0.29 ^{NS}	0.46 ^{NS}
<i>Genista linifolia</i>	-0.34	0.14	0.71	0.54 - 0.94	*	0.13 ^{NS}
<i>Sylibum marianum</i>	0.00	0.14	1.00	0.76 - 1.31	0.98 ^{NS}	0.98 ^{NS}
<i>Ulex parviflorus</i>	-0.01	0.17	0.99	0.71 - 1.38	0.96 ^{NS}	0.98 ^{NS}
Random effects	Variance	SD	Loglikelihood	Chi ²	<i>p</i> (> Chi ²)	
Invasion status ¹						
Transect / Seed hub ID	0.31	0.56	-9444.3	301.02	***	
Transect	0.25	0.50	-9594.9	173.97	***	
Plant species (Invaded) ²						
Transect / Seed hub ID	0.13	0.36	-4081.6	44.742	***	
Transect	0.00	0.02	-4081.6	44.739	***	
Plant species (Non-invaded) ²						
Transect / Seed hub ID	0.42	0.65	-4868.4	212.05	***	
Transect	0.70	0.84	-4944.1	363.47	***	

¹Model1 (~Invasion status+(1|Transect/Seed hub ID)

²Model2 (~Plant species+(1|Transect/Seed hub ID)

q = Benjamini-Hochberg corrected *p* values

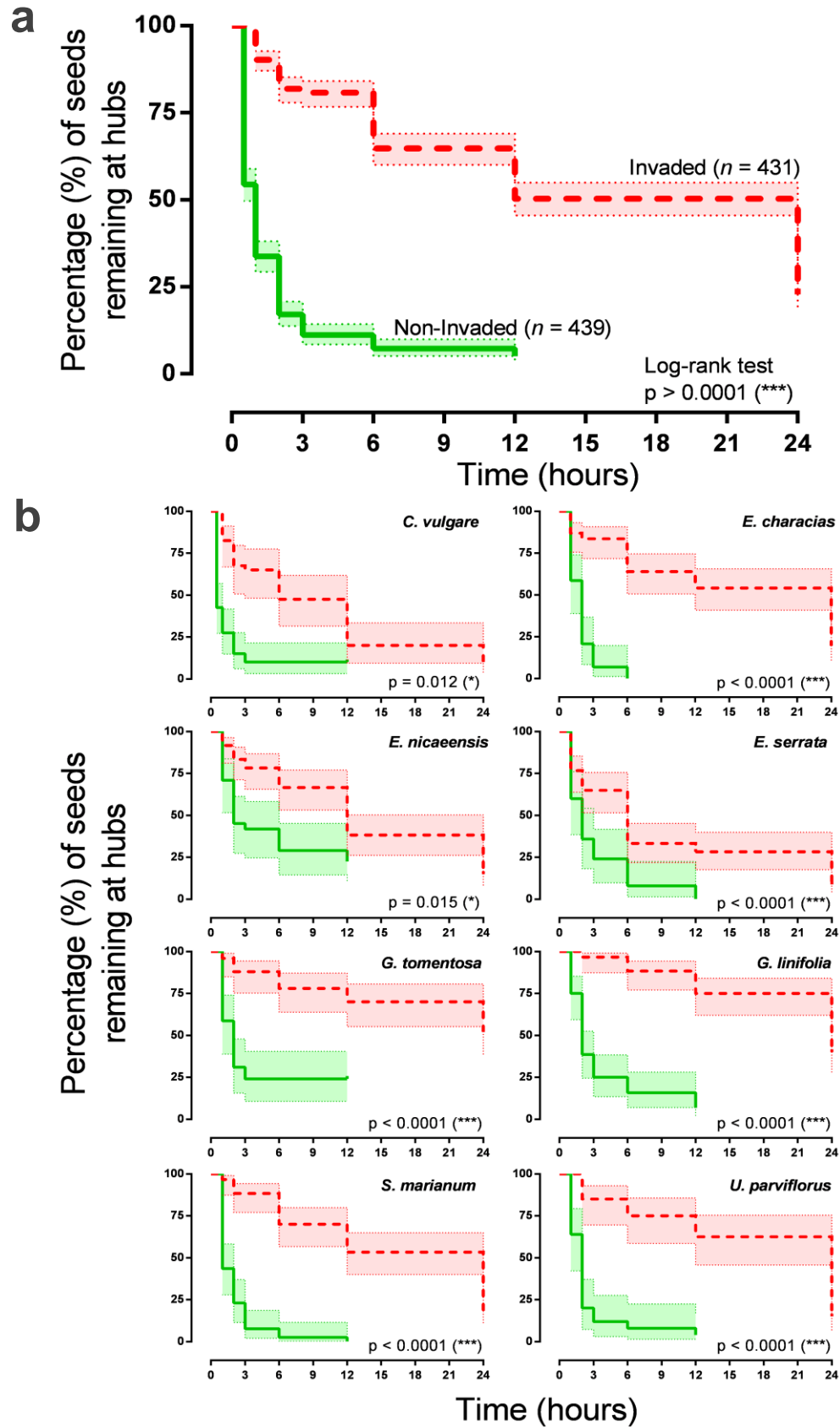


Figure 4.2: Seed removal rate differed significantly between invaded (*L. humile* present) and non-invaded (*L. humile* absent) sites. Kaplan-Meier survival curves comparing the seed removal rate of seeds placed in *L. humile* invaded (dashed) and non-invaded (solid) sites using a Cox Proportional Hazard model [$\pm 95\%$ CI]. Effect of invasion status for all plants (a) and for each plant species in turn (b) on seed removal rates shown with the Log-rank test for trend result [$\pm 95\%$ CI].

Plant species selection was shown to have a significant effect on whether a seed was likely to be removed or not in invaded sites (Log-rank test for trend: $X^2 = 65.77$, $DF = 1$, $p < 0.0001$). By contrast, plant species selection did not have any significant effect on seed removal in non-invaded sites (Log-rank test for trend: $X^2 = 2.432$, $DF = 1$, $p = 0.1188$). The fastest rates of removal in invaded sites were for the plant species *Cirsium vulgare* (52% [\pm SEM 7.9%] of seeds removed after 6 h) and *Euphorbia serrata* (65% [\pm SEM 6.2%] of seeds removed after 6 h); however, these removal rates were still significantly lower than seeds of the same plant species (*C. vulgare* 90% [\pm SEM 4.7%] and *E. serrata* 92% [\pm SEM 2.1%] dispersed (removed after 6 h) in non-invaded sites (Fig. 4.2b). By contrast, the lowest rates of removal of seeds in invaded sites were for *Galactites tomentosa* (22% [\pm SEM 6.2%] of seeds removed after 6 h) and *Genista linifolia* (12% [\pm SEM 4.1%] of seeds removed after 6 h); these rates were significantly less than the lowest rates of removal for any species in non-invaded sites (Fig. 4.2b).

4.4.2 Hypothesis 2: *L. humile* nests in invaded sites are less evenly distributed than *P. pallidula* nests in non-invaded sites

There were significant differences in the distribution of nests of the primary seed dispersing ant species in invaded and non-invaded sites. In the invaded sites, *L. humile* nests were clumped (mean index of dispersion score of $7.962 \pm$ SEM 0.75, $n = 10$). This was significantly different to the sympatric *P. pallidula* (GLMM: $F = 31.546$, $DF = 1$, $p < 0.001$), which exhibited a uniform distribution (mean index of dispersion score of $0.9375 \pm$ SEM 0.04, $n = 10$) in the non-invaded sites (Fig. 4.3).

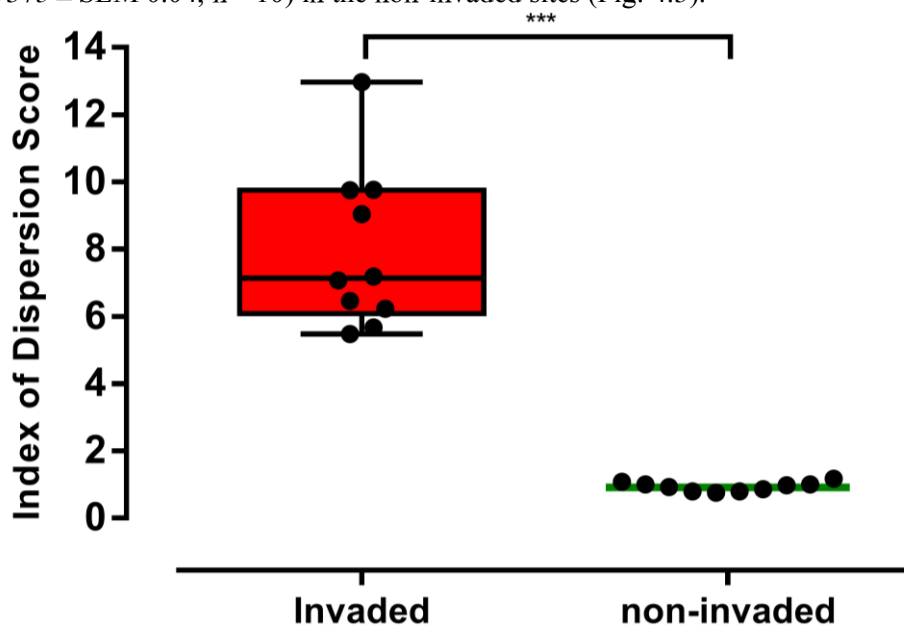


Figure 4.3: Nests of the primary seed disperser in invaded sites (*L. humile*) were more clumped than the primary seed disperser in non-invaded sites (*P. pallidula*). Boxplot of index of dispersion scores (Y-axis) across 20 grids placed in invaded ($n = 10$) and non-invaded ($n = 10$) sites.

4.4.3 Hypothesis 3: Seeds in sites with *L. humile* invasions are less likely to be placed in locations favourable for germination and establishment compared with non-invaded sites

Invasive *L. humile* ants were less effective at taking seeds into their nests. *Linepithema humile* ants ($n = 20$, $63\% \pm \text{SD } 30.6\%$) retrieved significantly fewer of the 40 presented seeds into their nest compared with the native *P. pallidula* ant ($n = 20$, $100\% \pm \text{SD } 0\%$) species, which retrieved all presented seeds into their nest (GLMM: $F = 208.84$, $\text{DF} = 1$, $p < 0.0001$). Furthermore, *L. humile* was more selective over which plant species it took into the nest, retrieving significantly fewer seeds of the smaller-seeded *Genista monspessulana* ($n = 20$, $35\% \pm \text{SD } 13.1\%$) than the larger-seeded *Sarothamnus arboreus* ($n = 20$, $92\% \pm \text{SD } 6.3\%$) (GLMM: $F = 213.27$, $\text{DF} = 1$, $p < 0.0001$) (Fig. 4.4a).

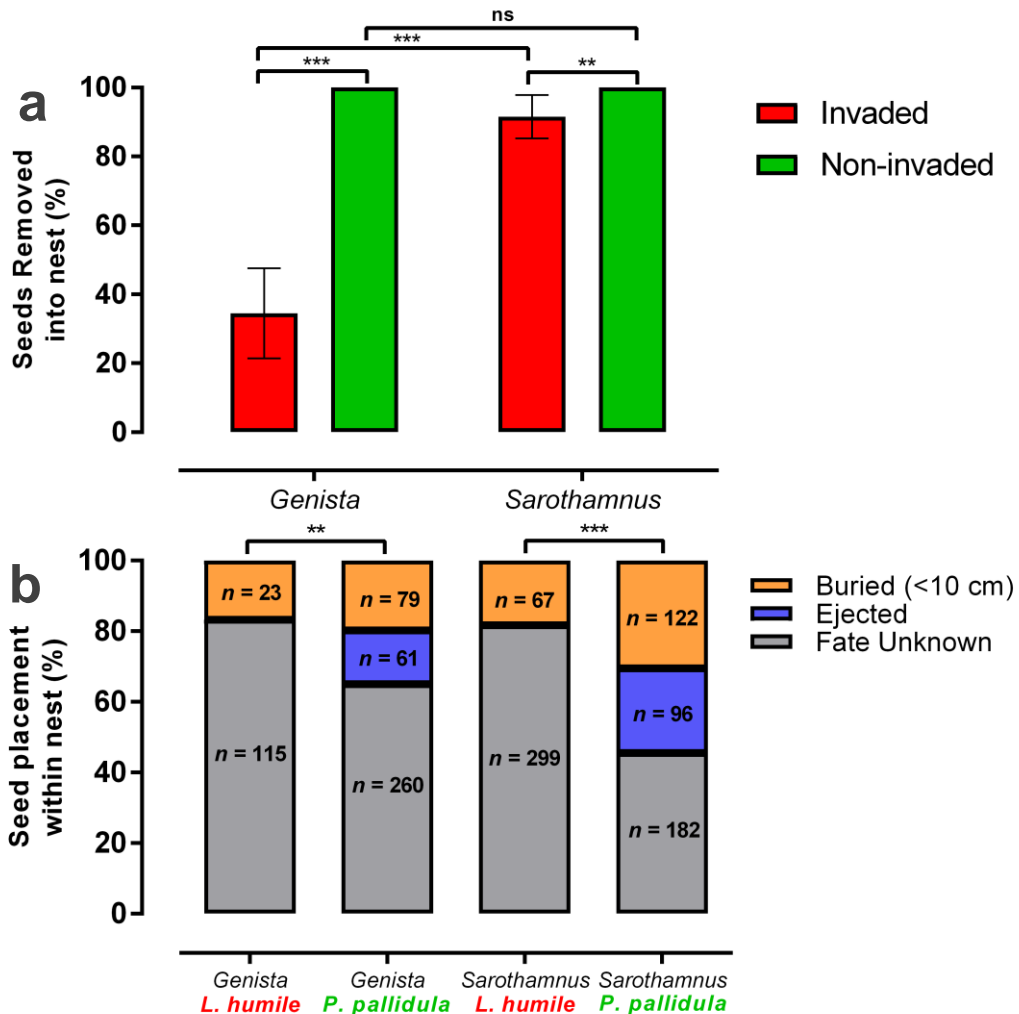


Figure 4.4: Seeds were less likely to be taken into nests and placed in conditions favourable for germination and establishment in invaded (*L. humile* present) than non-invaded (*L. humile* absent) sites. (a) Percentage of seeds from two plant species (*Sarothamnus arboreus* and *Genista monspessulana*) retrieved into nests of invasive *L. humile* or native *P. pallidula* ant species ($n = 40$ nests; $n = 40$ seeds per nest (20 per species)). (b) Fate of the seeds removed into nests (categorised as: *ejected* above-ground, *buried* <10 cm below-ground and *fate unknown*) for the two-plant species in invasive *L. humile* ($n = 20$; red) and native *P. pallidula* ($n = 20$; green) ant nests [$\pm 95\%$ CI].

Invasive *L. humile* ants were less likely to place seeds at depths suitable for germination and seedling establishment than the native ant *P. pallidula*. After 72 h, fewer seeds were detected at < 10 cm depth (i.e. suitable for seedling emergence) in *L. humile* nests than in native *P. pallidula* nests (GLMM: $F=20.605$, $DF=1$, $p<0.0001$). In addition, no seeds were observed to be ejected from *L. humile* nests after they had been taken into the nest (Fig. 4.4b), supporting my assumption that seeds not found through excavation were buried deeper than 10 cm (see Methods).

By contrast, at least 15% (\pm SD 14.7%, $n=20$) of *G. monspessulana* and 24% (\pm SD 19.6%, $n=20$) of *S. arboreus* seeds were ejected from *P. pallidula* nests and deposited in waste dumps < 5 cm from their nest entrance. All ejected seeds lacked elaiosomes (putatively removed by the ants for nutritional needs) and a few (5%) showed signs of granivory (damaged seed coats). In *P. pallidula* nests the proportion of seeds buried and ejected did not differ significantly for either *G. monspessulana* (Mann–Whitney U-test: $U=146$, $DF=1$, $p=0.14$) or *S. arboreus* (Mann–Whitney U-test: $U=149$, $DF=1$, $p=0.17$) (Fig. 4.4b). Seeds not accounted for in non-invaded sites (classified as fate unknown; Fig. 4.4b) were either buried deeper within the nest or ejected and moved beyond the 20 cm search boundary.

4.5 DISCUSSION

The invasion of ecosystems by exotic organisms is threatening long-established mutualistic relationships between native species, including those associated with ant-plant interactions. In this study, I found evidence to suggest that across all three key phases in the seed dispersal process, the seed-handling behaviour of ants within sites invaded by Argentine ants differed significantly from that of the non-invaded native seed-dispersing ant community (Fig. 4.5).

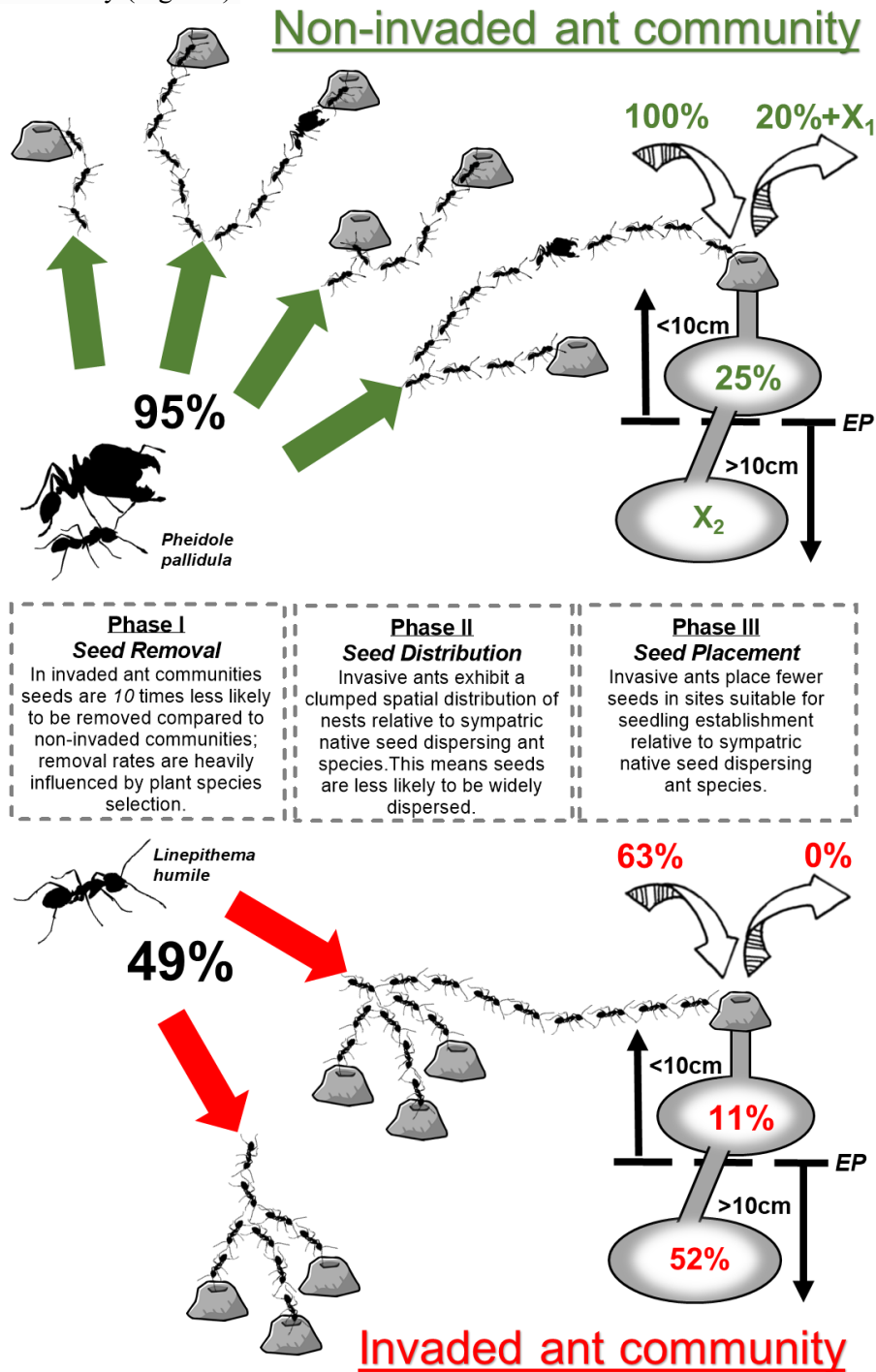


Figure 4.5: Invasive Argentine ants (*L. humile*) disrupt seed dispersal across three key phases of the dispersal process. Labels: Excavation Point (EP) and seed with *fate unknown* (X₁, X₂)

These data provide further evidence of the detrimental impacts of invasive ants on seed dispersal processes. Moreover, this study highlights the importance of quantifying seed dispersal over all stages of the dispersal process, to avoid under-estimating the impact of invasive ants on this important ecosystem service. Overall, seeds in the invaded regions (compared with seeds in non-invaded regions) were: less likely to be removed by ants; were potentially distributed over a smaller area, and likely to be placed at depths less favourable for germination and establishment. These results suggest that invasive ants may cause significant disruption to seed dispersal processes due to their seed-handling behaviour, and that this deficiency may lead to a decline in floral biodiversity and composition within invaded sites. The cascade of ecological impacts resulting from these invasions is in turn also likely to disrupt interactions among other biota which rely on these ant-plant mutualisms.

The first experiment suggests that seeds in sites invaded by *L. humile* were less likely to be removed and transported than in non-invaded sites. The loss of the primary seed disperser (*P. pallidula*) in invaded habitats is associated with a reduction in both the rate and number of seeds being removed and dispersed. This supports previous studies (Gómez and Oliveras 2003; Bas et al. 2009) that found *L. humile* to be a poor-quality seed disperser, with slower rates of uptake and removal relative to that of the native seed disperser, *P. pallidula*. As a result, seeds scattered in invaded sites will remain on the soil surface for longer, leading to either an increased risk of predation by vertebrates (Bennet and Krebs 1987; Rey et al. 2002; Carney et al. 2003), or loss by other means. My data support the findings of other studies on ant-plant mutualisms, which identified the native sympatric species (e.g. *P. pallidula*) as a high-quality seed disperser, whereas the exotic species *L. humile* is a low quality seed disperser that is only pre-adapted to a narrow range of phenotypic traits (Gomez and Oliveras 2003; Witt et al. 2004). This can be further seen by the fact that within invaded regions, seed removal rates varied considerably between the eight selected plant species. This study therefore showcases how seed dispersal within invaded regions may favour selected plant species.

Importantly, this experiment suggests that seed removal success in invaded sites may vary significantly among native plant species. In sites invaded by *L. humile* the removal rate of seeds of different myrmecochorous plant species varied. By contrast, in non-invaded sites, *P. pallidula* ants removed seeds from all plant species in the study equally well. This difference is likely to be related to the morphology of both the ants (Gorb and Gorb 1995; Ness et al. 2004) and the seeds (Hughes and Westoby 1992; Mark and Olesen 1996; Garrido et al. 2002; Edwards et al. 2006; Gómez et al. 2005; Rowles and O'Dowd 2009; Boieiro et

al. 2012). Since *P. pallidula* is a dimorphic ant species, with a range of worker sizes, it may be better able to handle a wide range of seed sizes and shapes, compared with the monomorphic *L. humile* (Oliveras et al. 2005b). Invasion by *L. humile* therefore is likely to lead not only to a decrease in overall seed dispersal efficiency, but also to a shift in the types of plant species being dispersed, resulting in a loss of viable seeds, and a long-term change in seed bank dynamics (Bond and Slingsby 1984; Christian 2001). Such alterations of seed bank composition may limit possibilities for the regeneration of myrmecochorous plant species.

The second experiment showed that *L. humile* nests in invaded sites were less evenly distributed than *P. pallidula* nests in non-invaded sites. Both *L. humile* and *P. pallidula* are recognised as short distance seed dispersers (Gómez and Oliveras 2003), meaning that seed dispersal is usually localised to ant nests in close proximity to the parent plant. While removal of seeds does not necessarily correlate with dispersal success or seedling establishment, spatial distribution of ant nests is likely to be a good indicator for seed dispersal patterns. The results show that *L. humile* nests were more clumped, and thus occupied a smaller spatial area than the more evenly dispersed native *P. pallidula* nests. Clumped nest aggregations are typical of *L. humile* populations in California, as well as in their native regions (Heller and Gordon 2006; Heller et al. 2008). Such nest aggregations suggest that seeds dispersed within invaded sites are likely to be placed within a smaller spatial area than seeds removed in the non-invaded sites. This could lead to increased intra- and inter-specific competition among seeds and consequently lower seedling survival, which can result in a failure to colonise distant and/or newly available habitats (Quilichini and Debussche 2000; Gorb and Gorb 2003). Changes in ant nest distribution within a site may therefore alter the population genetic structure of plant species over time (Lesica and Kannowski 1998; MacMahon et al. 2000). This aspect of the seed removal process remains relatively poorly resolved within myrmecochorous systems for both non-invaded and invaded ecosystems. While I have found some evidence to support this hypothesis, further work is required to account for the true spatial distribution of seeds within invaded and non-invaded ecosystems.

The third experiment illustrated that seeds in sites with *L. humile* invasions were less likely to be placed in locations favourable for germination and establishment compared with non-invaded sites. Seed placement within a nest is beneficial because it decreases post-dispersal seed predation (Beattie 1985). However, placement within a nest may be detrimental if seeds are buried at a depth that increases seedling mortality (Bas et al. 2007; Narbona et al. 2007a; Renard et al. 2010). A large portion of the seeds used in the trial

were unaccounted for; however, the results still indicate that *L. humile* removed fewer seeds into their nests relative to the native *P. pallidula* ant species, and that a smaller proportion of those seeds were being placed at a depth deemed suitable for plant establishment.

Several assumptions (see Methods) have been made in interpreting my results. More research is still required to more accurately track the placement of seeds post-dispersal and the rate of seed removal over time. This could include: improved seed tracking (Bologna and Detrain 2015), more extensive nest excavations (using plaster) techniques, over longer time periods (beyond the 72 h), and using a larger number of plant species. Nonetheless, this study does highlight that, in addition to removing all presented seeds into their nest and placing more seeds at a depth suitable for plant establishment, *P. pallidula* ants also ejected as many seeds from their nests as they deposited within it. Although this behaviour has been observed before in *P. pallidula* (Gómez et al. 2003; Oliveras et al. 2005a; Bas et al. 2007), I found this behaviour to be absent in *L. humile* ants. This behaviour is likely to be driven by the morphology of the seed, and the ant ecology (Gómez et al. 2005), and could be an important and often overlooked factor in myrmecochorous seedling survival: spreading seeds across two different micro-habits (above and below-ground) could maximise the chance of seedling establishment in a variable environment (Gremer and Venable 2014). For example, smaller seeds dispersed above-ground in waste piles could be advantageous for survival, as seeds this size cannot withstand deep burial depths (Baskin and Baskin 1998). Deposition in waste piles above-ground could potentially also benefit seeds when there is a higher localised level of nutrients in the soil (Higashi et al. 1989) and it may further allow seeds to imbibe water more readily in low rainfall years (Merino-Martín et al. 2017). There is, however, an increased risk of predation (Bennet and Krebs 1987; Rey et al. 2002). Indeed, many *Euphorbia* seeds have non-deep physiological dormancy that is not enhanced by periods of darkness (Baskin and Baskin 1998; Narbona et al. 2007b); thus they do not require burial within an ant nest to germinate.

Despite compelling findings of this study, it is important to acknowledge the limitations of this experiment, specifically the low level of replication *across* sites. Although there is replication *within* sites (and this pseudo-replication is accounted for statistically), only two invaded and two non-invaded sites were studied. It is conceivable that the reported impacts of *L. humile* on the seed dispersal process here are peculiar to the specific ant communities within these two sites (relative to the two non-invaded sites). Such replication issues are common in this field, as it is challenging to obtain data on independent replicated studies of invasions in regions that have similar biomes, similar invasion history whilst employing

similar sampling methods. For example, Hughes and Westoby (1990) seed removal by ants in 3 plots within a single National Park, separated by 2-10km. They were unable to take account of plot as the three sampling plots is too low a level of replication to be included as a random factor in analyses. Similarly, Rodriguez-Cabal et al. (2012) studied disruption of seed removal by the invasive Asian needle ant; they sampled all within a single National Park (one site), and they did not take site into account although they could have done with a (pseudo-) replication of 29 sampling points in areas where they found (or did not find) invasive ants. These studies highlight the difficulties in balancing demanding field sampling experiments with high levels of community-level replication. Thus, with respect to my study here, the levels of replication and the issues with psuedoreplication are not unusual. However, further work is encouraged across a larger number of invaded and non-invaded sites, before we can confidently extrapolate these findings to the wider Iberian Peninsula.

In conclusion, this study adds to the rich and growing literature on the impacts of invasive ants on seed dispersal processes. Specifically, my results suggest how differences in seed-handling behaviour between invasive and native ants can be detected across several stages of the seed dispersal process. More broadly, these findings suggest that the displacement of native species by invasive species has the potential to lead to ecological cascades of displacement across taxa. In fact, it has been shown that invasion can lead to an unravelling of important and often closely co-evolved interactions that underpin the wider structure and stability of ecosystems (Rogers et al. 2017). Future studies on assessing the impacts of invasive ant species on a key ecosystem service, such as myrmecochory, should consider the full dynamic nature of the mutualism, not just a single stage in the interaction.

CHAPTER FIVE

5. INTERACTIONS BETWEEN MULTIPLE INVASIVE ALIEN SPECIES DRIVE CASCADES IN AN ANT-PLANT MUTUALISM

5.1 ABSTRACT

Invasive species pose a serious threat to the biological diversity, complexity and function of native ecosystems. Invasion cascades, whereby one invasive species facilitates and/or enables the secondary invasion of another species, are becoming increasingly prevalent. Despite this threat, we still understand remarkably little about the processes of secondary invasions and the potential interaction outcomes of multiple invasive species; this knowledge gap limits our ability to accurately assess current and future threats of invasive species. In this study I assess how ant communities invaded by the non-native invasive Argentine ant (*Linepithema humile*) interact with both native and invasive myrmecochorous plant species and demonstrate how these altered interactions disrupt seed dispersal services. I found evidence to suggest that sites invaded by *L. humile* show preferential dispersal of invasive myrmecochorous *Acacia* seeds over those of the sympatric and endemic native plant species seeds. By contrast, non-invaded sites were found to exhibit a degree of resilience to these invasive plant species, as comparatively fewer invasive *Acacia* seeds were placed in sites deemed favourable for seedling establishment. These results suggest that habitats invaded by *L. humile* are likely to be more permeable to invasion by invasive non-native plant species. Such cascading interactions among invasive species are likely to threaten the wider ecosystem and biodiversity within it.

5.2 INTRODUCTION

Biodiversity is declining at a precipitous rate worldwide, and invasive species are recognised as one of the leading drivers of animal and plant extinctions (Sala et al. 2000; Clavero and García-Berthou 2005; Pyšek et al. 2012; Bellard et al. 2016; D’Antonio and Kark 2017). In order to assess the impacts of invasive species, researchers typically focus on a simple linear paradigm that compares invaded vs. non-invaded systems, with attention directed at potential interactions between native and non-native invasive agents within these systems. While these studies are important, it should be noted that many ecosystems are likely to be affected by several non-native invasive species at the same time (Preston et al. 2012). In such situations invasive species may facilitate each other, either directly due to specialist interactions, or indirectly by reducing the resilience of native ecological networks. Such positive interactions between invaders have only been demonstrated in a few oceanic island and freshwater ecosystems, with their combined or synergistic effects often being unpredictable and variable (Grosholz 2002; Ross et al. 2004; Cope and Winterbourn 2004). In extreme cases, these invader-invader interactions modify the biotic and abiotic environment, leading to an ‘invasional meltdown’ scenario, causing a reconfiguration of the community dynamics, which may reduce ecosystem function and biodiversity (Simberloff and Von Holle 1999; Green et al. 2011). It is therefore vital to predict when and where antagonistic and/or mutualistic invader-invader interactions are likely to occur, as only then may a realistic estimate be made as to the impact these invasive species may have on ecosystem biodiversity and functionality.

Ants are among the world’s most invasive alien species. Of the 13,000 described ant species (Agosti and Johnson 2005), 19 are listed on the Global Invasive Species Database, with five ant species in the ‘Top 100 Worst Alien Invaders’ (Global Invasive Species Database 2018). This notoriety arises because these ants not only cause negative impacts within their own taxon (e.g. competitive exclusion of native ant species (Holway et al. 2002; Holway and Suarez 2006; Lessard et al. 2009)), but they also have effects on many different taxa, in particular by disruption of ecologically important mutualisms (Holway 1998; Christian 2001; Ness and Bronstein 2004; Davis et al. 2010). For example, O’Dowd et al. (2003) showed that the invasive yellow crazy ant (*Anoplolepis gracilipes*) alters plant community structure through direct mutualism with the invasive yellow lac scale insect (*Tachardina aurantiaca*), and also indirectly through exclusion of the native Christmas Island land crabs (*Gecarcoidea natalis*). This, in turn, facilitated the invasion of a third species, the giant African land snail (*Achatina fulica*), which leads to perhaps the best example of an ‘invasional meltdown’ (Green et al. 2011). The potential for diverse forms

of species interactions with cascading biotic and abiotic impacts make ants an excellent focal group of organisms to explore invader-invader interactions.

Ants contribute to plant dispersal through active transportation of their seeds, a process called myrmecochory. This mutualism is found in at least 11,000 (4.5% of all) angiosperm plant species, across 77 (17% of all) families (Bronstein et al. 2006; Lengyel et al. 2010). Plant species that rely on myrmecochory have an oily appendage on each seed (known as an elaiosome), which attracts an ant, enticing it to move the seed back to its nest (Beattie 1985). Once at the nest, the ant larvae consume the elaiosome. The ant workers then discard the intact seed in or around the ant nest (Culver and Beattie 1980; Gómez et al. 2005). This ant behaviour is often vital for plant survival, particularly where seed germination is challenged by spatially or temporally variable conditions, or by low levels of soil nutrition and water. This is because myrmecochorous dispersal may provide seeds with a potentially nutrient-rich microsite for germination, and may also reduce seed predation and exposure to other destructive forces (e.g. fire) (Davidson and Morton 1981; Bond and Slingsby 1983; Gibson 1993; Cautle et al. 2005).

Not all ant species offer the same quality in their seed dispersal services. Effective seed dispersers are usually large scavenger/omnivorous ant species, with activity periods that correspond to seed release periods (review in Warren and Giladi 2014). These ant species need to be able to quickly and effectively remove seeds (Ness and Bressmer 2005; Warren et al. 2014), but they must also leave the seed intact and unharmed, and be able to deposit it in sites suitable for germination and survival (Culver and Beattie 1980; Hanzawa et al. 1988). While several ants may be interacting with seeds in a given environment, not all their interactions may be mutualistic. For example, the ant *Aphaenogaster senilis* takes seeds into its nest, but deposits these seeds at soil depths where they will not survive (Gómez and Espadaler 1998). The specificity of myrmecochorous plants makes them highly sensitive to ecological disruptions, or local extinctions. This is particularly relevant if invasion by non-native ant species leads to displacement of the primary native seed-dispersing ant species (Christian 2001; Gómez and Oliveras 2003; Rodríguez-Cabal et al. 2012), as this may modify patterns of (and typically reduce) seed dispersal for the invaded plant community. For example, sites invaded by the non-native Argentine ant (*Linepithema humile*) in the Cape Floristic Region led to the exclusion of the native pugnacious ants (*Anoplolepis custodians*), resulting in a loss of seed dispersal services for large seeded plant species (Bond and Slingsby 1984; Christian 2001). However, not all ant species were negatively affected by the presence of *L. humile*, and those that were able to co-exist were able to disperse a range of small seeded plant species (Christian 2001). However, the

quality of seed dispersal services in invaded ant communities, relative to non-invaded ant communities, has so far not been fully assessed.

Invasive species may also interact with other invasive species to increase rates of spread. Most of the studies on how invasive ants affect seed dispersal services have focused either directly on the invasive ants (e.g. attention being given to the dispersal ability of the invader) or indirectly (e.g. the dispersal abilities of the native ant species that are able to coexist within an invaded community) (Christian 2001; Gómez and Oliveras 2003; Rodriguez-Cabal et al. 2012). So far, only one study has examined the effects of ant invasions on invasive plant species: Prior et al. (2015) showed that the non-native invasive European fire ant (*Myrmica rubra*) preferentially dispersed co-introduced seeds of the invasive greater celandine (*Chelidonium majus*) in Canada over those of the sympatric native myrmecochorous plant species. Such a close interaction may be expected given that *M. rubra* already disperses seeds of this and other myrmecochorous plant species in its native ranges in Central Europe (Servigne and Detrain 2008; Bologna and Detrain 2015). Consequently, novel myrmecochorous invader-invader interactions between ant and plant species remain relatively unresolved, especially given that many invasive ant species originate from different geographic regions and are thus unlikely to have innate co-evolved associations with the native plants in the regions they invade.

The Cape Floristic Region (CFR) is one of the world's global biodiversity hotspots (Myers *et al.* 2000), with 559 nationally recognised non-native invasive species (South Africa Government Gazette 2014). This list of non-native invasive species includes the Argentine ant (*L. humile*), as well as a number of invasive myrmecochorous Australian wattle (*Acacia*) plant species. The CFR therefore provides a model environment in which to further explore and investigate potential invader-invader interactions. In this study, I assess the impact of invader-invader interactions on native ecosystems by determining how *L. humile* ants influence the seed dispersal of both native and invasive plant species. I test the hypotheses that sites invaded by *L. humile* will show: (1) an overall decrease in seed dispersal services (Hypothesis 1) and (2) a shift in seed preference of invaded ant communities towards non-native invasive plant species (Hypothesis 2). I also expect the effects observed (in 1 and 2) to be visible across all stages of the seed dispersal process (Hypothesis 3) relative to non-invaded ant communities.

5.3 METHODS AND MATERIALS

5.3.1 Study system

Experiments were conducted over two field seasons (November 2014 – February 2015, and January 2017 – February 2017) at times when myrmecochorous seeds were being dispersed naturally. The research was conducted at six sites within Jonkershoek Nature Reserve (33°55'51"S, 18°51'16"E), itself part of the Boland Mountain Complex within the Cape Floristic Region (CFR) of South Africa. This location was chosen because: (a) it has an established population of the invasive ant *L. humile*, first detected in the 1980s (Patrick Shone, pers. comm.), and (b) it has a high density of invasive myrmecochorous *Acacia saligna* and *A. pycnantha* plant species at the edges.

5.3.2 Plant species

Twelve plant species (seven native and five invasive) were selected. These species were chosen because they showed variable seed morphology (e.g. size and shape) and genealogy (i.e. eight genera from four plant families) and were locally abundant (Table 5.1). Seeds of native plant species were collected from the six field localities within Jonkershoek Nature Reserve; however, due to eradication programs, seeds from the invasive *Acacia* population could not be collected. Instead, seeds of all five invasive plant species used in this experiment were collected near the University of Cape Town Campus (33°57'27"S 18°27'38"E) and surrounding areas.

Table 5.1: Plant species and their respective seed weights. Seeds collected from Jonkershoek Nature Reserve and University of Cape Town campus, between November 2015 and January 2016. *These species are known to be secondarily dispersed by ants in their native range.

Plant Species	Status	Collection Date	Mean Seed Weight (mg)	Dispersal mechanism
<i>Acacia cyclops</i> A Cunn. Ex G.Don.	Invasive	Dec-14	80.5 [±2.5]	Birds*
<i>Acacia longifolia</i> (Andrews) Willd.	Invasive	Jan-15	16.9 [±1.0]	Ants
<i>Acacia melanoxylon</i> R. Br.	Invasive	Dec-14	18.7 [±3.1]	Birds*
<i>Acacia pycnantha</i> Benth.	Invasive	Jan-15	14.7 [±0.9]	Ants
<i>Acacia saligna</i> (Labill.) H. L. Wendl.	Invasive	Dec-14	20.5 [±0.4]	Ants
<i>Hypodiscus aristatus</i> (Thunb.) C. Krauss.	Native (large-seeded)	Dec-14	45.4 [±4.5]	Ants
<i>Leucospermum conocarpodendron</i> H. Buek	Native (large-seeded)	Jan-15	101.6 [±7.8]	Ants
<i>Phyllica pubescens</i> Aiton.	Native (small-seeded)	Dec-14	21.4 [±1.1]	Ants
<i>Podalyria calyprata</i> (Retz.) Willd.	Native (small-seeded)	Dec-14	20.9 [±1.8]	Ants
<i>Psoralea pinnata</i> L.	Native (small-seeded)	Dec-14	9.8 [±4.8]	Ants
<i>Serruria krausii</i> Meisn.	Native (small-seeded)	Dec-14	6.4 [±0.9]	Ants
<i>Willdenowia teres</i> Thunb.	Native (large-seeded)	Dec-14	24.6 [±1.0]	Ants

For each species, fruits and cones near maturation were collected from at least 25 plants (from at least three independent locations) before being left to dehisce naturally in the laboratory. Seeds were handled with forceps and gloves throughout the study, and stored

in sealed plastic bags at -15°C to preserve the elaiosome and maintain its attractiveness to ants (Ness and Morin 2008; Clark and King 2012). To prevent post-dispersal germination, all invasive seeds used in the trials were selected from either damaged seed stock (e.g. signs of beetle damage) and/or they were mechanically damaged to ensure that the seeds were no longer viable. In order to ensure that this damage did not modify the ants' seed preference, a pilot study was conducted in which both intact and damaged seeds were offered to a range of ant species. This study showed that all seeds were removed by the ants irrespective of seed condition (i.e. intact or damaged).

5.3.2.1. Pilot study to determine the viability of damaged invasive *Acacia* seeds.

Prior to undertaking field trials, viability tests were performed on seed stocks of all five invasive plant species (listed in Table 5.1). This was done to ensure that the seeds utilised in field trials did not germinate (post-dispersal). Per plant species, 100 seeds were selected from each of the following three treatment groups: intact, insect damaged (embryo consumed by granivorous beetles), and mechanically damaged (seed punctured with a heated nail). For all seeds the elaiosome was detached and the testa was partially removed with a scalpel. Seeds (20 per dish) were placed on Petri dishes lined with filter paper (Whatman No. 1), which were then placed in a 20°C incubator, with an 8-hour light and 16-hour dark cycle. Filter paper was kept damp throughout the study and replaced once a week. During this time seeds were periodically cleaned to limit the fungal growth. Seedling counts were performed once a week for five weeks, with the emergence of a radicle being taken as the criterion for germination.

5.3.2.2. Pilot study to determine the attractiveness of damaged invasive *Acacia* seeds.

Prior to undertaking field trials, preferences trials were performed on both intact and damaged seeds of all five invasive plant species (listed in Table 5.1). This was done to ensure that damaged seeds utilised in field trials did not differ in their attractiveness to that of intact seeds. Seed hubs were setup (Chapter Five) and a seed mix consisting of all five invasive plant species (3 seeds per plant species) were placed on each seed hub. Seed hubs were observed for up to 1 hour or until all seeds were removed. As soon as a seed was moved beyond 5 cm from the hub it was collected using a hand aspirator. Six seed hubs were placed across each of the sites described in Chapter Five, with both intact and damaged seed mixes being placed at least once per hub.

5.3.3 Ant communities

Two distinct ant communities were used in this research. These communities were selected based on the presence/absence of the invasive *L. humile* ant, and the abundance of native large and small seed dispersing ant species (see Chapter Three):

- Invaded ($n = 2$ sites) – Presence of *L. humile*; relative high abundance of *Tetramorium sericeiventre* and *Meranoplus peringueyi*.
- Non-invaded ($n = 4$ sites) – Absence of *L. humile*; relative high abundance of *Pheidole capensis*, *Lepisiota capensis* and *Anoplolepis* sp.

Previous research by Christian (2001) demonstrated that these native ant species can be broadly classified as either large-seed (i.e. *Anoplolepis* sp.) or small-seed (i.e. *P. capensis*) dispersing guilds. Non-invaded sites appeared to vary in terms of their abundance of these seed dispersing guilds (see Chapter Three); I have included more non-invaded sites than invaded sites into the experimental design in order to ensure the natural range of seed dispersal responses in non-invaded sites was represented.

5.3.4 Field experiments

5.3.4.1. Hypothesis 1: Seeds placed in sites invaded by *L. humile* are less likely to be removed from seed hubs than those in non-invaded sites

Based on the findings of Chapter Four, I predicted that fewer seeds would be dispersed in sites where *L. humile* was present, relative to those where *L. humile* was absent. I used observations of the removal of seeds from experimental hubs as a measure of seed dispersal. At each site, one transect (previously used in ant community assessment trials; see Chapter Three) was selected, and 10 seed hubs were set up at 10 m intervals. Each seed hub consisted of a 10 cm² white card with a dome wire mesh placed on top. Ants (and other small invertebrates) were able to access the seeds, but larger vertebrates were not. In total, 60 seed hubs (10 per site) were set up in each of the two invaded and four non-invaded sites. Each hub consisted of four seeds from two randomly paired plant species, with two seeds per plant species.

Seeds were placed on the seed hubs at 08:00 hours and surveyed at one-hour intervals, up to a maximum of three hours, after which any seeds left on the hubs were collected. This timing was selected as naturally dispersed seeds left longer than three hours tend to be consumed by rodents rather than being dispersed by ants (Jeremy Midgley pers. comm.). At each survey time point, the total number of seeds from each species remaining on the hub was recorded. The experiment was repeated again at 13:00 hours to test for any

potential temporal effects on ant activity. Seed choice experiments were run every day, for three consecutive weeks, with seeds from each plant species being placed once on each hub.

All statistical analyses were carried out in R (version 2.3.2) (R Core Team 2017). I compared seed removal rates between and within invaded and non-invaded sites using a Cox Proportional Hazards Model (Cox 1972) and Log-rank test (Bland and Altman 2004) in the *survival* (version 2.42-3) and *coxme* (version 2.2-10) packages (Therneau and Grambsch 2000; Therneau 2018). Generalised linear mixed models (GLMM) using a Poisson error distribution in the *lme4* (version 1.1-17) package (Bates et al. 2015) were then used to analyse seed removal frequencies (portion of seeds removed from seed hubs after three hours) in invaded and non-invaded sites (Crawley 2012). In both the survival and GLMM analyses, the fixed effects were invasion status (invaded vs. non-invaded), sampling period (morning vs. afternoon), and plant species ID (n = 12; Table 5.1); seed hub ID (nested within transect) was included as a random factor, which controls for the effects of site and repeated sampling of seed hubs within sites. All generated *p* values were subjected to the Benjamini-Hochberg correction method (Benjamini and Hochberg 1995) to control for false discovery rates.

5.3.4.2. Hypothesis 2: Non-invaded and invaded ant communities are equally likely to remove seeds of invasive plants as the seeds of native plant species

Whilst Hypothesis 1 considered overall seed removal rates, Hypothesis 2 unpicks the origins of the seeds to determine whether invaded ant communities were systematically dispersing seeds from invasive plants over seeds from native plants. Using the data collected for hypothesis 1, seed removal preference for each ant community was determined using survival analysis (Cox Proportional-Hazards model). *Hazard Ratios (HR)* scores are widely used in time to event studies (review in Ergon et al. 2017) and provide a powerful statistical tool for understanding the effects of different covariates (e.g. different community properties) on survival probabilities. In this instance, *HR* scores represent the likelihood of a seed dispersal event occurring for each plant species relative to that of the population mean of the respective ant community (n = 12; all plant species); seed hub ID (nested within transect) was included as a random factor, which controls for the effects of site and repeated sampling of seed hubs within sites.

5.3.4.3. Hypothesis 3: Removal of invasive and native plant species into ant nests and their placement location within the nest will vary by ant species and ant community

I predicted that the propensity for ants to remove seeds into nests and place them at depths likely to be favourable for germination and establishment would vary by ant species, and (importantly) by whether it was an ant community invaded with *L. humile* or not. To test this hypothesis, I selected the three most abundant ant species detected at ant baits in invaded regions (*Linepithema humile*, *Meranoplus peringueyi*, and *Tetramorium sericeiventre*) and non-invaded regions (*Anoplolepis custodiens*, *Lepisiota capensis*, and *Pheidole capensis*) (data given in Chapter Three; Table 3.4). For each ant species, twenty independent nests (at least 10 m apart) from six sites were used. Each nest was presented with a seed mix consisting of three seeds from each of six plant species previously used in Hypothesis (1) and (2). This seed mix consisted of seeds from three invasive (*A. longifolia*, *A. pycnantha*, and *A. saligna*) and three native (*Leucospermum conocarpodendron*, *Phyllica pubescens*, *Podalyria calyprata*) plant species. The seed mix was scattered evenly around the nest entrance (within 5 cm). Perforated Petri dish lids (150 mm × 15 mm) were placed over both the seeds and the nest entrance; each lid was weighed down by a rock, with soil compacted around them to ensure that only the target ant nest/species was likely to access and interact with these seeds. The seeds were left for 24 hours, at which point seed removal into the nest was assessed by collecting and recording any seeds remaining under the petri dish. I compared the transport of individual seeds into ant nests. In this instance I consider the seeds to have only two possible discreet outcomes (transport into the nest [1] vs. abandonment above-ground [0]); as such, a binomial GLMM model was selected where the fixed effects were ant species (n = 6) and plant species (n = 6), with nest ID (n=120) included as a random factor.

The ultimate location of where seeds were placed in the nest ('seed placement') was assessed by taking plaster casts of nests, excavating them and measuring the depth of seeds found within the caste. This is a standard approach used to study nest size and architecture. To do this, dental plaster was poured into the nest, fixing seeds in the location within the nest that the ants had placed them in (methods described in Tschinkel (2010)). Nest casts were then excavated completely and transported back to the laboratory. Seeds were then retrieved by soaking in water in the laboratory and using physical abrasion of the plaster casts to release the seeds. Nest were carefully dismantled in this way in sections that corresponded to three depth criteria: 0 – 12 cm; 13 – 24 cm; > 25 cm (see Figure 5.1).



Figure 5.1: A plaster cast of an *Anoplolepis custodiens* nest, excavated from Jonkershoek Nature Reserve in South Africa (© Adam Devenish).

The cut off for a viable depth of seed placement was determined from the literature. Survival of myrmecochorous seeds within fynbos habitats is enhanced substantially through the process of transporting the seeds below ground, as this protects them from vertebrate seed predators and other destructive forces (Heithaus 1981; Breytenbach 1986). However, while deeper burial depth may ensure a greater degree of protection from predators (Reichman 1979; Fuchs et al. 2000) it may come at a significant cost to the seedling survival. This is because seeds that are buried too deep may either fail to emerge, or emerge with insufficient reserve to survive (Seiwa et al. 2002; Guo et al. 2010). Given these factors, I considered seeds that were not transported below ground, or buried too deep

(>12cm) as being placed in locations deemed detrimental to the seedling survival. This depth of 12 cm was selected on the basis that previous studies have shown this depth to be the natural range at which some myrmecochorous seedlings emerge (Christian and Stanton 2004). Unfortunately, the range of plant species for which these data are available is limited: germination depths were available for only one of the six plant species used in this study. In addition, seeds used in nest excavation trials were split into three broad groups: invasive (*A. longifolia*, *A. pycnantha*, *A. saligna*), native small-seeded (*P. pubescens*, *P. calypttrata*), and native large-seeded (*L. conocarpodendron*). With the latter size classes for native plant species is based on the Christian (2001) classification system, with seeds that weight more than 32 mg classified as large-seeded. In order to determine whether ant species and therefore ant community status was having effect on seed placement, I compared the placement (optimal vs. sub-optimal) of individual seeds within ant nests. In this instance I consider the seeds to have only two possible discreet outcomes (optimal placement [1] vs. sub-optimal placement [0]); as such, a binomial GLMM model was selected where the fixed effects were ant species (n = 6) and plant species (n = 6), with nest ID (n = 120) included as a random factor.

5.4 RESULTS

5.4.1 Viability and attractiveness of damaged *Acacia* seeds.

After five weeks no seeds had germinated in either the insect damaged or mechanically damaged treatment groups, across all five invasive plant species (Fig. 5.2). This differed significantly from the intact treatment group in which germinated seedlings were recorded (Kruskal-Wallis test: $H = 13.36$, $DF = 2$, $p < 0.001$).

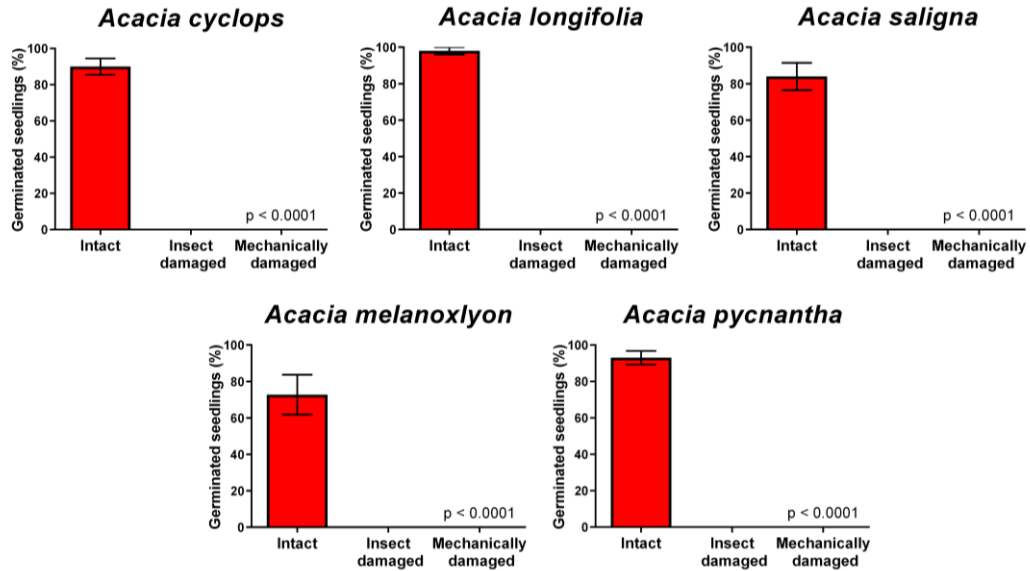


Figure 5.2: Cumulative seedling germination (after five weeks) across three treatment groups: intact ($n = 5$), insect damaged ($n = 5$), and mechanically damaged ($n = 5$) [± 1 SE].

Seed removal from hubs, was not influenced by treatment type (Mann–Whitney U-test: $U = 144$, $DF = 1$, $p = 0.55$) (Fig. 5.3). In other words, non-viable seeds were no less attractive to ants than viable seeds.

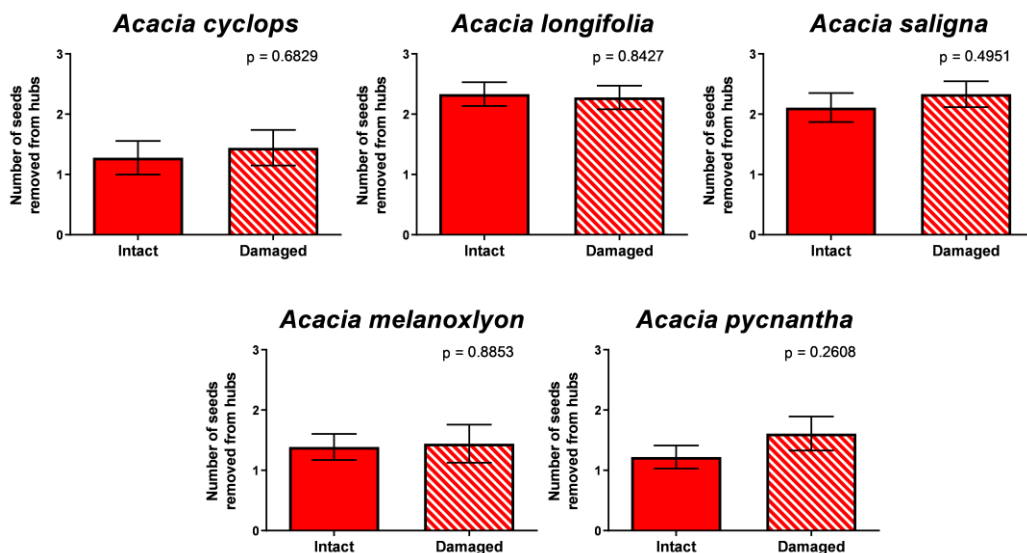


Figure 5.3: Number of seeds removed from hubs ($n = 72$) using both intact (solid; $n = 36$) and damaged (hatched; $n = 36$) seed mixes [± 1 SE].

5.4.2 Hypothesis 1: Seeds placed in sites invaded by *L. humile* are less likely to be removed from seed hubs than those in non-invaded sites

Fewer seeds were removed from seed hubs in *L. humile* invaded sites than non-invaded sites. After 3h, only 30% (\pm SEM 1.7%, $n = 250$) of seeds had been removed from seed hubs placed in invaded sites, compared with 45% (\pm SEM 1.6%, $n = 495$) in non-invaded sites (GLMM: $F = 5.138$, $DF = 1$, $p < 0.05$; Fig. 5.4).

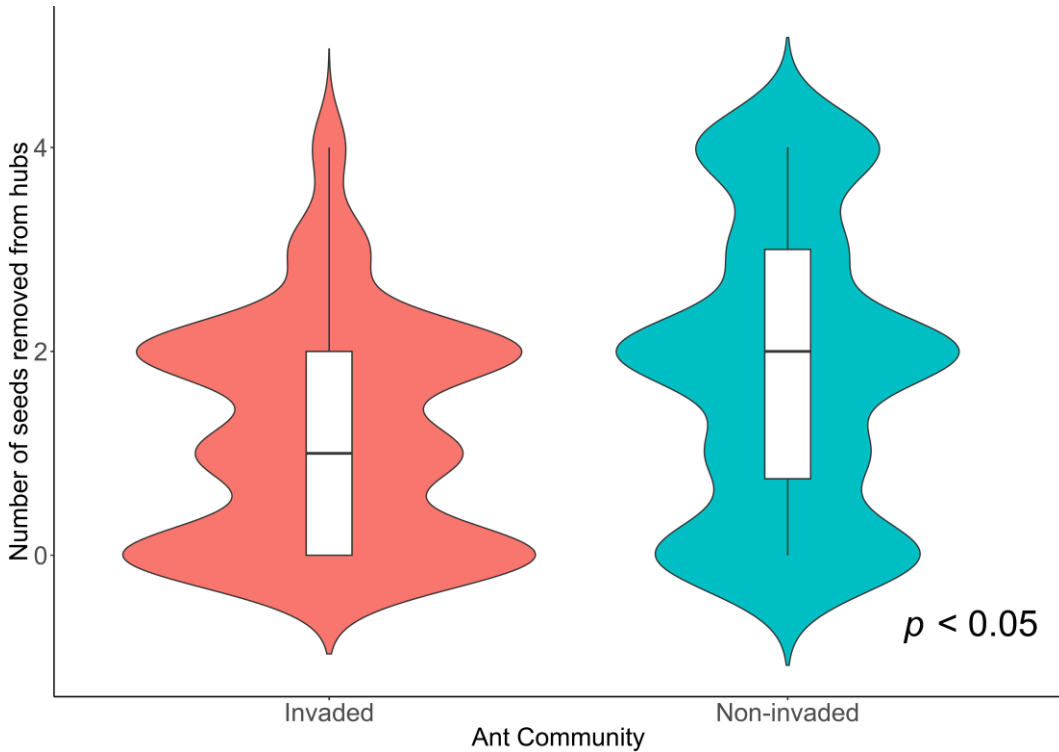


Figure 5.4: Violin and box plots of the number of seeds removed from hubs placed invaded ($n = 250$) and non-invaded ($n = 495$) sites.

These results were corroborated by the survival analysis: seeds placed in invaded sites were 1.7 times ($n = 2924$, 95% CI [1.01, 2.98]) less likely to be removed compared with seeds placed in non-invaded sites (Table 5.2). Furthermore, sampling period had no significant effect on the total number of seeds removed from hubs (GLMM: $F = 2.1466$, $DF = 1$, $p = 0.128$)

Table 5.2: Effects of invasion status (Model 1), period (Model 2) and plant species (Model 3) on the removal rate of seeds from hubs using a Cox's regression analysis. *Hazard Ratio (HR)* is determined by the difference between the slopes of the corresponding treatments and represents the likelihood of a seed dispersal event occurring. Hubs within transects are included as random factors, to control for the non-independence of replicate hubs within sites.

Fixed effects	Coefficient	SE	Hazard Ratio (HR)	95% Confidence intervals for HR	p	q
Invasion status ¹	0.55	0.27	1.73	1.01 - 2.98	*	*
Sampling period ²	-0.05	0.06	0.96	0.85 - 1.07	0.43 ^{NS}	0.43 ^{NS}
Within invaded sites ³						
<i>Acacia cyclops</i>	-1.96	0.50	0.14	0.05 - 0.39	***	***
<i>Acacia longifolia</i>	1.40	0.14	4.07	3.06 - 5.42	***	***
<i>Acacia melanoxylon</i>	-0.12	0.22	0.89	0.57 - 1.38	0.59 ^{NS}	0.59 ^{NS}
<i>Acacia pycnantha</i>	1.14	0.15	3.13	2.34 - 4.18	***	***
<i>Acacia saligna</i>	1.01	0.15	2.73	2.03 - 3.68	***	***
<i>Hypodiscus aristatus</i>	-3.41	1.00	0.03	0.00 - 0.24	***	***
<i>Leucospermum conocarpodendron</i>	-1.96	0.50	0.14	0.05 - 0.39	***	***
<i>Phyllica pubescens</i>	-0.18	0.22	0.84	0.54 - 1.29	0.41 ^{NS}	0.44 ^{NS}
<i>Podalyria calyptрата</i>	-1.25	0.36	0.29	0.14 - 0.59	***	***
<i>Psoralea pinnata</i>	-0.36	0.24	0.70	0.43 - 1.13	0.13 ^{NS}	0.15 ^{NS}
<i>Serruria krausii</i>	0.51	0.17	1.67	1.18 - 2.36	**	**
<i>Willdenowia teres</i>	-1.24	0.36	0.29	0.14 - 0.59	***	***
Within non-invaded sites ³						
<i>Acacia cyclops</i>	-0.24	0.13	0.78	0.60 - 1.02	0.06 ^{NS}	0.08 ^{NS}
<i>Acacia longifolia</i>	0.48	0.11	1.32	1.03 - 1.45	***	***
<i>Acacia melanoxylon</i>	-1.02	0.18	0.36	0.25 - 0.52	***	***
<i>Acacia pycnantha</i>	0.23	0.11	1.25	0.99 - 1.57	*	0.07 ^{NS}
<i>Acacia saligna</i>	0.10	0.12	1.10	0.87 - 1.40	0.42 ^{NS}	0.50 ^{NS}
<i>Hypodiscus aristatus</i>	-1.28	0.19	0.28	0.19 - 0.41	***	***
<i>Leucospermum conocarpodendron</i>	0.40	0.10	1.70	1.21 - 1.95	***	***
<i>Phyllica pubescens</i>	0.03	0.12	1.03	0.81 - 1.30	0.79 ^{NS}	0.79 ^{NS}
<i>Podalyria calyptрата</i>	-1.06	0.18	0.35	0.24 - 0.49	***	***
<i>Psoralea pinnata</i>	0.08	0.12	1.08	0.85 - 1.36	0.52 ^{NS}	0.56 ^{NS}
<i>Serruria krausii</i>	0.98	0.09	2.67	2.22 - 3.22	***	***
<i>Willdenowia teres</i>	0.24	0.11	1.28	1.02 - 1.59	0.26 ^{NS}	*
Random effects	Variance	SD	Loglikelihood	Chi ²	p (> Chi ²)	
Invasion status ¹						
Transect / Seed hub ID	0.02	0.15	-9145.5	4.564	*	
Transect	0.09	0.30	-9188.1	85.187	***	
Period ²						
Transect / Seed hub ID	0.02	0.15	-9146.7	4.4677	*	
Transect	0.17	0.41	-9227.5	161.62	***	
Plant species (Invaded) ³						
Transect / Seed hub ID	0.01	0.11	-4220.2	0.99	0.31 ^{NS}	
Transect	0.01	0.09	-4220.2	1.1286	0.28 ^{NS}	
Plant species (Non-invaded) ³						
Transect / Seed hub ID	0.08	0.28	-14067	57.402	***	
Transect	0.15	0.39	-14067	186.02	***	

¹ Model1 (~Invasion status+(1|Transect/Seed hub ID)

² Model2 (~Sampling period+(1|Transect/Seed hub ID)

³ Model3 (~Plant species+(1|Transect/Seed hub ID)

q = Benjamini-Hochberg corrected p values

5.4.3 Hypothesis 2: Non-invaded and invaded ant communities are equally likely to remove seeds of invasive plants as the seeds of native plant species

Comparison of the Hazard Ratios within and between invaded and non-invaded sites (Table 5.2) suggested that the invaded ant communities (Fig. 5.5a) (but not the non-invaded ant communities; Fig. 5.5b) are preferentially dispersing the invasive seeds, over those of the native plant species (Fig. 5.5).

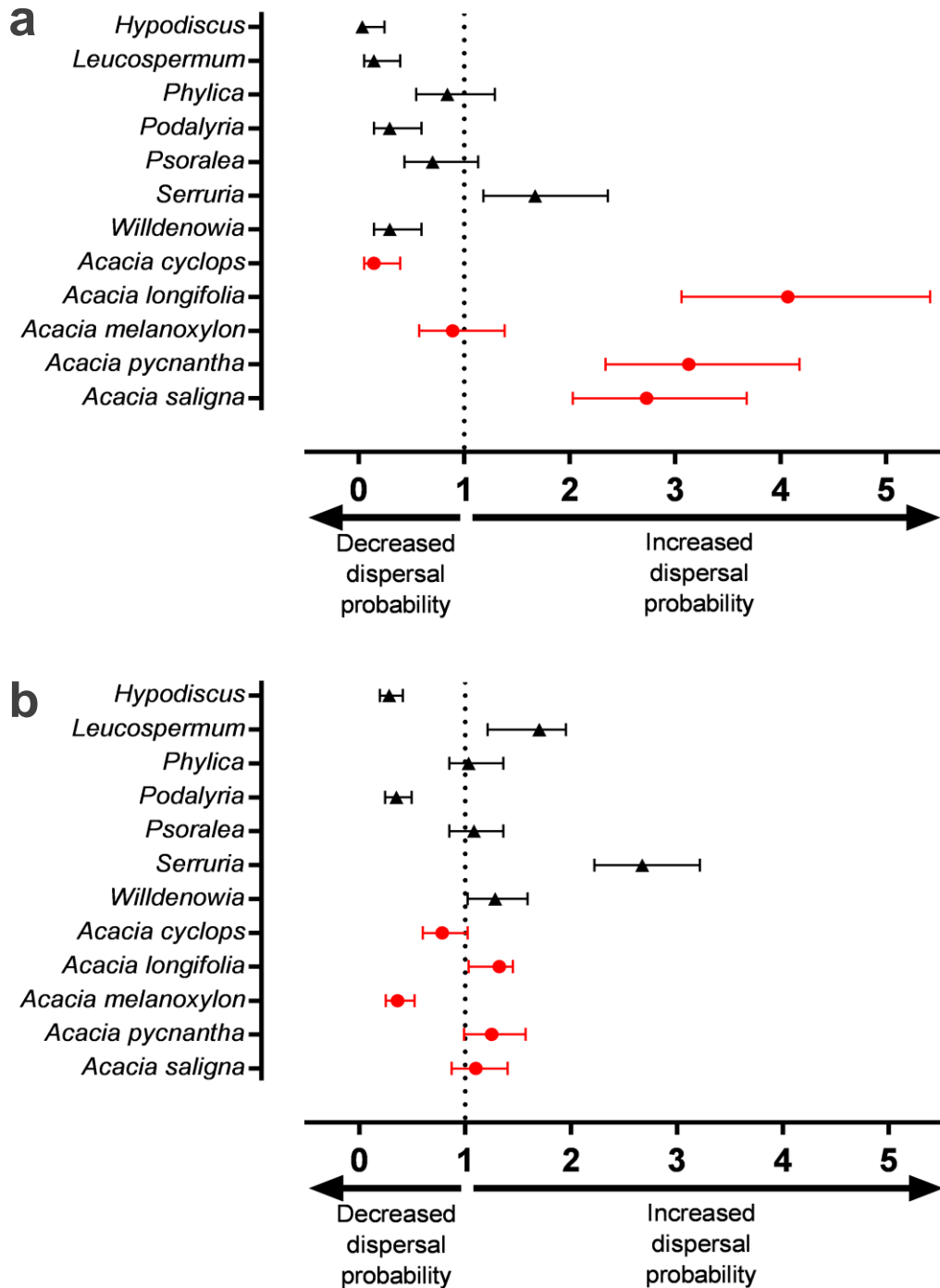


Figure 5.5: Seed preferences (*HR* scores with 95% confidence intervals) for twelve plant species removed from seed hubs placed in invaded (a) and non-invaded (b) sites. Invasive seeds (red circle) are preferentially dispersed over native seeds (black triangle) in sites invaded by *L. humile*.

The three *Acacia* sp. (*A. saligna*, *A. longifolia* and *A. pygmaea*) were between 2.7 and 4 times more likely to be dispersed in invaded ant sites (Table 5.2). By contrast, in non-invaded sites ants preferentially dispersed seeds of native plants (both the native large-seeded (e.g. *Leucospermum*) and small-seeded (e.g. *Serruria*) Proteaceae species (Fig. 5.5)).

5.4.4 Hypothesis 3: Removal of invasive and native plant species into ant nests and their placement location within the nest will vary by ant species and ant community

Seed removal into nests differed significantly among the six-ant species (GLMM: $F = 26.898$, $DF = 5$, $p < 0.0001$). Out of the six ant species, two of ants in the invaded communities (*Linepithema humile* [$2.0 \pm SE 0.31$ $n = 20$], and *M. peringueyi* [$1.6 \pm SE 0.47$ $n = 20$]) and one of the species in the non-invaded community (*Lepisiota capensis* [$1.4 \pm SE 0.65$ $n = 20$]) seldom transported more than two seeds into their nest over the 24 hour period (Fig. 5.6). By contrast, two of the dominant ants of the non-invaded communities (*P. capensis* (12.6 seeds $\pm SE 0.65$, $n = 20$), and *A. custodiens* ($7.0 \pm SE 0.49$, $n = 20$)) and one of the dominant ants in the invaded communities (*T. sericeiventris* ($8.5 \pm SE 0.64$, $n = 20$)), transported 52% ($\pm SD 16.1\%$) of all presented seeds into their nest (Fig. 5.6).

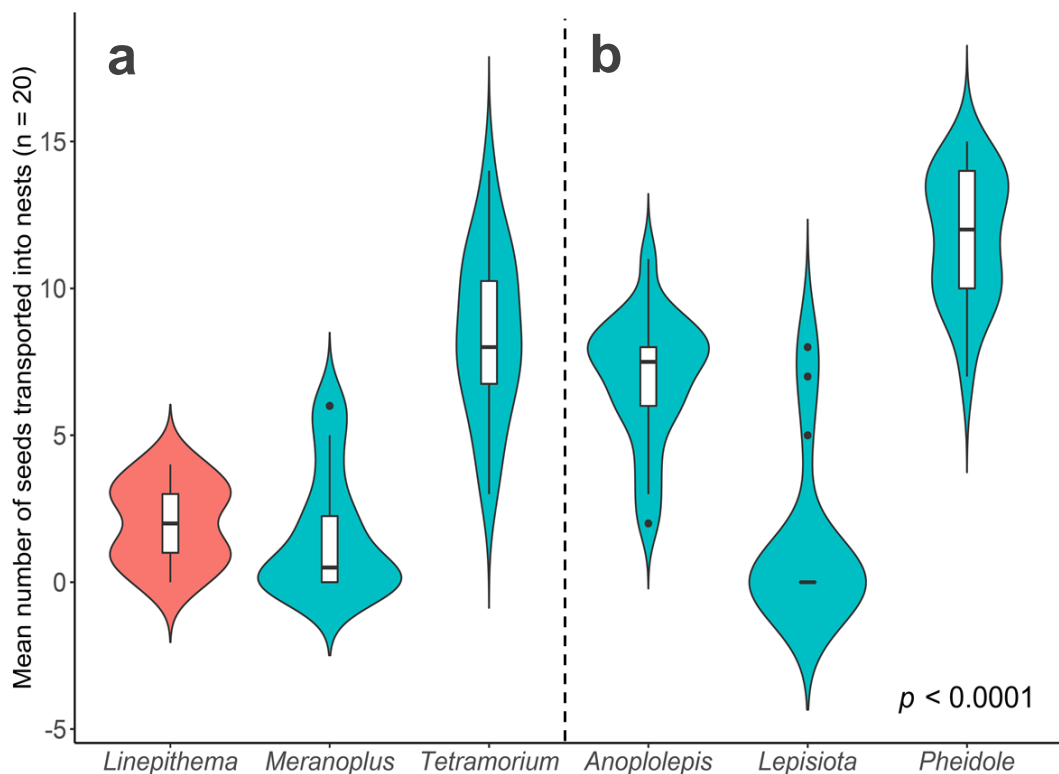


Figure 5.6: Seed removal into nests varied across all six-ant species. Violin and box plots of the mean number of seeds removed into nests across a range of native (blue) and invasive (red) ant species present in (a) invaded and (b) non-invaded ant communities.

There was a significant effect of plant species on the depth of seed placement in nests (GLMM: $F=14.534$, $DF=5$, $p<0.0001$). With the exception of the native large-seeded endemic *Leucospermum* (LC in Table 5.3) plant species, the other smaller-seeded plant species (both invasive and native) seeds were often left at depths deemed unfavourable for seedling germination and establishment (i.e. >12 cm).

Table 5.3: Transport of seeds into nests and placement at depths deemed favourable (0 – 12 cm) for seedling germination and establishment varied according to both ant (n = 6) and plant (n=6) species. Mean percentage of seeds retrieved from plaster casts of six different ant species [\pm SD].

Species	Seed placement (%)			
	Soil surface	Within nest		
	-	0-12 cm	13-24 cm	> 25 cm
<i>Anoplolepis custodiens</i>				
AL ¹	30.0 \pm 35.7	18.3 \pm 22.9	51.7 \pm 33.3	-
AP ¹	78.3 \pm 24.8	-	21.7 \pm 24.8	-
AS ¹	75.0 \pm 26.2	10.0 \pm 15.7	15.0 \pm 14.7	-
PP	81.7 \pm 33.3	5.0 \pm 12.2	13.3 \pm 27.4	-
PC	95.0 \pm 12.2	3.3 \pm 10.3	1.7 \pm 7.5	-
LC	8.3 \pm 14.8	91.7 \pm 14.8	-	-
<i>Lepisiota capensis</i>				
AL ¹	81.7 \pm 38.2	15.0 \pm 31.5	3.3 \pm 10.3	-
AP ¹	93.3 \pm 13.7	6.7 \pm 13.7	-	-
AS ¹	96.7 \pm 10.3	3.3 \pm 10.3	-	-
PP	91.7 \pm 38.2	8.3 \pm 17.4	-	-
PC	100 \pm 0.0	-	-	-
LC	100 \pm 0.0	-	-	-
<i>Linepithema humile</i> ¹				
AL ¹	43.3 \pm 37.6	40.0 \pm 27.8	16.7 \pm 22.9	-
AP ¹	95.0 \pm 12.2	5.0 \pm 12.2	-	-
AS ¹	95.0 \pm 12.2	5.0 \pm 12.2	-	-
PP	100 \pm 0.0	-	-	-
PC	100 \pm 0.0	-	-	-
LC	100 \pm 0.0	-	-	-
<i>Meranoplus peringueyi</i>				
AL ¹	80.0 \pm 29.4	11.7 \pm 19.6	8.3 \pm 18.3	-
AP ¹	85.0 \pm 27.5	10.0 \pm 21.9	5.0 \pm 12.2	-
AS ¹	96.7 \pm 10.3	3.3 \pm 10.3	-	-
PP	95.0 \pm 12.2	5.0 \pm 12.2	-	-
PC	91.7 \pm 18.3	5.0 \pm 16.3	-	3.3 \pm 10.3
LC	98.3 \pm 7.5	-	-	1.7 \pm 7.5
<i>Pheidole capensis</i>				
AL ¹	0.0 \pm 0.0	41.7 \pm 30.3	58.3 \pm 30.3	-
AP ¹	26.7 \pm 29.8	21.7 \pm 16.3	51.7 \pm 31.5	-
AS ¹	25.0 \pm 34.0	35.0 \pm 20.2	40.0 \pm 26.7	-
PP	13.3 \pm 16.8	65.0 \pm 22.8	21.7 \pm 22.4	-
PC	50.0 \pm 33.3	43.3 \pm 30.1	6.7 \pm 13.7	-
LC	93.3 \pm 17.4	6.7 \pm 17.4	-	-
<i>Tetramorium sericeiventre</i>				
AL ¹	21.7 \pm 27.1	73.3 \pm 25.6	5.0 \pm 12.2	-
AP ¹	56.7 \pm 26.7	43.3 \pm 26.7	-	-
AS ¹	51.7 \pm 38.2	48.3 \pm 38.2	-	-
PP	48.3 \pm 38.2	46.7 \pm 33.2	5.0 \pm 12.2	-
PC	43.3 \pm 36.0	50.0 \pm 31.5	6.7 \pm 13.7	-
LC	96.7 \pm 10.3	1.7 \pm 7.5	-	1.7 \pm 7.6

¹Non-native invasive species

Plant species code: AL (*Acacia longifolia*); AP (*Acacia pycnantha*); AS (*Acacia saligna*);

PP (*Phylica pubescens*); PC (*Podalyria calyptrata*);

LC (*Leucospermum conocarpodendron*)

However, the portion of seeds left at depths deemed unfavourable for seedling germination and establishment varied significantly between ant species (GLMM: $F=31.541$, $DF=5$, $p<0.0001$). For example, *A. custodiens* placed significantly more seeds of the invasive *Acacia* at depths likely to be unfavourable for germination and seedling establishment relative to that of the native endemic *Leucospermum* (large-seeded) plant species (GLMM: $F=20.545$, $DF=5$, $p<0.0001$).

Importantly, although all native ant species transported invasive *Acacia* seeds into their nests (result above), seeds from invasive *Acacia* species were more likely to be found at a suitable depths for germination and establishment within the nests of *T. sericeiventris* ant species (abundant in invaded ant communities), than that of the primary seed dispersers (*A. custodiens* and *P. capensis*) in non-invaded ant communities (GLMM: $F=29.129$, $DF=2$, $p<0.0001$ (Fig. 5.7).

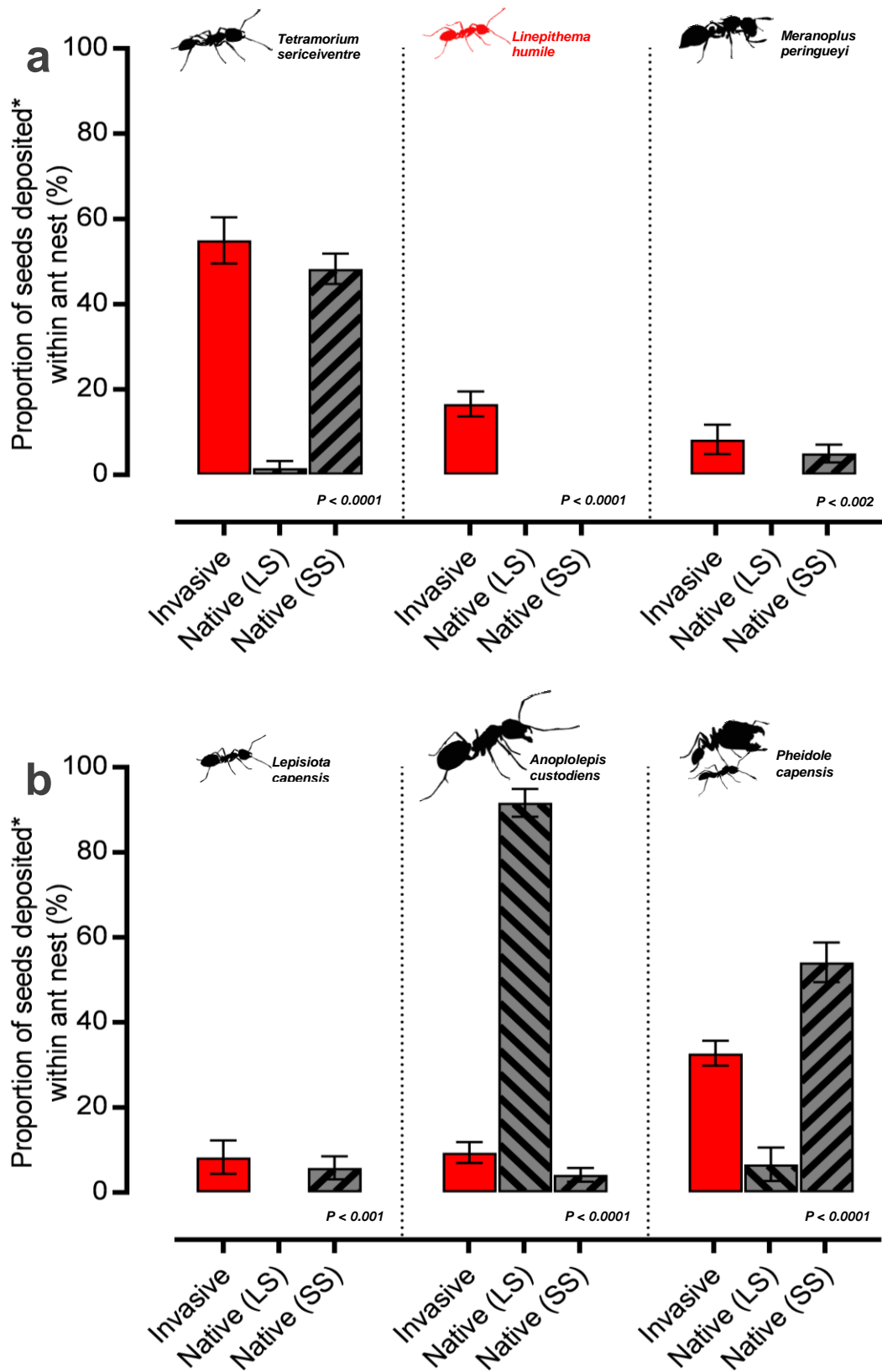


Figure 5.7: Proportion of seeds placed at depths deemed favourable for seedling germination and establishment within nests of the six most abundant ant species: across invaded (a) and non-invaded (b) ant communities. [\pm SEM], p values based on results from a binomial GLMM. [LS = large-seeded; SS = small-seeded]

5.5 DISCUSSION

The results of this study demonstrate some of the potential mechanisms by which an ant invader can make plant ecosystems more susceptible to invasion by non-native plant species. Sites where *L. humile* were present were associated with a discernible decline in seed dispersal potential, relative to sites where *L. humile* was absent. Furthermore, not only did these invaded ant communities disperse fewer seeds, but they also showed a strong relative preference for non-native invasive *Acacia* seeds, rather than seeds of sympatric endemic native plant species. This study therefore provides some early stage evidence that areas invaded by *L. humile* may suppress populations of some native plant species, whilst enhancing the spread of non-native invasive plant species. This may result in positive feedback and an invasion cascade, hastening the modification of the native plant community and eventually leading to altered or reduced ecosystem functionality and reductions in the biodiversity of both native plant and insect communities. While these findings build upon and support previous findings regarding the impacts of *L. humile* and other ant invaders on myrmecochorous interactions (Bond and Slingsby 1984; Christian 2001; Gómez and Oliveras 2003; Rowles and O'Dowd 2009; Rodriguez-Cabal et al. 2012; Prior et al. 2015). They also extend beyond previous research in South Africa that has traditionally focused solely on the impact of the invasive ant on native plant species alone (Bond and Slingsby 1984; Christian 2001), and therefore provide some of the first evidence of invader-invader myrmecochorous interactions within South Africa.

There were three key findings. Firstly, seeds placed in sites invaded by *L. humile* were less likely to be removed from seed hubs than those in non-invaded sites. This finding mirrors the results obtained in Spain (Chapter 4) and previous studies undertaken in South Africa (Bond and Slingsby 1984; Christian 2001) and elsewhere in the world (Carney et al. 2003; Gómez and Oliveras 2003; Bas et al. 2009; Rowles and O'Dowd 2009). Taken together, these results suggest that more seeds will remain on the soil surface in invaded ant communities than non-invaded ones, leading to seeds perishing from either rodent predation (Heithaus 1981), or loss by other means (e.g. desiccation and bush fires). Interestingly, unlike the findings obtained in Spain (Chapter 4), where the invaded ant community was comprised of only two ant species (invasive *L. humile* and native non-seed dispersing *Plagiolepis pygmaea*), in South Africa the ant community remained relatively intact, despite the displacement of the primary seed dispersers (e.g. *A. custodiens* and *P. capensis*). The results are therefore more reflective of an invaded community response, where a number of native ant species interact and transport seeds, rather than *L. humile* itself.

The second key finding was that invaded ant communities were preferentially dispersing the three invasive plant species seeds, whereas non-invaded ant communities showed no clear preference for either invasive nor native seeds. As with the findings in Spain (Chapter 4) it is likely that this preference for seeds is a result of morphological difference in both the ants (Gorb and Gorb 1995; Ness et al. 2004) and the seeds (Hughes and Westoby 1992; Mark and Olesen 1996; Garrido et al. 2002; Edwards et al. 2006; Gómez et al. 2005; Rowles and O'Dowd 2009; Boieiro et al. 2012). This study is also some of the first evidence to my knowledge to show the preference of ants for dispersing invasive *Acacia* seeds within invaded ant communities. Interestingly, this finding differs from some earlier work on *L. humile* in New Zealand (Rowles and O'Dowd 2009) and other invasive ant species such as *Myrmica rubra* in Canada (Prior et al. 2015) where the preference for invasive myrmecochorous seeds was driven directly by the invasive ant species itself. Rather, instead in my study the preference for invasive seeds appears to be driven indirectly by the native ant species that co-exist with *L. humile*, and therefore a consequence of the altered ant community. Furthermore, the findings of this study also contrast some of the earlier work by Christian (2001), which showed that *L. humile* invasions in CFR were negatively impacting large-seeded native species only. Whereas, my results indicate that sites where *L. humile* were recorded showed evidence of reduced native seed dispersal, irrespective of seed size. This means that their impact of *L. humile* on native endemic myrmecochorous fauna may be greater than what had been initially predicted.

The third key finding was that the likelihood that seeds were removed into ant nests, and their placement location within the nest, depended on the ant species and ant community. Overall, these results suggest that ant communities in sites invaded by *L. humile* were likely to place seeds from invasive plants at more favourable depths than native plant species. There are two components that contribute to this. Firstly, the remaining native ant species that disperse seeds in invaded communities (e.g. *T. sericeiventris* and *M. peringueyi*) appeared to treat the invasive *Acacia* seeds more favourably than it did the seeds of native plants. Furthermore, a significant portion of invasive seeds dispersed in non-invaded regions were placed at depths that were not viable for germination. Native ant species (in both invaded and non-invaded sites) appear therefore to transport the seeds above ground, but they appear to be placing them at depths that will be detrimental to seedling survival. Secondly, the invasive *L. humile* ant failed to transport any native plant seeds (large-seeded or small-seeded) into their nests; instead, they only transported the seeds of the invasive *Acacia* and placed them at favourable depths for germination. By contrast, the non-invaded ant communities were more likely to place seeds from native plants at favourable depths than those from invasive plant species. Taken together, these

differences in seed handing behaviour by ants in the two communities suggest that seeds of invasive plants are likely to do better in areas where the invasive ant *L. humile* is present.

Overall, the emerging picture from this study is that native ants are effective dispersers of non-native seeds: invasive *Acacia* seeds were actively dispersed within all sites, irrespective of whether the ant community was entirely native, or included the invasive ant *L. humile*. These findings support earlier work done by Holmes (1990), which suggested that native ant species may be playing a key role in the success of *Acacia* invasion in South Africa. This pre-adaption of *Acacia* seeds for dispersal by ants outside its native range, is likely driven by the high plant performance in its native range (Schlaepfer et al. 2010). Broadly speaking, success of many invasive plant species appears to be driven by reproductive traits that favour the rapid recruitment and spread of their offspring (Pyšek and Richardson 2007; Van Kleunen et al. 2010); accordingly, *Acacia* species have a greater seed set relative to endemic native plant species (Wright 1994; Marchante et al. 2010). Responses seen in this study therefore suggests that the invasive plant species possess potential seed traits that are more attractive to a broad range of ant species, relative to that of the endemic native plant species. The role of seed traits in seed preference by ants in invaded and non-invaded communities is explored further in Chapter 6.

Despite the compelling story implied by these results, it is important to note that these data are derived from a limited number of sites, located within only one nature reserve. In particular the spatial arrangement of invaded and non-invaded sites within Jonkershoek was not ideal. Unfortunately, the invasion front within Jonkershoek has remained relatively locked into one area of the nature reserve. This means I was not able to find other invaded sites beyond the Swartboskloof area. Every attempt was made to ensure that sites were at the same altitude, distance from the road and within a similar floristic and habitat structure. Furthermore, I have also attempted to control for these shortcomings using models that incorporate a robust random factor, that can control for potential spatial and pseudo-replication. Beyond these limitations of sample size (only two invaded sites vs. four non-invaded sites) and spatial arrangement of sites, the other aspects of the methods were either similar (Rowles and O'Dowd 2009) or indeed more rigorous (Christian 2001; Gómez and Oliveras 2003) than some previous studies. In fact, the distance between seed hubs within my ant communities is greater than most studies of this nature (e.g. Rowles and O'Dowd (2009) used two metre intervals between hubs); in fact this distance was chosen as previous studies have suggested that it is sufficient to make them independent (King and Porter 2005). Nonetheless, further work would be required to establish whether the trends observed here are more widespread, or if they are just an artefact of the ant communities

within Jonkershoek Nature Reserve. However, it is worth noting that earlier research by Christian (2001) in Kogelberg Nature Reserve (over 70 km away) found that the same native ant species (e.g. *T. sericeiventre* and *M. peringueyi* among others) co-existing with *L. humile* in invaded regions of Kogelberg Nature Reserve. This means that despite the relatively small sample size of this study, my results have the potential to be reflective of other *L. humile* invaded ant communities in the CFR.

A comparison of the impact of *L. humile* on seed dispersal in Spain (Chapter 4) and South Africa (this Chapter) highlights some intriguing and potentially important points about how the same invasive species can have quite different effects in contrasting ecosystems. In the South African sites, the majority of the seed dispersal services in invaded communities were fulfilled instead by native ant species, that co-exist with *L. humile* (e.g. *T. sericeiventre*), rather than the invasive species itself (as observed in Spain). This indicates the importance of taking a community-based approach, rather than just targeting a specific ant species, which often appears to be the norm in other similar studies of this nature. Also, despite interacting with invasive seeds in non-invaded sites, there does appear to be some degree of biotic resistance occurring in non-invaded ant communities, with the primary seed dispersers (e.g. *A. custodiens* and *P. capensis*) failing to place the majority of invasive seeds in sites favourable for seedling germination and establishment. Further research is still required to bolster these findings and to establish: a) the optimal burial depth for these native and invasive plant species, and b) whether the placement of seeds found in this study is truly representative of the long-term placement of seeds (i.e. seeds are not transported after 24 hours). While seed fate was inferred (based on burial depth) in this study, further work to quantify the soil seed bank and plant community across invaded and non-invaded ant communities would be desirable, as this would enhance the reliability of future predictions regarding the potential fate of both invasive and native plant species.

In conclusion, interactions between invaded ant communities and invasive plants present a considerable challenge to native ecosystems. Restructuring of the network of ant-plant interactions as a result of invasion by *L. humile* may be facilitating the spread and subsequent invasion by myrmecochorous *Acacia*. Invasion by *L. humile* may also be decreasing the abundance of native myrmecochorous ants and consequently native myrmecochorous plant species, especially those plants with large seed phenotypes. By significantly extending previous estimates that focused solely on the impact of the invasive ant on native plant species alone (Christian 2001), this study has identified a potential invasive synergy that indicates that invasion by one species will often facilitate and enhance the spread of another invasive species.

CHAPTER SIX

6. VARIATION IN MYRMECOCHOROUS SEED TRAITS IN INVASIVE ACACIA TREES AND NATIVE PLANT COMMUNITIES: EVIDENCE FROM CHEMICAL SELECTION AND ANT COMMUNITY PREFERENCE

6.1 ABSTRACT

Predicting how changes in community assemblage will influence ecosystem function and stability is a fundamental challenge in ecology and invasion biology. In order to achieve this, a sound understanding of how species interact and the mechanisms that govern these biotic interactions is required. Such an understanding is particularly crucial as many ecosystems are invaded by non-native species that differ in their traits to those of the native species they displace. These differences alter the ecological community, generating a shift in the strength and/or direction of the biotic interactions. Here I use mutualistic ant-seed interactions as a model system to test how differences in seed traits of native and invasive species influence seed selection across both invaded and non-invaded ant communities. Firstly, I found that invasive *Acacia* plants do not differ markedly in their physical and chemical seed traits to those of the sympatric native plant species. Secondly, I found evidence to suggest that native ant species can discern plant species based on their chemical profiles alone. Finally, when the trait data was combined with data previously collected in Chapter 5, it appeared to suggest that seed dispersal within invaded ant communities is dependent on a different combination of physical and chemical seed traits, compared with that of non-invaded ant communities. Therefore, by altering the native ant community structure, ant invaders may reduce the range of seed traits that persist in the environment. Invasions by non-native ants are therefore likely to reduce phenotypic variation in seed traits, as well as alter plant biodiversity.

6.2 INTRODUCTION

Biotic interactions are some of the fundamental elements upon which our ecosystems depend. Biotic interactions can be defined as any interaction between species, where the action of one species alters the population growth of another (Abrams 1987). These can take many forms, such as interactions related to predation, competition, host-parasite, and mutualisms (Bascompte 2009). For all species, survival depends on the presence of another organism, for example, in the form of a particular food source or pollinator (Linder et al. 2012). The strength, direction and specificity of these biotic interactions are governed by the surrounding environment and the associated traits of the interacting organisms (Thompson 2005). These interactions determine species' distributions at local and regional scales (Kissling et al. 2007; Wiens 2011). Understanding biotic interactions, therefore, is essential for managing, conserving and predicting changes in ecological communities. This is especially pertinent in the context of a rapidly changing global environment, particularly one that will generate combinations of environmental parameters never before experienced.

Biotic interactions are governed by the traits of the interacting partners. The characteristics or traits of organisms are often shared across many species and are often used as a means by which to distinguish organisms from one another, beyond just phylogenetic relatedness. Such traits also relate directly to how an organism interacts in communities, beyond its relatedness to other organisms. For example, phenotypic traits such as body size and beak shape are often used to categorise birds and as a tool to predict diet, habitat suitability or extinction risk (Bennett and Owens 1997). Using trait-based approaches for understanding ecological interactions is a powerful method for comprehending functional interactions in ecosystems (Hevia et al. 2017), as it is more successful at predicting interaction outcomes (i.e. strength and direction) than measures of relative species abundance (Stang et al. 2009).

Global ecosystems are undergoing rapid environmental change, either through climate alteration, habitat loss (fragmentation), land-use change, or species invasion (Sala et al. 2000). These modifications to the existing network of biotic interactions have been associated with the loss of ecosystem stability and function (Balvanera et al. 2006; Memmott et al. 2007; Perrings et al. 2011). In mutualistic networks, the loss of one partner can alter the function of the mutualism and could lead to the co-extinction of the dependent partner (Dunn et al. 2009b). However, in many cases the loss of biotic interactions will precede the extinction of the mutualistic partner. For example, a non-native invasive

species might disrupt a pollination network by discouraging the native pollinator from interacting with flowers, leading to a functional extinction (Galetti et al. 2013) before actual extinction of the species occurs. However, understanding the rapid disruption of biotic interactions due to environmental demands the collection and analysis of large amounts of data (Valiente-Banuet et al. 2015).

Ant-mediated seed dispersal services (myrmecochory) are sensitive to changes in species assemblages. Myrmecochory is a diffuse mutualism between ant and plant species (Giladi 2006). In this biotic interaction, plants produce seeds with a specialised appendage (the elaiosome) which attracts ants to remove the seed back to their nest. The elaiosome is then consumed and the seed is discarded intact in or around the ant nest (Beattie 1985). This process benefits the plant by dispersing the seed and placing it in a location likely to enhance seedling survival, while the ant is rewarded with a nutrient-rich food source. However, not all ant species interact with seeds in the same way. Many ant species will consume the elaiosome *in situ* or consume the seed itself in their nest (Warren and Giladi 2014), resulting in the disruption of the seed dispersal service. In any given environment only a fraction of the ant species present will offer a high-quality seed dispersal service (Gove et al. 2007). Any displacement of high-quality dispersers has the potential to alter seed survival prospects and may deliver negative impacts throughout the wider plant (and insect) community (Christian 2001).

The effectiveness of ants to interact, remove and disperse seeds is determined by a wide range of traits (Garrido *et al.* 2002; Gorb and Gorb 2003; Gómez, Espadaler, and Bas 2005; Boulay, Coll-Toledano, and Cerda 2006; Servigne and Detrain 2008). For example, the willingness of an ant to interact with seeds may be governed by the seed's traits (Fig. 6.1), but it is also governed by the ability of the ant to transport the seed (i.e. the distance moved), which is governed by the ant's traits (Fig. 6.1). Therefore, studies tend to classify ants as either low or high quality seed dispersers, based on the strength (i.e. propensity to transport seeds) and direction (i.e. positive mutualistic, rather than antagonistic) of their biotic interactions (Warren and Giladi 2014).

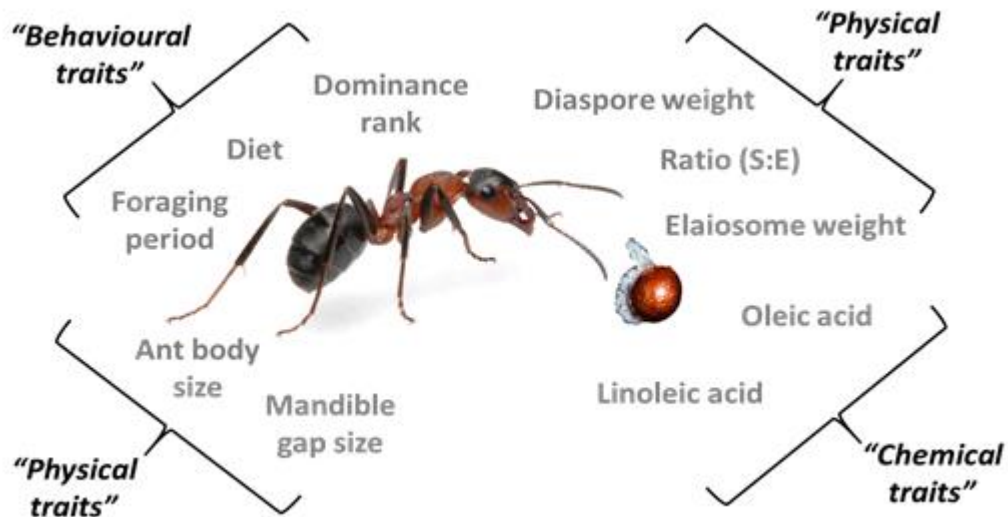


Figure 6.1: Myrmecochorous interactions are mediated by a range of ant and seed traits.

To date, much of our understanding as to the nature of myrmecochorous interactions stems from physical seed traits, such as diaspore size, elaiosome size, and seed-elaiosome ratio (Hughes and Westoby 1992a; Mark and Olesen 1996; Gorb and Gorb 1999a). However, these traits alone have often proven to be unsuccessful at predicting interaction outcomes. For example, although a larger elaiosome provides a higher reward per ant trip, experimental increases in elaiosome size relative to the seed size resulted in a reduction in the ants' removal response (Hughes and Westoby 1992a). This indicates that the trait response function is not a linear relationship, and that the interaction between ants and seeds is more complex than the simple size of the elaiosome. The role of chemical traits is another consideration which has been largely overlooked, despite the likely importance of these signals in the dispersal process. The elaiosome is a lipid rich appendage which contains fatty acids that appear to elicit both a 'carry-back' response (oleic acid) and a 'feeding' response (linoleic acid) in ant mutualists (Lanza et al. 1992; Hughes et al. 1994). Chemical traits can vary both within and between plant species (Lanza et al. 1992; Boulay et al. 2006; Boieiro et al. 2012), and these differences have been shown to directly influence dispersal success. For example, seeds with higher oleic acid content are preferentially removed by ants, irrespective of reward size (Turner and Frederickson 2013). A more holistic assessment of the seed traits that ants are responding to is therefore obviously needed.

Invasive species modify myrmecochorous interactions. Non-native plants and ants present widespread threats to global ecosystems because invasive species often differ from the sympatric native species in key biotic traits (Drenovsky et al. 2012). For example,

invasive ant species tend to be smaller than the seed-dispersing ant genera they displace, resulting in a reduction in the mean seed dispersal distance (Ness and Bronstein 2004). The presence of these smaller invasive ant species leads to a shift in the quality of seed-dispersal services (Gómez and Oliveras 2003; Rodríguez-Cabal et al. 2012) resulting in a change in plant community assemblages (Bond and Slingsby 1984; Christian 2001). These changes in ant community structure, as a result of invasion, are therefore likely to modify the underlying network of interactions that occur between both ants and seeds. Most of our understanding surrounding the mechanisms of these interactions stems from studies of the ant-plant interactions of native species, or studies in non-invaded ecosystems. However, we often lack clear assessment of whether invasive ant species are responding to seed traits in the same manner as native species. Comparisons of traits of non-native and native seeds, and the responses of native and non-native ants to these seed traits, will provide important information for understanding how global ant and plant invasions alter ecosystems.

In this study, I identify some of the key traits of the ant and plant partners involved in myrmecochory. I determine how the traits of both native and invasive plant and ant species differ, and how these traits are associated with variation in ant-plant interactions. This study was based in the South African Fynbos of the Cape Floristic Region (CFR), which offers high levels of both endemic (native) and invasive myrmecochorous fauna and flora. This study therefore had three major aims:

- *Aim 1: Construct a database of, and compare, the physical and chemical traits of invasive Acacia and native seeds from nine genera.* This allows for comparison of seed traits among and between native and invasive *Acacia* plant species. I use this information to test the hypothesis that invasive *Acacia* and native plant species differ in their physical and chemical seed traits (Hypothesis 1).
- *Aim 2: Conduct a series of ant-seed choice experiments in regions with and without invasive ants to determine how native and invasive ants respond to the traits of native and invasive Acacia seeds.* I used these data to test two hypotheses. First, I test whether elaiosome presence enhances seed removal rate in both native and invasive ants (Hypothesis 2); specifically, I predicted that all ants would respond to the presence of an elaiosome, because it provides the ant with a chemical lure by which to locate a seed and a physical appendage by which to hold a seed. Second, I tested whether the chemical traits of the elaiosome alone are sufficient to promote seed dispersal by six native and invasive ant species (Hypothesis 3); specifically, I predicted that high-quality seed dispersers would respond more strongly to the seed's elaiosome chemicals than low-quality seed dispersers

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- *Aim 3: Determine the seed traits that drive seed dispersal by native or invasive ant communities.* I use the data from Aims 1 & 2 to test the hypothesis that differences in seed removal rates between invaded and non-invaded ant communities are driven by contrasting sets of seed traits (Hypothesis 4).

6.3 METHODS AND MATERIALS

6.3.1 Database construction to compare the physical and chemical traits of invasive *Acacia* and native seeds (for Aim 1)

6.3.1.1. Seed collection

Seeds from twenty plant species (across 10 genera) were collected for both field and laboratory trials (Table 6.1). These species were selected as they represented some of the most commonly encountered myrmecochorous plant species within the CFR. For each species, fruits and seeds near maturation were collected from at least 25 plants, which were then left to dehisce naturally in the laboratory. All seeds were handled with forceps and gloves throughout the study; upon dehiscing the seeds were stored in sealed plastic bags at -15°C to preserve the chemical and physical properties of the elaiosome and thus the traits to which ants may respond. Choice of plant species was firstly based on regional abundance (i.e. commonly encountered species within the search area), and secondly by ensuring that the species chosen represented a wide diversity of seed morphologies and genealogies. Seeds of native plant species were collected from the Jonkershoek Nature Reserve ($33^{\circ}55'51''\text{S}$, $18^{\circ}51'16''\text{E}$) or the neighbouring Kogelberg Nature Reserve ($34^{\circ}12'07''\text{S}$, $18^{\circ}51'05''\text{E}$) in South Africa. Seeds of invasive *Acacia* plant species were collected from the University of Cape Town Campus ($33^{\circ}55'07''\text{S}$, $18^{\circ}25'23''\text{E}$). Non-native invasive *Acacia* are arguably one of the most destructive and widely distributed invasive plant species within the CFR (Le Maitre et al. 2011). Unfortunately, while it would have been desirable to collect seeds from a wider array of invasive genera (outside of *Acacia*) there were relatively few myrmecochorous invasive genera in all of the sites sampled. Of the few that we did find they were either comprised of a small population (i.e. one plant) or were not dispersing seed in these locations or at the time of seed collecting, consequently I was limited to only one genus of invasive plants in this study.

Table 6.1: Plant species used in the construction of the seed trait database. Seeds were collected from three localities: UCT – University of Cape Town; JNR – Jonkershoek Nature Reserve; KNR – Kogelberg Nature Reserve. All seeds are known to be primarily dispersed by ants, with the exception of two species (*) which are secondarily dispersed by ants in their native ranges. Diaspore and elaiosome weights given in milligrams [± 1 SD].

Plant Species	Collection site	Status	Dispersal mechanism	Diaspore weight (mg)	Eelaiosome weight (mg)	Seed-elaiosome Ratio
<i>Acacia cyllops</i> G.Don	UCT	Invasive	Bird*	80.5 [± 2.5]	30.4 [± 1.44]	1.7 [± 0.1]
<i>Acacia longifolia</i> (Andrews) Willd.	UCT	Invasive	Ant	16.9 [± 1.0]	2.3 [± 0.1]	7.2 [± 0.5]
<i>Acacia melanoxylon</i> R. Br.	UCT	Invasive	Bird*	18.7 [± 3.1]	4.9 [± 1.5]	2.7 [± 1.6]
<i>Acacia pycnantha</i> Benth.	UCT	Invasive	Ant	14.7 [± 0.9]	1.7 [± 0.2]	8.8 [± 1.0]
<i>Acacia saligna</i> (Labil.) Wendl.	UCT	Invasive	Ant	20.5 [± 0.4]	1.4 [± 0.1]	12.9 [± 1.0]
<i>Hypodiscus aristatus</i> (Thunb.) C.Krauss	JNR	Native (large-seeded)	Ant	45.4 [± 4.5]	8.4 [± 1.6]	4.9 [± 0.3]
<i>Hypodiscus willdenowia</i> (Nees) Mast.	KNR	Native (large-seeded)	Ant	26.7 [± 0.5]	4.4 [± 0.4]	6.0 [± 0.5]
<i>Leucospermum conocarpodendron</i> H. Buek	JNR	Native (large-seeded)	Ant	101.6 [± 7.8]	11.0 [± 0.7]	8.4 [± 0.4]
<i>Leucospermum lineare</i> R. Br.	KNR	Native (large-seeded)	Ant	71.7 [± 3.2]	6.2 [± 1.2]	12.0 [± 2.8]
<i>Leucospermum oleaefolium</i> R. Br.	JNR	Native (large-seeded)	Ant	57.0 [± 3.2]	6.3 [± 0.3]	10.1 [± 0.8]
<i>Paranomus spicatus</i> (Thunb.) Kuntze	KNR	Native (small-seeded)	Ant	6.1 [± 1.0]	0.4 [± 0.0]	15.1 [± 2.1]
<i>Phyllica lasiocarpa</i> Sond.	JNR	Native (small-seeded)	Ant	18.6 [± 0.9]	2.0 [± 0.2]	9.9 [± 0.6]
<i>Phyllica pubescens</i> Aiton	JNR	Native (small-seeded)	Ant	21.4 [± 1.1]	2.0 [± 0.3]	10.4 [± 1.9]
<i>Podalyria calyptata</i> (Retz.) Willd.	JNR	Native (small-seeded)	Ant	20.9 [± 1.8]	1.2 [± 0.1]	20.1 [± 3.8]
<i>Psoralea pinnata</i> L.	JNR	Native (small-seeded)	Ant	9.8 [± 4.8]	0.7 [± 0.1]	18.2 [± 4.9]
<i>Serruria elongata</i> R. Br.	JNR	Native (small-seeded)	Ant	7.7 [± 0.4]	0.6 [± 0.1]	12.3 [± 1.6]
<i>Serruria krausii</i> Meisn.	JNR	Native (small-seeded)	Ant	6.4 [± 0.9]	0.7 [± 0.1]	7.5 [± 1.4]
<i>Spatalla ericoides</i> Phillips	KNR	Native (small-seeded)	Ant	3.0 [± 0.8]	0.2 [± 0.1]	15.9 [± 2.9]
<i>Willdenowia sulcata</i> Mast.	KNR	Native (large-seeded)	Ant	45.4 [± 1.2]	5.0 [± 0.1]	8.1 [± 0.2]
<i>Willdenowia teres</i> Thunb.	JNR	Native (large-seeded)	Ant	24.6 [± 1.0]	1.6 [± 0.3]	14.6 [± 2.6]

6.3.1.2. Seed traits

Data were collected on a range of physical and chemical seed traits. Physical seed traits included total diaspore weight, elaiosome weight and seed-elaiosome ratio. These traits are cited in the literature as the most important physical characteristics that ants target during seed removal (Hughes and Westoby 1992a; Mark and Olesen 1996; Gorb and Gorb 1999a). Seeds were weighed on a GR-202 five place balance (A&D Instruments Ltd, Abingdon, UK) following the Millennium Seed Bank Partnership (MSBP) standard protocol. Mean diaspore weight (seed plus elaiosome) was calculated from the mean weight of five replicates of 50 randomly selected seeds. By contrast, elaiosome weights were obtained by calculating the difference between the five replicates of 50 randomly selected seeds before and after the elaiosome was detached. The seed-elaiosome ratio was calculated by dividing the seed weight by the elaiosome weight. The lower the seed-elaiosome ratio, the higher the reward to the ant transport:cost ratio.

Chemical seed traits were assayed using Gas Chromatography - Mass Spectrometry (GC-MS) on both intact diaspores (elaiosome present) and seed only (elaiosome absent) samples using methods described in Colville et al. (2016). In both instances, three replicates ($n = 3$) of 10 randomly selected diaspores (with or without elaiosomes attached) were transferred to 20 mL glass vials containing 50 mg L⁻¹ butylated hydroxytoluene in 6.4 mL of isopropanol and 10 µL of 10 mg mL⁻¹ heptadecanoic acid (internal standard). In order to extract the fatty acids from the suspended diaspores, these vials were sealed and vortex-mixed for 2 minutes prior to centrifuging at 2300 rpm at 4°C for 2 minutes. The supernatant was transferred to a fresh, pre-weighed vial and stored at -20°C, whilst the pellet was re-extracted with 4 mL isopropanol containing 50 mg mL⁻¹ and 4 mL chloroform overnight (at room temperature with constant shaking (150 rpm)). The vial was then centrifuged, and the supernatants combined and dried by evaporation under a nitrogen stream, in a sand bath heated to 45°C. The residue was dissolved in 2 mL chloroform:methanol (2:1 (v/v)) and 0.5 mL of 0.88 % (w/v) potassium chloride and shaken before removing the upper phase. The lower phase was washed with 0.7 mL methanol:potassium chloride (0.88% (w/v), 1:1 (v/v)) and dried under nitrogen at 45°C. The mass of the vial was recorded to obtain the mass of the oil extracted, and the residue was re-suspended in 1 mL toluene. Fatty acids were derivatised with 2 mL of 1% sulphuric acid in methanol overnight at 50°C. In order to remove excess derivatising agent and other impurities, the solution was washed with 5 mL hexane and 5 mL 5 % (w/v) sodium chloride. The hexane phase was transferred to a fresh vial and the lower phase was re-washed with 5 mL hexane. The hexane phases were combined and evaporated under nitrogen at 45°C. The residue was dissolved in 1mL

hexane and transferred to a 2mL auto sampler vial for GC-MS analysis. 1 μ L was injected into the Gas Chromatographer (Thermo Finnigan Trace GC Ultra) and fatty acid methyl esters were separated using a Rt-2560 column (100 m length, 0.25 mm internal diameter; Restek) running a temperature program (5 min at 140°C, 4°C min⁻¹ to 230°C, 15 min hold; helium carrier gas at constant flow rate of 1 mL min⁻¹). The compounds were detected using MS (Thermo Finnigan Trace DSQ; ionization energy 70 eV, scan frequency range m/z 10-650 per 0.2 s) and identified through comparison with the NIST mass spectral database and analytical standards (F.A.M.E. Mix C4-C24, Supelco). Fatty acid methyl esters were quantified using standard curves of quantitative standard mixtures (F.A.M.E. Mix GLC-10, -30 and -50, Supelco). Relative abundances of fatty acids in seeds from each species were obtained by comparing normalised chromatograph peak areas for both diaspore (seed and elaiosome) and seed only samples. The difference between these two groups represents the chemical composition of the elaiosome.

6.3.1.3. Hypothesis 1: Invasive Acacia and native plant species differ in their chemical and physical seed traits

The chemical and physical characteristics were compared across the twenty-plant species using a non-parametric Kruskal Wallis test. Each plant species was assigned to one of three groups: invasive, native large-seeded and native small-seeded. The two seed size classifications used for native species was based on a similar metric used by Christian (2001); with diaspores classified as large-seeded if they had a mean weight of 24.5 mg (see Table 6.1) or more. Additionally, a Phylogenetic Principal Component Analysis (PPCA) in the *phylotools* (version 0.1.2) package (Revell 2012) was used to identify the key fatty acid differences between species and origin. The loadings were then used to perform a hierarchical clustering analysis in the *factominer* (version 1.39) package (Lê et al. 2008); optimal number of clusters were determined using gap statistic on *k*-means a clustering algorithm (Hartigan and Wong 1979; Tibshirani et al. 2001).

6.3.2 Field experiments to determine how native and invasive ants respond to the traits of native and invasive *Acacia* seeds (for Aim 2)

Two focal ant communities were used in this study. These ant communities were defined by the presence/absence of *L. humile* and had been previously used in seed cafeteria experiments described in Chapter Five. I conducted cafeteria experiments (seed choice) to determine the effects of elaiosome presence (i.e. physical and chemical traits) on seed removal (hypothesis 2). I also tested the effects of elaiosome chemical traits (hypothesis 3) on ant preference for seeds.

6.3.2.1. Hypothesis 2: Elaiosome presence enhances seed removal rate by native and invasive ants

To determine whether elaiosome presence enhances seed dispersal across all ant communities, the dispersal rate for both elaiosome-present and elaiosome-detached seeds was measured across sites previously sampled in both Spain (see Chapter Four) and South Africa (see Chapter Five). Using methods described in previous chapters, at each site, seed hubs were arranged at 10 m intervals along a 100 m transect. Each seed hub consisted of a 10 cm² white card with a dome wire mesh placed on top. Ants were able to access the seeds, but vertebrates were not. In total, 60 seed hubs (10 per site) were used, with 20 classified as invaded and 40 as non-invaded.

To determine the effects of elaiosome presence on seed removal, twelve seeds (from two randomly-paired plant species; six seeds per plant species) were placed on seed hubs. Half of the seeds on the seed hubs had their elaiosomes manually removed with a scalpel in the laboratory, whilst the other half were handled but elaiosomes were left intact. All seeds were handled with gloves throughout the study. The seeds were placed on the seed hubs at 08:00 hours and 13:00 hours. During each period seeds were surveyed hourly up to a maximum of three hours; at each survey time point, the total number of seeds from each species remaining on the hub was recorded. At the end of this period any seeds left on the hubs were collected. The experiments were limited to three hours because seeds left longer than three hours are likely to be consumed by rodents rather than dispersed by ants (Jeremy Midgley pers. comm.).

The seed choice experiments were run over two consecutive weeks (4th – 16th January 2015), with seeds from each plant species being placed at least once on each hub. Rates of seed removal were compared across invaded and non-invaded sites using a Cox Proportional-Hazard model (Cox 1972) and Log-rank test (Bland and Altman 2004) in the *survival* (version 2.42-3) and *coxme* (version 2.2-10) packages (Therneau and Grambsch 2000; Therneau 2018). The fixed effects were invasion status (invaded vs. non-invaded) and elaiosome (present vs. absent); seed hub ID (nested within transect) was included as a random factor, which controls for the effects of site and repeated sampling of seed hubs.

6.3.2.2. Hypothesis 3: Chemical compounds within the elaiosome facilitate seed dispersal by native and invasive ants

Given that elaiosomes provide both physical and chemical cues, behavioural assays were conducted using a neutral decoy ‘seed’ (filter paper) to determine the effect of elaiosome chemistry on diaspore selection in the six most abundant ant species (Table 6.2). These included ant species with different levels and qualities of dispersal (see Chapter 5). High-quality seed dispersers were classified as ant species that rapidly recruit and remove seeds from hubs. By contrast, poor-quality dispersers were classified as ant species that seldom recruit and remove seeds from hubs. In total, twenty trials were performed per ant species at 20 independent ant nests (at least 5 metres apart and located across all six field sites).

Table 6.2: Seed disperser quality (high/poor) of the six most abundant ant species, across invaded and non-invaded sites in Jonkershoek Nature Reserve. Categorisation of disperser quality is based on the results from Chapter 5.

Ant species	Ant community		Disperser Quality
	Invaded	Non-invaded	
<i>Anoplolepis custodiens</i> Smith	Absent	Present	High
<i>Lepisiota capensis</i> Mayr	Absent	Present	Poor
<i>Linepithema humile</i> Mayr	Present	Absent	Poor
<i>Meranoplus peringueyi</i> Emery	Present	Present	Poor
<i>Pheidole capensis</i> Mayr	Absent	Present	High
<i>Tetramorium sericeiventre</i> Emery	Present	Present	High

Behavioural assays were tested for seven different plant species: *Acacia cyclops*, *A. saligna*, *A. pycnantha*, *Leucospermum conocarpodendron*, *Podalyria calyprata* and *Phylica pubescens*. These plant species were previously used in both the database construction (aim 1) and seed removal trials (aim 2; hypothesis 2). Behavioural assays were restricted to these seven-plant species because a few months before the trials, a bush fire burnt much of the Jonkershoek Nature Reserve, so depleting available seed stocks.

Prior to each trial, 5 g of elaiosomes were detached from a random selection of seeds within a species and added to 10 ml of hexane. The solution was left for 12 hours to extract the chemical compounds (following methods in Midgley and Bond 1995). To generate filter paper ‘seeds’, the chemical compounds (i.e. fatty acids) were transferred to circular filter paper (3 mm diameter) by submerging the filter paper in the hexane solution. At the start of each trial, a food bait (five to one tuna and honey mix) was placed 30 cm away from the focal ant nest entrance. Once the bait was discovered, the ants were allowed to forage on the food source for 15 minutes before the behavioural assays were performed. This was to increase the likelihood of contact with the filter paper ‘seeds’, because ants require direct physical contact to detect the chemical compounds in elaiosomes (Midgley and Bond

1995). Filter paper ‘seeds’ (imbibed with either chemical compounds extracted from elaiosomes in hexane or a hexane control) were placed individually 5 cm away from the nest entrance, in the path of the foraging ants. Each filter paper was observed for 5 minutes from the point of first contact, with interactions being scored accordingly (Table 6.3). This duration was chosen from a practical perspective, as previous tests have shown that temperature can influence temporal activity (and hence foraging behaviour) of ants. I therefore choose this time as an efficient means to complete the trial within a suitable time frame.

Table 6.3: Scoring system deployed in behavioural assay trials to determine the effects of chemical cues on ‘seed’ removal response. Based on methods described in Midgley and Bond (1995).

Score	Ant behaviour
0	<i>Ignored</i>
1	<i>Antennated</i> ('seed' is antennated for at least 3 seconds)
2	<i>Interacted</i> (attempt to remove 'seed', transport < 5cm)
3	<i>Removed</i> (transport the 'seed' back to the nest, transport > 5cm)

If, after 5 minutes, the filter paper was not removed or interacted with, it was replaced, and a new randomly selected filter paper was put into position. In total seven hexane (blank) controls and 21 elaiosome-impregnated filter paper pieces (three per plant species) were offered to each nest. Ant species’ response to the chemical cues were compared using a Kruskal Wallis test with a post-hoc Dunn’s Multiple Comparisons test (Dunn 1964).

6.3.3 Quantifying effects of seed traits on seed removal in order to determine the seed traits that drive seed-dispersal by non-invaded or invaded ant communities (for Aim 3)

6.3.3.1. Hypothesis 4: Seed removal rates between invaded and non-invaded ant communities are associated with contrasting sets of seed traits

To address this hypothesis, I combined data from Chapter 5 (seed removal from hubs) with trait data collected for Aim 1. This combined data was analysed using a GLMM in the *lme4* (version 1.1.14) package (Bates *et al.* 2015) with a Poisson error distribution (O’Hara and Kotze 2010) in order to measure the association between seed trait exposure and dispersal outcome for the total number of seeds removed from hubs placed in invaded and non-invaded ant communities (see Chapter Five). The fixed effects were four continuous outcome variables (seed traits: oleic acid concentration, linoleic acid concentration, diaspore weight and seed:elaiosome ratio); seed hub ID (nested within transect) was included as a random factor, which controls for the effects of site and repeated sampling of seed hubs. For this analysis I excluded both *A. cyclops* and *A. melanoxydon* samples as they are primarily bird-dispersed, and only secondarily dispersed by ants. *Odds Ratios* (OR)

estimates were extrapolated from the resulting GLMM models; this information represents the odds of an event occurring (in this case seed removal) given a particular exposure (seed trait).

6.4 RESULTS

6.4.1 Seed trait database

Aim 1: Construct a database of, and compare, the physical and chemical traits of invasive Acacia and native seeds from nine genera.

Chemical and physical traits were collected from seeds of 15 native and five invasive plant species (Table 6.1)

6.4.1.1. Physical traits

Plant species (both invasive and native combined) showed significant variation in their diaspore weight (Kruskal-Wallis test: $H = 97.47$, $DF = 19$, $p < 0.0001$), elaiosome weight (Kruskal-Wallis test: $H = 96.77$, $DF = 19$, $p < 0.0001$), and seed-elaiosome ratio (Kruskal-Wallis test: $H = 91.84$, $DF = 19$, $p < 0.0001$) (See overleaf Fig. 6.3). These traits were highly correlated with one another (Fig. 6.2): increased diaspore weight is associated with a significant increase in elaiosome weight (Spearman's rho: $r_s = 0.74$, $n = 20$, $p < 0.001$), and a significant decrease in seed-elaiosome ratio (Spearman's rho: $r_s = -0.59$, $n = 20$, $p < 0.01$).

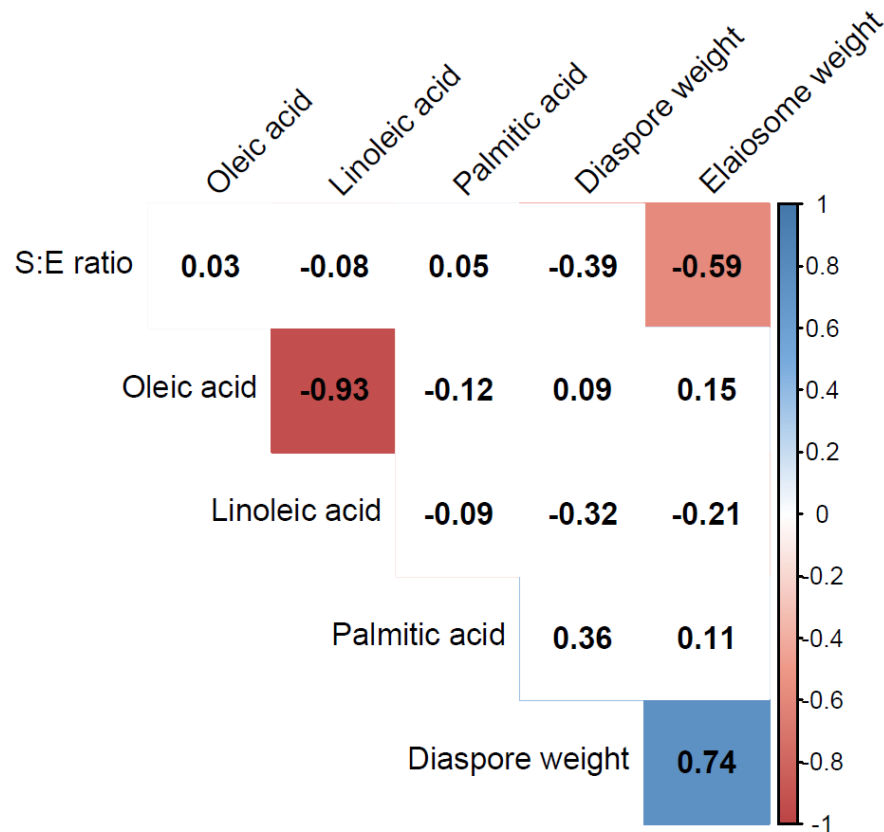


Figure 6.2: Correlation matrix of a range of physical ($n = 3$) and chemical ($n = 3$) seed traits taken from twenty different plant species. Significant values are delimited by colour squares: blue (positive correlation) and red (negative correlation). Vertical bar indicates level of correlation.

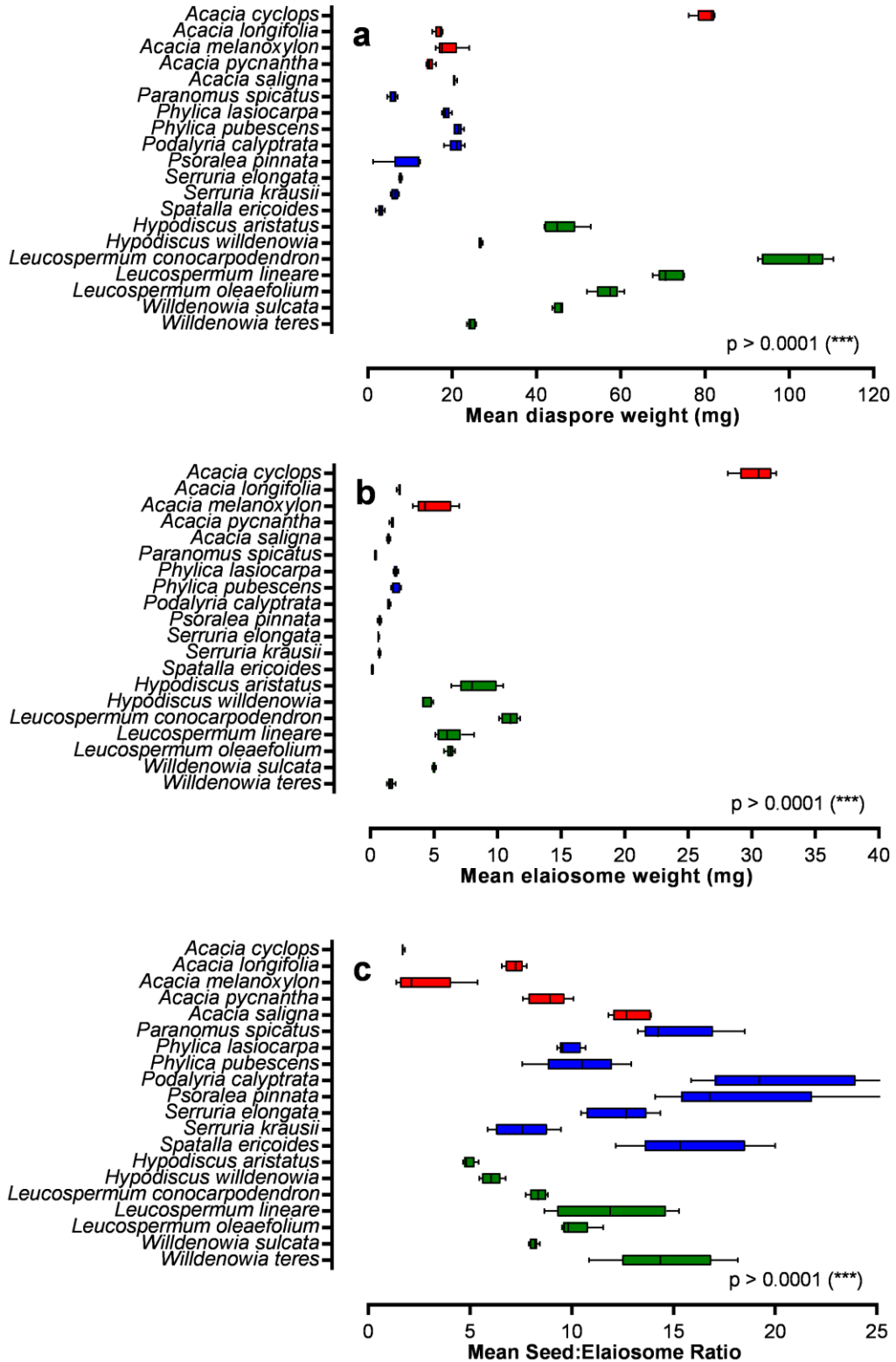


Figure 6.3: Box-plots of physical seed traits of 20 different plant species (Y-axis) with invasive species in red ($n = 5$ species), native small-seeded species in blue ($n = 8$ species) and native large-seeded species in green ($n = 7$ species) [$\pm 95\%$ CL].

6.4.1.2. Chemical traits

At least eleven different fatty acids across all 20-plant species were identified from the chromatograms. Elaiosomes showed relatively homogeneous fatty acid composition, with the most abundant fatty acids across all species being palmitic, oleic, and linoleic (Fig. 6.4).

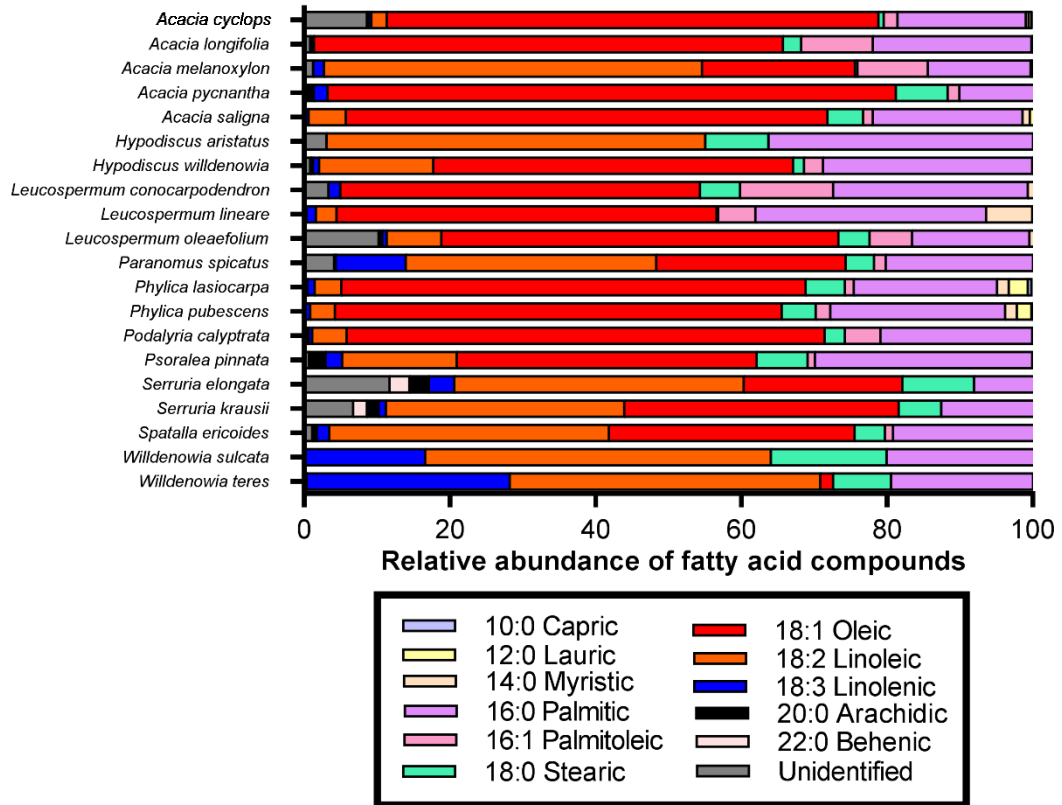


Figure 6.4: Relative abundance of fatty acids in elaiosomes from 20 different Invasive (n = 5), native small-seeded (n = 8) and native large-seeded (n = 7) plant species. Each fatty acid is supplied with its C:D number, where C is the number of carbon atoms in the fatty acid and D is the number of double bonds in the fatty acid.

Interestingly, plant species that exhibited a high amount of oleic acid tended to have a significantly lower amount of linoleic acid (Spearman's rho: $r_s = -0.93$, $n = 20$, $p < 0.001$) in their elaiosomes (Fig. 6.4). Further exploration of the relative abundance of fatty acids by means of a phylogenetic principal component analysis (PPCA) revealed that most of the differences between plant species could be explained by the first principal component (PC 1, See overleaf Fig. 6.5), which explained 89% of the variance: 57.1% of this was due to the differences in two key fatty acids - oleic acid and linoleic acid (See overleaf Table 6.4).

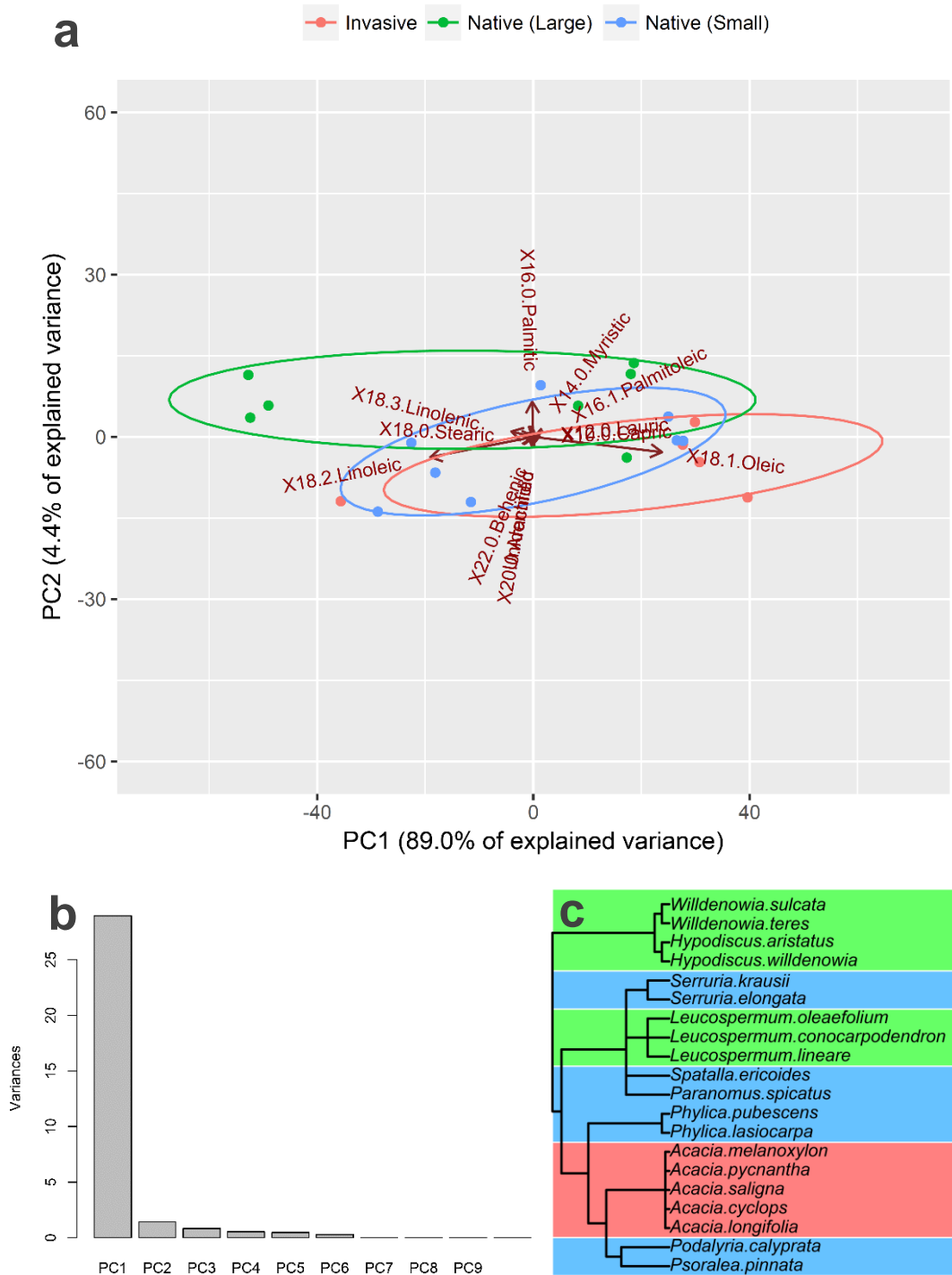


Figure 6.5: Phylogenetic Principal Component Analysis of relative abundance in fatty acids of elaiosomes from 20 different plant species (Top panel); Scree plot of eigenvalues of first 10 principal components (Bottom-left panel); Coloured phylogeny of the Invasive (red; $n = 5$), native small-seeded (blue; $n = 8$) and native large-seeded (green; $n = 7$) plant species.

Table 6.4: Loadings taken from a Phylogenetic Principal Component Analysis of the relative abundance of fatty acids of 20 different plant species (see Figure 6.5).

Fatty acid	Principal Component				
	1	2	3	4	5
Capric 10:0	0.04	-0.13	0.05	0.01	-0.06
Lauric 12:0	0.29	0.08	0.03	0.21	-0.3
Myristic 14:0	0.11	0.33	-0.05	0.09	0.1
Palmitic 16:0	0.07	0.97	-0.02	0.12	0.05
Palmitoleic 16:1	-0.39	0.36	0.01	-0.71	0.22
Stearic 18:0	0.07	-0.27	-0.2	0.75	0.43
Oleic 18:1	0.99	-0.08	0.1	-0.02	0.02
Linoleic 18:2	-0.99	-0.09	0.11	-0.01	0.02
Linolenic 18:3	-0.16	-0.46	-0.83	-0.19	-0.02
Arachidic 20:0	0.31	-0.44	-0.07	0.1	0.03
Behenic 22:0	0.04	-0.37	0.07	0.21	-0.07
Unidentified	0.02	0.01	0.06	0.17	-0.95
Standard deviation	5.38	1.19	0.91	0.74	0.68
Proportion of Variance	0.89	0.04	0.03	0.02	0.01
Cumulative Proportion	0.89	0.93	0.96	0.98	0.99

Given the results of the PPCA, further chemical trait analysis was restricted to these two key fatty acid compounds (oleic and linoleic), which are recognised as the main drivers of elaiosome selection in other studies (Pfeiffer et al. 2010).

6.4.1.3. Hypothesis 1: Invasive *Acacia* and native plant species differ in their chemical and physical seed traits

There were no systematic differences in the overall chemical ($n = 2$) and physical ($n = 3$) traits of native seeds and invasive *Acacia* seeds. Hierarchical cluster analysis based on both PPCA of these five chemical and physical seed traits identified four distinct clusterings (k -means = 4). Out of the four identified clusters, invasive plant species were spread over three of them (Fig. 6.6). In particular, the three invasive plant species that rely on ants as their primary disperser (*A. longifolia*, *A. pycnantha* and *A. saligna*) were found in the second cluster. This cluster contained both large and small-seeded native plant species. Furthermore, the invasive plant species *A. cyclops*, which is primarily bird dispersed and only secondarily ant dispersed, was placed in its own cluster (Fig. 6.6). As such, *A. cyclops* was excluded from further analyses.

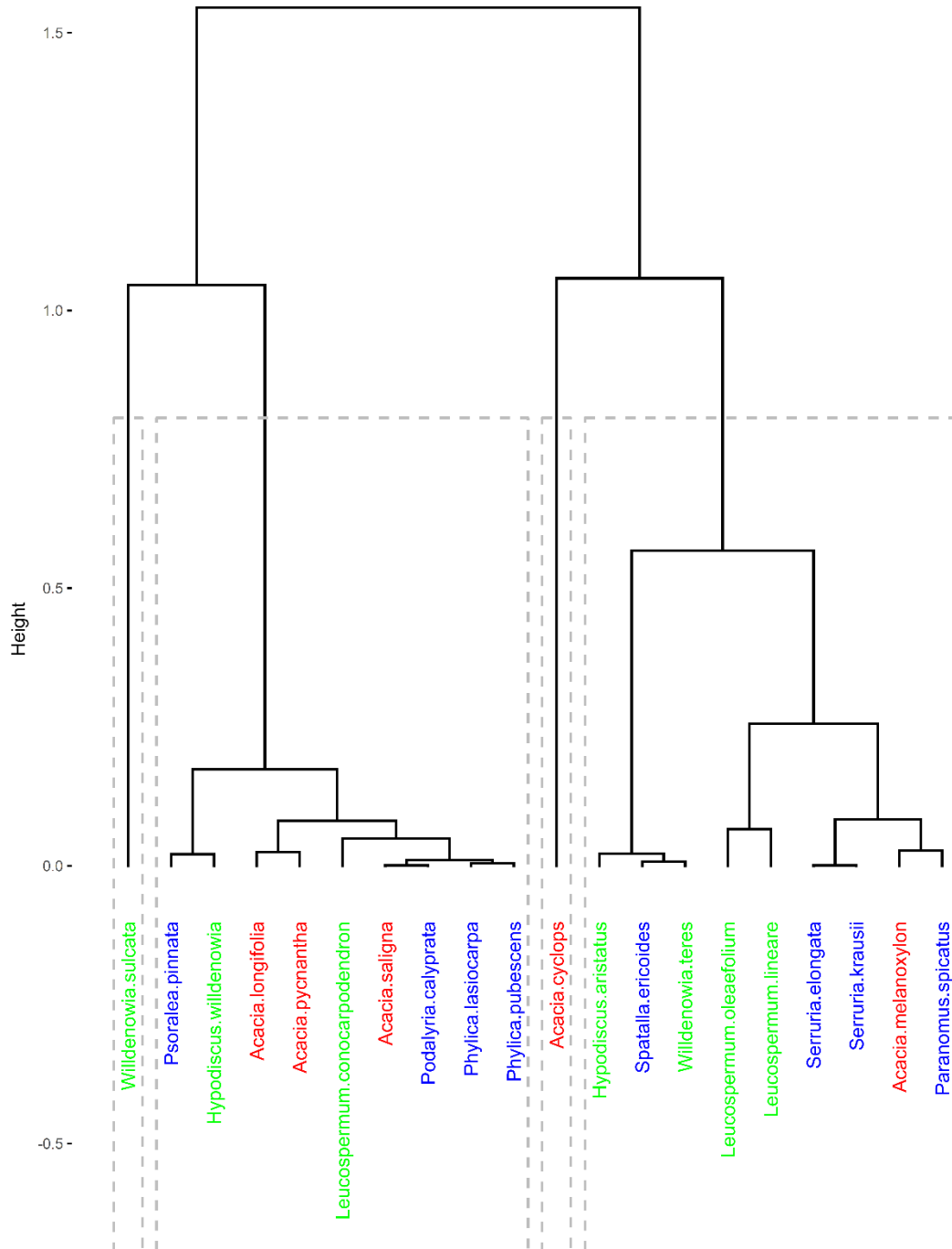


Figure 6.6: Dendrogram showing results of Hierarchical Cluster Analysis (k-means = 4) of the Phylogenetic Principle Component Analysis loadings of chemical and physical seed traits taken from 20 different Invasive (n = 5; red), native small-seeded (n = 8; blue) and native large-seeded (n = 7; green) plant species.

This lack of clear clustering by seed type category (invasive, native small-seeded, native large-seeded) is driven by the fact that there is considerable variation within each of the five recorded traits, as well as within the invasive *Acacia* genus itself. For example, invasive/*Acacia* plant species showed higher mean relative abundance of oleic acid in their elaiosomes (Fig. 6.7); however, they did not differ significantly from that of either large-seeded or small-seeded native plant species (Kruskal-Wallis test: $H = 5.357$, $DF = 2$,

$p < 0.0637$), suggesting that the invasive plant seeds did not differ per se in their physical and/or chemical traits from those of the native plant seeds.

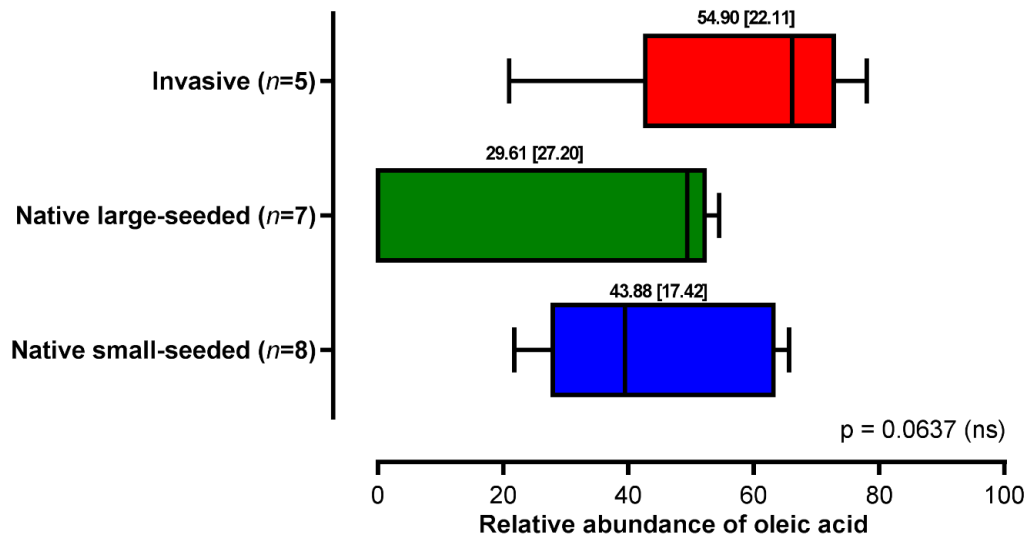


Figure 6.7: Box-plots of relative abundance (percentage representation in all identified compounds) of oleic acid in elaiosomes of invasive (red, $n = 5$ species) native small-seeded (Blue, $n = 8$ species) and native large-seeded (Green, $n = 7$ species) plants [$\pm 95\%$ CL].

6.4.2 Role of elaiosome in seed dispersal

Aim 2: Determine how native and invasive ants respond to the traits of native and invasive *Acacia* seeds.

6.4.2.1. Hypothesis 2: Elaiosome presence enhances seed removal rate by native and invasive ants

Elaiosome presence significantly increased the seed removal rate from seed hubs within invaded (Log rank-test for trend: $X^2 = 188.4$, $p < 0.0001$), and non-invaded (Log rank-test for trend: $X^2 = 156.7$, $p < 0.0001$) ant communities. The biggest effect was seen in the invaded ant communities, where elaiosome presence increased the likelihood of seed removal by 5.6 times (HR 5.6, 95% CI [4.21, 7.60]; Fig. 6.8a). By contrast, the smallest effect was seen in the non-invaded ant communities, where elaiosomes increased the likelihood of seed removal uptake by only 3.3 times (HR 3.3, 95% CI [2.24, 4.64]; Fig. 6.8b).

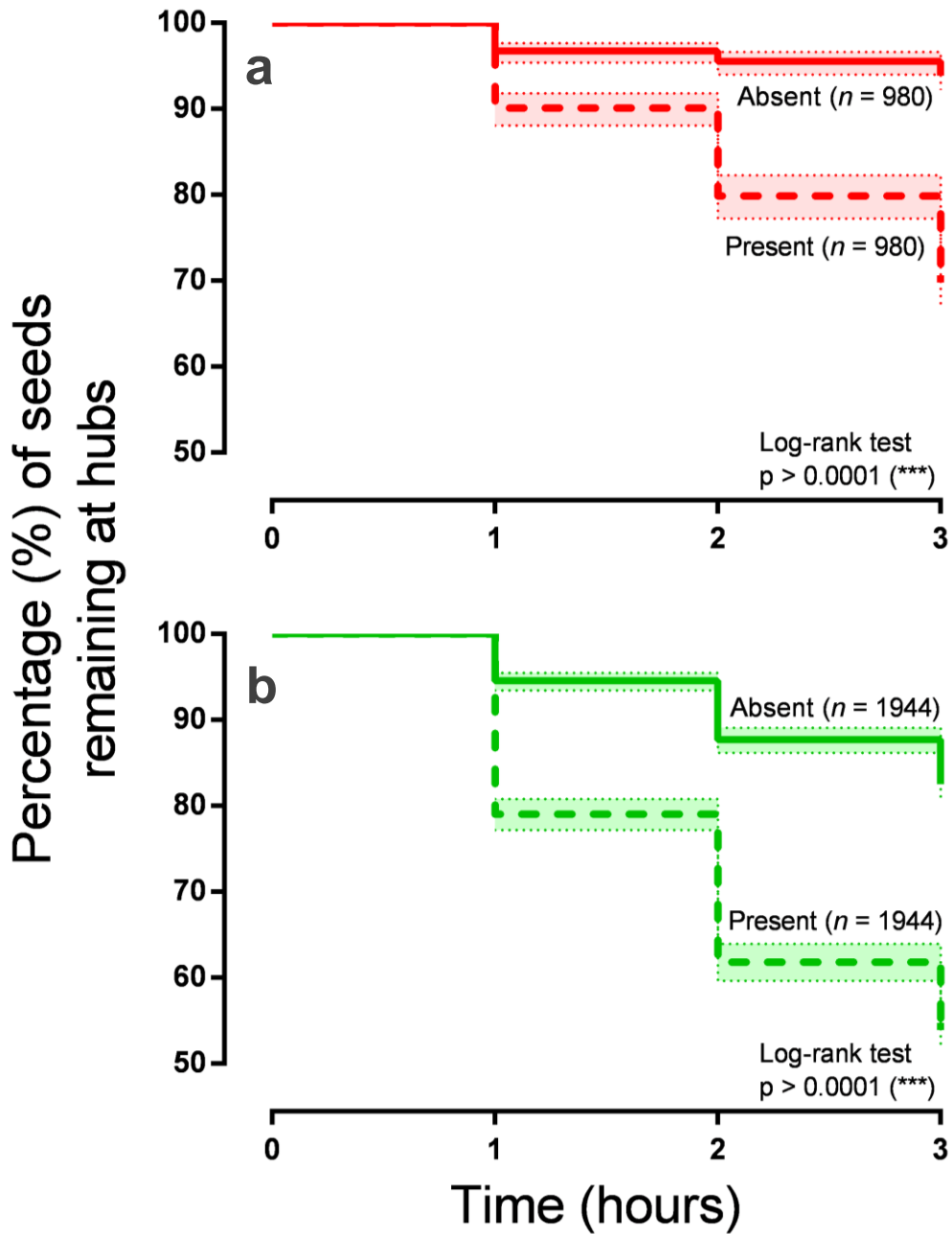


Figure 6.8: Presence of elaiosome significantly increases seed removal in all ant communities. Kaplan-Meier survival curves comparing the seed removal rate of seeds with elaiosome present (dashed line) and absent (solid line) in invaded (a) and non-invaded (b) sites, using a Cox Proportional Hazard Model [± 95 CI]. Effect of elaiosome presence on seed removal rates shown with the Log-rank test for trend result.

6.4.2.2. Hypothesis 3: Chemical compounds within the elaiosome facilitate seed dispersal by native and invasive ants

The response to filter paper ‘seeds’ varied across the six-ant species (Fig. 6.9). Hexane (blank) control filter paper ‘seeds’ were not removed, with the exception of behavioural

assays involving *Pheidole capensis* ants, which did occasionally transport blank filter paper ‘seeds’ (Fig. 6.9e).

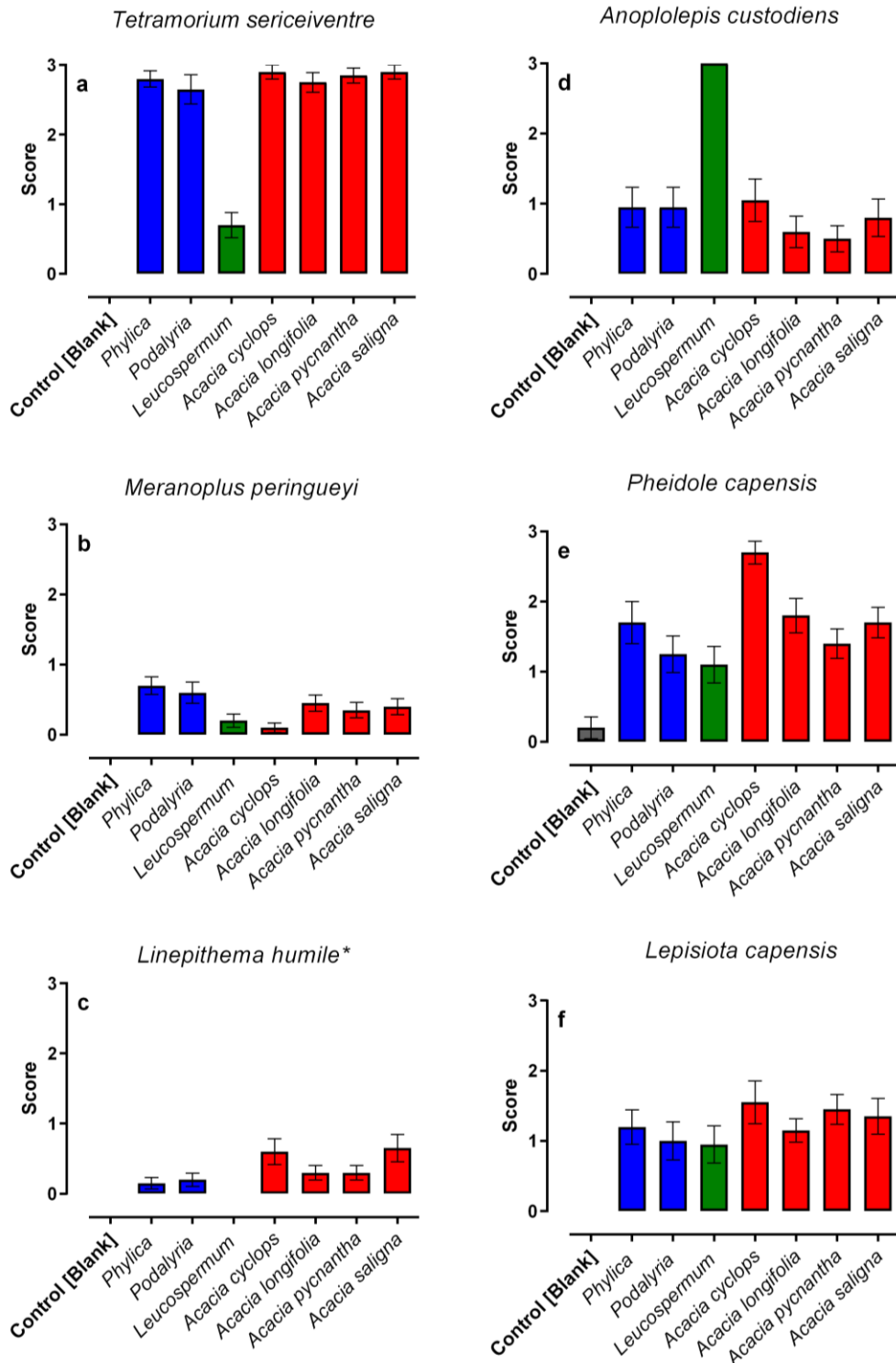


Figure 6.9: Reaction of six ant species to elaiosome filter paper ‘seeds’ with fatty acid compounds extracted from a range of Invasive (red), native small-seeded (blue) and native large-seeded (green) plant species [± 1 SEM]. Mean scores are based on a four-point scale (see Table 6.3), higher score corresponds to an increased likelihood of dispersal (3). Ant species are grouped according to their abundance in invaded (a-c) and non-invaded (d-f) ant communities. All species are native, except for *L. humile* (*) which is restricted to invaded ant communities only. Scoring system: 0 = no interaction; 1 = ‘seed’ antenattated briefly for under 3 seconds; 2 = ‘seed’ interacted with or transported less than 5 cm; 3 = ‘seed’ removed into the nest.

Of the three primary ant species found in invaded communities (*L. humile*, *T. sericeiventre*, and *M. peringueyi*), only *T. sericeiventre* ants removed filter paper ‘seeds’ back to their nest (Fig. 6.9a), whereas *M. peringueyi* and *L. humile* interacted with the filter paper ‘seeds’ only rarely and apparently at random with respect to the category of the ‘seeds’ (Fig 6.9b and 6.9c). *T. sericeiventre* showed no preference between filter paper ‘seeds’ of either invasive or small-seeded native plant species (Dunn’s multiple comparisons test: $X^2 = 2.427$, $p = 0.7875$); they investigated filter paper ‘seeds’ of the large-seeded *Leucospermum* species they did not transport them at all (Dunn’s multiple comparisons test: $X^2 = 76.36$, $p < 0.0001$).

Out of the three primary ant species found in non-invaded ant communities (*A. custodiens*, *L. capensis*, and *P. capensis*), only *A. custodiens* and *P. capensis* ant species were observed removing filter paper ‘seeds’ back to their nests (Fig. 6.9d and 6.9e). *Pheidole capensis* showed no preference to filter paper ‘seeds’ of either invasive or small-seeded native plant species (Dunn’s multiple comparisons test: $\chi^2(3) = 8.344$, $p = 0.2311$). By contrast, *A. custodiens* only transported filter paper ‘seeds’ of the large-seeded *Leucospermum* species (Kruskal-Wallis test: $H = 43.92$, $DF = 7$, $p < 0.0001$) and seldom interacted with either small-seeded or invasive filter paper ‘seeds’ (Fig, 6.9d).

6.4.3 Effects of ant community on seed trait selection

Aim 3: Determine the seed traits that drive seed-dispersal by non-invaded or invaded ant communities

6.4.3.1. Hypothesis 4: Seed removal rates between invaded and non-invaded ant communities are driven by contrasting sets of seed traits

Seed dispersal outcomes between invaded and non-invaded ant communities were found to be contingent upon a different set of seed traits. Effects of four seed traits on seed removal from hubs showed that in non-invaded ant communities, seed dispersal potential increased as both oleic (OR: 1.02, 95% CI [1.01, 1.03] $p < 0.001$) and linoleic acid (OR: 1.03, 95% CI [1.01, 1.04], $p < 0.001$) concentration increased (Fig. 6.10a). Neither diaspore weight (OR: 1.00, 95% CI [1.00, 1.01] $p = 0.182$) nor seed to elaiosome ratio (OR: 0.99, 95% CI [0.97, 1.00] $p = 0.09$) had any significant effect on seed removal potential. In other words, seed dispersal within non-invaded ant communities is driven more by changes in chemical seed properties than by changes in physical traits.

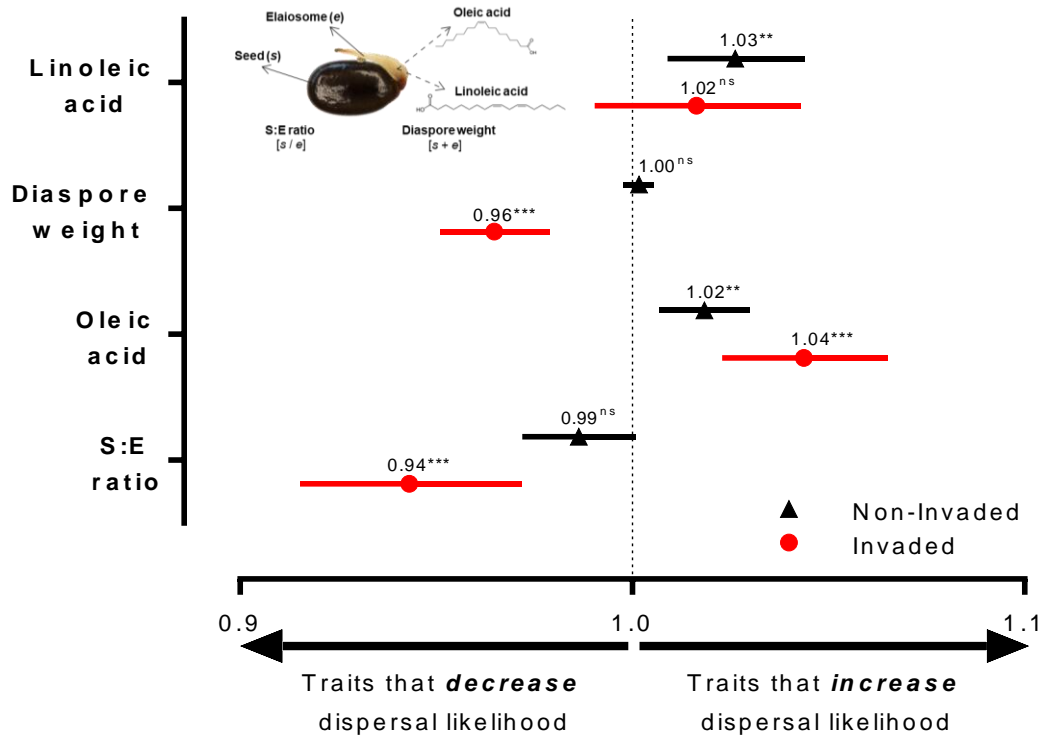


Figure 6.10: Effects of physical and chemical seed traits on seed removal from hubs placed in invaded (red circle) and non-invaded (black triangle) sites in South Africa. Odds ratio with 95% confidence intervals estimates derived from a GLMM model.

By contrast, seed removal potential of invaded ant communities increased with increasing concentrations of Oleic acid (OR: 1.04, 95% CI [1.02, 1.07], $p < 0.0001$) but decreased with increased diaspore weight (OR: 0.96, 95% CI [0.95, 0.98], $p < 0.0001$). and seed to elaiosome ratio (OR: 0.94, 95% CI [0.92, 0.97], $p < 0.0001$). Interestingly, unlike non-invaded ant communities, changes in concentrations of linoleic acid had no significant effect on seed dispersal outcomes (OR: 1.02, 95% CI [0.99, 1.04], $p < 0.110$). In other words, seed dispersal within invaded ant communities is dependent on a different set of both chemical and physical seed traits compared with that of non-invaded ant communities (Fig. 10a).

6.5 DISCUSSION

Predicting the outcome and trajectory of biotic interactions is an important tool in identifying which mutualisms are sensitive or resilient to anthropogenic changes. However, in order to achieve this task, one needs both an understanding of how species interact, as well as of which phenotypic traits mediate these interactions. This study addresses this knowledge gap for myrmecochorous interactions in several ways. Firstly, by generating a database of physical and chemical characteristics for seeds from five species of invasive *Acacia* plants and native seeds from 15 myrmecochorous plant species from nine genera, across five plant families. These data suggest that, although seeds vary in their chemical and physical traits among species, this variation was not explained by invasive or native status. Secondly, by determining the importance that elaiosome chemical characteristics have for seed selection by ants, I showed that some ant species exhibited different preferences according to seed chemical profiles of differing myrmecochorous plant species. Finally, by using the specific chemical signatures in a series of ant-seed choice experiments, I found evidence to suggest that seed dispersal within invaded ant communities is contingent on both physical and chemical traits of the seeds. These changes in seed traits could be used to explain seed dispersal rates at the community level and may have utility in predicting how the plant community may be altered in regions where the invasive ant is present. Furthermore, I found evidence that invaded ant communities may be responding to a narrower range of phenotypic seed traits compared with that of non-invaded ant communities. This suggests that invasion of native ecosystems by non-native ants may lead to a degradation and restructuring of mutualistic interactions networks, and a potential loss of key phenotypic seed traits.

The invasive *Acacia* and native plant species did not differ markedly in their seed traits; this is likely to be reflective of the convergent evolution of shared traits between myrmecochorous plants species (i.e. shared chemical cues). This finding suggests that invasive plant species may be able to prosper in novel environments by ‘piggybacking’ on previous mutualistic networks. Indeed, previous studies have shown that a high similarity in traits between resident native and invasive species can be indicative of strong mutualistic interactions (Stang et al. 2009; Gibson et al. 2012). This similarity in traits could explain, in part, why in Chapter Five invasive *Acacia* species were shown to be dispersed across all ant communities. Trait matching between invasive and native species has been shown in other studies (e.g. Minoarivelo and Hui 2016) to be a good predictor of impact, where the invasive species with the highest impact often has traits that are similar to the native recipient networks.

A potential bias in this experiment is that all the invasive seed species were derived from a single genus (*Acacia*): it is possible that with a wider phylogenetic sampling of invasive plant genera, there may be more chemical differentiation between the native and invasive seeds. However, it is worth noting that the phylogenetic principle component analysis and hierarchical cluster analysis detected as much variation in the trait space within the five species of the *Acacia* genus, as was detected among the native endemic species.

Another potential explanation for the lack of chemical differentiation among native and invasive seeds is local adaptation. Previous studies in the Iberian peninsula have shown that elaiosomes can vary in chemical composition within one plant species, depending on their geographic location (Boieiro et al. 2012). *Acacia* populations have been present within South Africa for a considerable amount of time (>100 years); the similarity in chemical traits, therefore may be the product of recent local adaptation within ant and plant communities of South Africa. Finally, the current study was only able to sample seeds from a single season: further work would therefore be required to collect seeds over several seasons to determine whether this introduces more chemical differentiation among native and invasive seeds, although it would be surprising if seasonality affected native and invasive seeds systematically differently. Future work should certainly focus on sampling from a wider list of invasive plant species to see if the trends identified here are truly representative of myrmecochorous interactions. This could include combining seed traits from other global hotspots on myrmecochory (i.e. North America and Australia), as together this information may enhance our understanding of the evolution (and potential local adaptation) in chemical cues of myrmecochorous plant species. Indeed, it would also be interesting to see whether other plant species (other than *Acacia*) seeds in Australia (native region of the invasive plant species used in this study) also share similar chemical seed traits, especially as this country has climatic conditions comparable to South Africa (Cowling et al. 1996).

Despite the absence of a clear chemical differentiation among plant species, my second experiments suggest that some of the native ant species were able to clearly discern between plant species (both native and invasive), purely on the basis of specific chemicals detected in the plant species. While this could indicate that they have a preference for a certain chemical ratio (i.e. amount of linoleic or oleic acid), it could equally be possible that the ants are targeting another chemical compound within the elaiosome itself. Interestingly, a similar chemical study by Youngsteadt et al. (2009) found that obligate mutualisms in ant garden plants was not mediated by oleic acid at all, but rather by another unknown chemical compound. Alternatively, if ant-seed preference is mediated by oleic acid, it could be

dependent on a particular isomer of oleic acid. This latter point is important as our method of quantifying fatty acids were only able to resolve the C:D (*Chain length* and number of *Double bonds*). This means that although seeds were shown to have similar levels of oleic acid (18:1), they might actually be composed of different amounts of oleic acid isomers (e.g. the double bonds are in different positions). Unfortunately, this level of isomeric resolution was beyond the scope of this research. Despite these uncertainties, my behavioural assays indicated that some high-quality seed dispersers showed a preference for filter paper ‘seeds’ imbued with fatty acids. This suggests that either plants are targeting high quality seed dispersers, or that only high-quality seed dispersers possess olfactory chemoreceptors sensitive enough to detect the presence of these fatty acids (Sheridan et al. 1996). Interestingly, *L. humile* showed no response to the filter paper ‘seeds’, which suggests that any interaction between these ants and seeds is likely to be mediated only through physical traits. A key limitation of this approach, though, was that by separating out the chemical traits, I was unable to quantify the importance of chemical cues relative to that of physical traits, as both these traits are likely to be used during the process of selection. Future work would, therefore, need to incorporate the manipulation of real seeds by removing and modifying the chemical signature of the elaiosome. This could then be used to indicate which cue is more important for seed selection (physical or chemical) and whether it is the real driver for seed removal or just an element that enhances it.

Finally, my last experiment suggests that when both physical and chemical seed traits are taken into account, ants in invaded and non-invaded communities appear to differ markedly in their responses to the selected seed traits. For example, seed removal by ants from hubs in invaded ant communities was more heavily influenced by a wider combination of seed traits (three seed traits) than in the non-invaded ant communities (two seed traits). Notably, linoleic acid appears to be a key predictor of seed removal in non-invaded ant communities, but not in invaded ant communities. Ant-seed discrimination in relation in invasion state has previously been attributed solely to physical attributes (i.e. seed size; (Christian 2001)); my findings reveal that chemical traits are also likely to be important. This could explain why some native endemic plant genera, such as *Serruria*, do not readily appear in invaded ant communities, even though they have the appropriate physical characteristics. Importantly, this finding has some important implications for predicting which plant species are at greatest risk of extinction due to *L. humile* invasion and may have some applied utility in developing management plans for either *in situ* and *ex situ* conservation: future studies (both pure and applied) should consider both physical and chemical seed traits in determining ant-seed interactions.

In conclusion, in this study I found evidence to suggest that diaspore (seed and elaiosome) traits play an important role in ant seed selection process, within both invaded and non-invaded ant communities. Of paramount importance is the evidence highlighting that it is important to consider both chemical and physical seed properties of the seed and elaiosome when predicting seed dispersal success. Likewise, it appears that invasion by *L. humile* may make ant communities more sensitive to differences in seed traits, and over time this may result in a loss of key phenotypic seed traits within these communities.

CHAPTER SEVEN

7. GENERAL DISCUSSION

7.1 OVERVIEW

The overall aim of this thesis was to address some of the key questions surrounding the evolution and disruption of myrmecochorous interactions. Focus has been given to the effects of the invasive Argentine ant (*Linepithema humile* Mayr) on ant community structure and myrmecochorous processes within Mediterranean ecosystems. In this section I discuss some of the key findings and potential limitations of my research, along with suggestions for future research avenues.

7.2 QUESTION 1: HOW WIDESPREAD IS MYRMECOCHORY WITHIN THE ANT PHYLOGENY?

Firstly, in Chapter Two, I wanted to address one of the key outstanding and neglected questions in myrmecochory research today, namely how widespread is this trait in the ant phylogeny? To address this, I constructed a database on myrmecochorous records published over the last 50 years. I found that at this current resolution (genus level, across five of the main Formicidae subfamilies), that myrmecochory, as an adapted ant trait, has evolved independently several times within the ant phylogeny. This to some extent mirrors the findings of Lengyel et al. (2010) which showed that myrmecochory has evolved independently at least 100 times within the angiosperm (flowering plant) phylogeny.

These results provide some empirical evidence to support the long-standing assumption that myrmecochory is a diffuse interaction between ant-plant mutualists (Giladi 2006; Lengyel et al. 2010; Kiers et al. 2010; Warren and Giladi 2014). Until now, we lacked the ant-phylogenetic perspective on this dogma; thus, my work provides a much-needed part to the puzzle, whilst also highlighting some key limitations in our current understanding of myrmecochory. The paramount limitation is that much of our current understanding of the evolution and function of myrmecochorous processes is derived from a relatively few geographic regions. For example, despite finding over 160 papers relating to the study of myrmecochory, the clear majority (>70%) were in fact from only three distinct geographic regions: North America, Western Europe and Australia. This lack of diversity identified in the literature, especially from global hotspots of myrmecochory such as South Africa, means that our understanding of this mutualism is limited to a few well-studied systems. Furthermore, most of the research to date has focused almost exclusively on one stage of the seed dispersal process – specifically seed removal by ants – and largely

overlooks how ants treat seeds after removal and how this impacts on the likelihood of germination. This means that to truly quantitatively test and understand the evolution of myrmecochory, I would require a larger and more complete dataset, that accurately assesses all stages of the seed dispersal process. This information would increase the topology of the phylogeny used in the ancestral state reconstruction, therein allowing for a more accurate assessment of the evolution of myrmecochory within the ant phylogeny.

Further scientific effort to expand the geographic and taxonomic knowledge of ant-seed interaction would be rewarded by helping address several important questions. For example, what are the adaptive advantages for ants who disperse seeds? The adaptive advantage of myrmecochory is well understood from the plants perspective, but it is not so clear from the ants' perspective. As such, to what degree ants benefit from myrmecochory remains relatively unclear, especially as most of the work done in this respect has come purely from laboratory studies. This means that while some tentative evidence exists regarding the benefits of elaiosome collection in terms of colony productivity (Morales and Heithaus 1998; Gammans et al. 2005; Marussich 2006), these types of studies unfortunately fail to account for all the other costs associated with foraging for elaiosomes. There is, therefore, considerable potential and scope to undertake empirical research to quantify the costs and benefits of myrmecochory to ant mutualists.

A second question of importance is: to what effect does ant diet dictate dispersal efficiency? The propensity for any given ant species to interact and disperse seeds remains unclear. This has led some authors to suggest that myrmecochory favours carnivorous and scavenging ant species (Hughes and Westoby 1992b), yet no researcher has explicitly tested this assumption. Given the increased availability of studies on ant traits and phylogenies (Moreau and Bell 2013; Ward et al. 2015; Parr et al. 2017) it would, therefore, be interesting to investigate to what extent ant diet dictates dispersal efficiency.

7.3 QUESTION 2: HOW DO NATIVE ANT COMMUNITIES RESPOND TO ARGENTINE ANT INVADERS?

Ant invasions are typically characterised by a displacement of native ant species (Holway et al. 2002). In Chapter Three, I explored how native ant communities respond to Argentine ant invaders by comparing sites where *L. humile* are present vs. those where they were absent, across two distinct geographic regions: Spain and South Africa. Not surprisingly, sites where *L. humile* were present were found to be lower in ant species richness than sites where *L. humile* were absent. Notably, native ant species that were more commonly associated with seed dispersal were among those that were displaced.

Furthermore, while this effect was observed across two distinct geographic regions, the native ant communities showed differential responses to the introduction of *L. humile*. In Spain, sites where *L. humile* were present were characterised by a near complete displacement of native ant species, with just one native ant species able to survive. By contrast, sites in South Africa where *L. humile* were present, it was found that relatively few native ant species were displaced. This resilience was previously identified to be a key aspect in retaining myrmecochorous services within invaded areas of the Cape Floristic Region for small-seeded plant species (Christian 2001).

This variability in native ant response to invasion highlights the importance of empirical studies in accurately quantifying the effects of an invasive species such as *L. humile*. Understanding and quantifying the true impact of an invasive species on native community structure remains a contentious issue in the scientific literature (Fridley et al. 2007; King and Tschinkel 2013b; Stuble et al. 2013). In this thesis I found some tentative evidence to demonstrate variability in native ant community structure in response to the presence of *L. humile*. It is, however, important to note that these findings are limited due to the relatively limited number of sites investigated and by the restricted time period of the research (one year). Limitations are also linked to the fact that comparison were made using invaded vs. non-invaded sites, rather than using a pre/post invasion comparison (e.g. as a result of exclusion experiments). This means that it may be premature to extrapolate these findings beyond this specific study system. These findings, however, do highlight the importance of context specific empirical studies and they raise several potential future research directions, which I discuss below.

7.3.1 How important are abiotic and biotic factors in predicting *L. humile* invasion success?

Linepithema humile in Spain is widespread and is found frequently in disturbed and undisturbed environments. Yet, despite both Spain and South Africa having a long invasion history (>100 years), *L. humile* remains understudied in undisturbed and pristine environments in South Africa. This is problematic, as it means that it may be difficult to accurately predict where *L. humile* may or may not occur, and in turn, it makes the potential impact this ant has on native ant communities difficult to predict. This means that abiotic (e.g. temperature, water availability) and biotic (e.g. native ant species, predators) factors, using a larger number of sites, across several seasons, need to be researched in order to be able to more accurately predict the abundance, distribution and impacts of *L. humile* within these environments.

7.3.2 What effect is *L. humile* having on ant functional traits within invaded ant communities?

Functional traits are increasingly being used as means by which to predict competitive interactions between invasive and native species (Funk et al. 2016; Hajek et al. 2017). In Chapter Three, I identified several native ant species and genera which were able to co-exist with *L. humile*. Comparison of the functional traits (e.g. morphological, life history, ecological, etc) of native species assemblages within invaded ant communities would provide critical information for understanding how ant communities are responding to *L. humile* invasion, beyond relatively simple metrics of species richness and abundance.

7.4 QUESTION 3: WHAT EFFECT IS *L. HUMILE* HAVING ON SEED DISPERSAL SERVICES?

During my appraisal of the literature (Chapter Two), it became clear that current understanding of myrmecochorous processes is generally limited to above-ground seed transport events. This restricted level of information means that the separate study of antagonistic and mutualistic interactions is not possible, and neither can the researcher fully determine the quality of the seed dispersal processes. When placed within the context of ant invasions, this means the existing assessments on the impacts invasive species have on myrmecochorous processes may be insufficient. To address this shortcoming, I focused on the effects of *L. humile* on seed dispersal within the Iberian Peninsula, a region with a relatively well understood seed-dispersing ant community and a long invasion history. Using a series of field experiments (Chapter Four), I concluded that *L. humile* is a poor replacement for the primary sympatric native seed-disperser, and that this inefficiency was derived at several key stages of the dispersal process. I found, not only were seeds dispersed in invaded regions ten times less likely to be transported, but also that they were likely to be distributed over a potentially smaller spatial area and placed in sites that were less favourable for seedling establishment. This suggests that impacts of *L. humile* invasion are potentially greater than previously estimated by Gómez and Oliveras (2003). In fact, these results support the view that ant invasions are likely to lead to a disruptive rearrangement of native plant species, with a reduction in the gene flow potential between populations of native plant species, due to lower dispersion and seedling survival.

One challenge that emerged from this research was despite my best efforts, there are still several caveats and underlying assumptions that have been made in order to attempt to understand the seed dispersal process. For example, I never explicitly tested seedling

survival, and was limited to inferred it based on seed burial depth. This means further research on optimum seed planting depth for differing plant species would have been a valuable addition to this research, as it would have allowed for a more accurate prediction of the potential impacts *L. humile* may have on seed germination and seedling survival within invaded zones.

7.4.1 What is the *optimal* placement of myrmecochorous seeds?

Seedling survival curves often illustrate how few plant species survive despite the large number of seeds parent plants produce (Baskin and Baskin 1998; Moles and Westoby 2004). The term *optimal* is, therefore, potentially problematic, as what may appear optimal in one circumstance, may prove detrimental in another. For example, in Spain there is an underlying assumption that the optimal placement for seeds is to be buried below ground (Bas et al. 2007); however, burial depth and/or rainfall levels may in fact make this strategy detrimental to seedling survival (Baskin and Baskin 1998). It would therefore be beneficial to research and understand how much seed placement influences seedling survival of both native and invasive plant species, and how this *optimal* placement might change both temporally and spatially.

7.4.2 What effect is *L. humile* having on non-myrmecochorous seeds?

Most, if not all research undertaken into the impacts of *L. humile* on seed dispersal has focused on myrmecochorous seeds (Bond and Slingsby 1984; Christian 2001; Carney et al. 2003; Gómez and Oliveras 2003; Oliveras et al. 2005a; Rowles and O'Dowd 2009). However, many non-myrmecochorous plant species may also be affected. For example, many plant species within the Iberian Peninsula are potentially adapted for dispersal by granivorous ant species (Arnan et al. 2012). This includes several *Genista* plant species which have been shown to possess traits, such as thick seed coats (testa) to withstand mechanical damage by harvester ant species (e.g. *Messor* sp.). Given that in Chapter Three, I found that all granivorous ant species were displaced by *L. humile*, this suggests that these non-myrmecochorous species may be equally negatively affected, as more of their seeds may be left on the soil surface, rather than being dispersed away from the parent plants. Likewise, a thicker seed testa comes with a trade-off, as these seeds are less able to imbibe water and thus germinate (Baskin and Baskin 1998). Removal of this selection pressure means that presence of *L. humile* might be indirectly selecting for differential seed traits that favour thinner seed testas. Further work into exploring this understudied aspect of *L. humile* invasion may give an important insight into the evolutionary selection pressures

driving seed dispersal of both myrmecochorous and non-myrmecochorous plant species within the Iberian Peninsula.

7.4.3 What is the actual impact of *L. humile* on seedling survival?

Much of the research, including work undertaken in this thesis, has focused on proximate means by which to measure the effects of *L. humile* on seed dispersal. While these approaches can be informative, they are not without their challenges and limitations. For example, as mentioned above, the displacement of harvester ant species, such as *Messor*, as a product of invasion, might mean more seeds can persist (and survive) on the soil surface. Inferring impacts using proximal measures alone may, therefore, prove problematic. It would be advisable to also look for more direct impacts, such as evidence of this disruption in the plant community composition; for example, sampling of above-ground plant community structure and below-ground soil seed bank levels across an invasion gradient. This information would thereby provide more tangible and much needed direct evidence of the impacts of ant invaders, such as *L. humile*, on myrmecochorous processes.

7.5 QUESTION 4: DOES THE PRESENCE OF *L. HUMILE* FAVOUR OTHER INVASIVE SPECIES?

Most other studies on the impacts of invasive myrmecochorous ant species on the dispersal ability of native plant species have focused on the dichotomy of invaded vs. non-invaded ant communities. This approach, however, fails to take into account that ecosystems are often subjected to invasion by multiple species (Preston et al. 2012). To explore this, I conducted a series of field trials within both a pristine and an endemic-rich area of the Cape Floristic Region (Chapter Five). The aim of this research was to explore the potential for invader-invader interactions between invasive myrmecochorous *Acacia* seeds and invasive ant communities. Interestingly, unlike previous assessments by Christian (2001), I found that small-seeded plant species were indeed negatively affected by the presence of *L. humile*, and that their seeds were less likely to be transported. A key finding of this study was that invasive *Acacia* were transported at all sites, irrespective of the presence of *L. humile*. However, where *L. humile* were present, the seeds of three of the invasive *Acacia* species were preferentially being dispersed, compared with that of the native sympatric plant species. Furthermore, compared to the primary seed dispersers in non-invaded sites, one native ant species that co-exists with *L. humile* was found to also place relatively more seeds of the invasive *Acacia* at potentially suitable depths for seedling establishment. When considering the wider implication of this study, I found that invasion

by *L. humile* is leading to a decoupling of natural ant-plant mutualisms, and that they are potentially also increasing the permeability of regions to invasion by *Acacia* plant species.

Such invader-invader interactions, while not unique to this study system, hint at the perverse nature of impacts by invasive species. It is important to acknowledge that these findings (see Chapter Four) are still limited due to the fact that across one season, only a few sites within a single nature reserve were researched. Moreover, only one invasive plant genus (*Acacia*) was evaluated; whether this preferential treatment is true of other invasive plant species remains to be resolved. These findings, however, add to a growing body of evidence (Rowles and O’Dowd 2009; Prior et al. 2015) of myrmecochorous invader-invader interactions. Potential future key research questions include the following:

7.5.1 Could preferential dispersal of *Acacia* in turn enhance *L. humile* colonisation?

Previous research by Schoeman and Samways (2011) has shown that *L. humile* are more prevalent in plant communities invaded by non-native plant species, such as *Acacia*. Under the assumption that *Acacia* survival is enhanced in the presence of *L. humile* it would be interesting to see whether a form of invader-invader feedback loop exists. In other words, if *L. humile* presence enhances the survival of *Acacia*, maybe the presence of this plant species in turn facilitates the spread and survival of *L. humile* populations. It would, therefore, be useful to sample ant community structure (i.e. Chapter Three) and seed dispersal potential (i.e. Chapter Four and Five) across a disturbance gradient as this would help to identify whether abundance and seed dispersal services alter across both disturbed and undisturbed environments.

7.6 QUESTION 5: WHAT EFFECT IS *L. HUMILE* HAVING ON SEED TRAIT SELECTION?

In order to understand why *Acacia* were being preferentially dispersed in regions where *L. humile* were present, I investigated some of the key traits that mediate myrmecochorous interactions. In Chapter Six, I constructed a database on a range of physical and chemical seed traits for a variety of endemic native and invasive (*Acacia*) plant species. However, based on the traits collected, I found no clear evidence to suggest that invasive *Acacia* seeds differed substantially from the seeds of native sympatric species. Rather, it appears that invasive plant species were able to ‘piggy-back’ on pre-existing myrmecochorous interaction networks.

By delving further into this aspect of seed traits, it became clear to me that despite the large body of literature of myrmecochory, much of our understanding surrounding the

mechanisms that mediate myrmecochorous processes comes from the studies of physical attributes (Hughes and Westoby 1992a; Mark and Olesen 1996; Gorb and Gorb 1999a). I, therefore, wanted to determine whether variations of elaiosome chemical fatty acid profiles reflected potential dispersal preferences in the primary ant dispersers, across both invaded and non-invaded ant communities. I found through behavioural assays that ant species showed varied responses to the chemical profiles of the seeds of different plant species, and that clear biases or preferences between ant species existed. Specifically, some high-quality seed dispersing ant genera were more responsive to chemical cues than the lower-quality ant genera.

Finally, by combining the data collected in Chapters Five and Six I looked at the ant community response to different seed traits. I found that invaded communities were more heavily influenced by differences in seed traits, whereas dispersal success in non-invaded ant communities was less influenced by seed trait variation. These results suggest that invaded ant communities are effectively screening and consequently reducing the natural range of phenotypic seed traits. Invasion by *L. humile* is, therefore, potentially leading to not only a loss of both ant and plant species richness, but also a loss of phenotypic seed traits as well.

While these findings extend our understanding of myrmecochorous ant-seed interactions, it is important to note that the approach I have taken is not without its limitations. For example, my comparison between invasive and native plant species was limited to only one invasive genus (*Acacia*), and with seeds collected from one season. Further work would therefore be required to see whether these findings are truly representative of invasive myrmecochorous species, rather than an aspect limited to the *Acacia* genus. Nonetheless, there are some interesting future research avenues to consider, which I discuss below.

7.6.1 How static are myrmecochorous seed traits?

While this thesis has highlighted the importance of considering seed traits in the context of invasion, it should be noted that it is based on the implicit assumption that the seed traits measured are static. It would, therefore, be important to sample plant seed traits over a disturbance gradient, across several seasons, to see if they are able to adapt or respond to invasion and change in native ant community structure.

7.6.2 Which traits are more important in seed selection?

One aspect that remains unresolved in the study of myrmecochory is clarifying how important physical and chemical traits are during ant seed selection. In Chapter Six, I found that some high-quality seed dispersing ant species (e.g. *Pheidole capensis*) showed little to no response to chemical cues. Therefore, chemical cues may be potentially a less important factor in determining dispersal outcome in this ant species. Manipulating diaspores to alter their chemical signature may, therefore, be an effective approach in which to explore some of the key underlying preference mechanisms, and to determine the importance of varied seed traits during ant-plant interactions and seed selection.

7.7 CONCLUDING REMARKS

To conclude, in this thesis I have demonstrated and explored some of the key aspects of myrmecochory. In addition, I have constructed databases that can be used to gain greater insight into this important ecosystem service; and this, together with my research results, will support the understanding and the ongoing study of myrmecochorous processes. However, this thesis is but a step in the right direction, as further work is still needed to clarify, not only the many processes of this important interaction, but also the long-term implications of species invasion.

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APPENDICES

APPENDIX 1

Table A1: Records of Myrmecochory. Biogeographic distribution (taken from records on antwiki.org) types: AU-Australian; IM-Indo-Malayan; NA-Nearctic; NT-Neotropical; PA-Palearctic; PT-Paleotropical; WW-Worldwide.

No.	Ant Subfamily	Ant Genera	Geographic distribution	Granivory	Dispersal score	Dispers quality	Plant families	Plant genera	Reference
1	Dolichoderinae	<i>Anonychomyrma</i>	AU IM	Absent	0.07 (0.027)	Poor	Fabaceae, Proteaceae	<i>Acacia</i> , <i>Pultenae</i> , <i>Grevillea</i>	Auld & Denham 1999, Beaumont et al. 2011, Beaumont et al. 2013
2	Dolichoderinae	<i>Azteca</i>	NT	Absent	Insufficient data	Poor	Gesneriaceae, Passifloraceae	<i>Codonanthe</i> , <i>Turnera</i>	Marini-Filho 1999, Salazar-Rojas et al. 2012
3	Dolichoderinae	<i>Dolichoderus</i>	NA NT PA IM AU	Absent	0.04 (0)	Poor	Proteaceae, Rubiaceae, Zingiberaceae	<i>Grevillea</i> , <i>Anthorrhiza</i> , <i>Globba</i>	Auld & Denham 1999, Maeyama & Matsumoto 2000, Pfeiffer et al. 2004
4	Dolichoderinae	<i>Dorymyrmex</i>	NA NT	Absent	0.04 (0.01)	Poor	Asteraceae, Capparaceae, Celastraceae, Euphorbiaceae, Papaveraceae, Passifloraceae, Polygalaceae	<i>Cirsium</i> , <i>Maytenus</i> , <i>Cnidioscolus</i> , <i>Croton</i> , <i>Manihot</i> , <i>Jatropha</i> , <i>Dendromecon</i> , <i>Turnera</i> , <i>Piriqueta</i> , <i>Polygala</i>	Carney et al. 2003, Alba-Lynn & Henk 2010, Leal et al. 2007, Salazar-Rojas et al. 2012, Stuble et al. 2010
5	Dolichoderinae	<i>Forelius</i>	NA NT	Absent	0.03 (0)	Poor	Passifloraceae, Polygalaceae	<i>Piriqueta</i> , <i>Polygala</i> , <i>Turnera</i>	Stuble et al. 2010, Salazar-Rojas et al. 2012
6	Dolichoderinae	<i>Iridomyrmex</i>	AU IM	Absent	0.24 (0.19)	Good	Amaranthaceae, Euphorbiaceae, Fabaceae, Myrtaceae, Proteaceae	<i>Acacia</i> , <i>Pultenae</i> , <i>Grevillea</i> , <i>Sclerolaena</i> , <i>Adriana</i> , <i>Jatropha</i> , <i>Bertya</i> , <i>Bossiaea</i> , <i>Dillwynia</i>	Davidson & Morton 1981, Anderson 1988a, Hughes & Westoby 1990, Hughes & Westoby 1991, Hughes & Westoby 1992a, Hughes & Westoby 1992a, Andersen & Morrison 1998, Auld & Denham 1999, Bebawi & Campbell 2002, Bebawi & Campbell 2004, Scott & Gross 2004, Gove et al. 2007, Parr et al. 2007, Auld 2009, Beaumont et al. 2009, Beaumont et al. 2013, Pascov et al. 2015, Whitney 2002
7	Dolichoderinae	<i>Linepithema</i> *	NT (WW*)	Absent	0.09 (0.076)	Poor	Boraginaceae, Euphorbiaceae, Fabaceae, Papaveraceae, Polygalaceae, Rhamnaceae	<i>Anchusa</i> , <i>Euphorbia</i> , <i>Cytisus</i> , <i>Genista</i> , <i>Dendromecon</i> , <i>Polygala</i> , <i>Rhamnus</i> , <i>Phyllica</i>	Quilichini & Debussche 2000, French & Major 2001, Carney et al. 2003, Gomez & Oliveras 2003, Gomez et al. 2003, Witt & Giliomee 2004, Rowles & O'Dowd 2009
8	Dolichoderinae	<i>Papyrius</i>	AU	Absent	0.01 (0.016)	Poor	Fabaceae	<i>Acacia</i>	Andersen & Morrison 1998, Gove et al. 2007

9	Dolichoderinae	<i>Tapinoma</i>	WW	Absent	0.13 (0.25)	Poor	Boraginaceae, Euphorbiaceae, Cyperaceae, Ranunculaceae, Polygalaceae, Rhamnaceae, Violaceae	<i>Anchusa, Euphorbia, Carex, Adriana, Croton, Piriqeta, Polygala, Helleborus, Rhamnus, Viola</i>	Culver & Beattie 1978, Beattie & Culver 1981, Ganeshaiah & Shaanker 1988, Espadaler & Gomez 1996, Espadaler & Gomez 1997, Wolff & Debussche 1999, Quilichini & Debussche 2000, Garrido et al. 2002, Gomez & Oliveras 2003, Gomez et al. 2003, Boulay et al. 2005, Alcántara et al. 2007, Boulay et al. 2007b, Manzaneda et al. 2007, Beaumont et al. 2009, Manzaneda & Alcántara 2009, Stuble et al. 2010
10	Dolichoderinae	<i>Technomyrmex</i>	NT PT IM AU	Absent	0.002 (0)	Poor	Fabaceae	<i>Acacia</i>	Gove et al. 2007
11	Dorylinae	<i>Labidus</i>	NA NT	Absent	0.01 (0.0002)	Poor	Capparaceae, Phytolaccaceae	<i>Capparis, Seguieira</i>	Dominguez-Haydar & Armbrecht 2011
12	Ectatomminae	<i>Ectatomma</i>	NT	Absent	0.22 (0.16)	Good	Capparaceae, Caricaceae, Celastraceae, Euphorbiaceae, Fabaceae, Marantaceae, Passifloraceae, Phytolaccaceae, Poaceae, Zingiberaceae	<i>Capparis, Seguieira, Carica, Maytenus, Cnidoscolus, Croton, Jatropha, Manihot, Ricinus, Acacia, Calathea, Passiflora, Urochloa, Renealmia</i>	Le Corff & Horvitz 1995, Morrone et al. 2000, Martins et al. 2006, Leal et al. 2007, Zelikova & Breed 2008, Garcia-Robledo & Kiprewicz 2009, Santamaria et al. 2009, Renard et al. 2010, Dominguez-Haydar & Armbrecht 2011, Almeida et al. 2013, Leal et al. 2013
13	Ectatomminae	<i>Rhytidoponera</i>	AU IM	Absent	0.37 (0.25)	Good	Amaranthaceae, Euphorbiaceae, Fabaceae, Dilleniaceae, Elaeocarpaceae, Rhamnaceae, Proteaceae	<i>Sclerolaena, Hibbertia, Tetratheca, Adriana, Bertya, Acacia, Pultenaea, Bossiaea, Daviesia, Dipogon, Grevillea, Hardenbergia, Trymalium</i>	Davidson & Morton 1981, Anderson 1988a, Brew et al. 1989, Hughes & Westoby 1992, Hughes & Westoby 1993, Westoby et al. 1991, Hughes & Westoby 1992a, Hughes & Westoby 1992b, Ireland & Andrew 1995, Peakall & Beattie 1995, Andersen & Morrison 1998, Auld & Denham 1999, Scott & Gross 2004, Gove et al. 2007, Parr et al. 2007, He et al. 2008, Beaumont et al. 2009, Lomov et al. 2009, Rowles & O'Dowd 2009, Beaumont et al. 2011, Majer et al. 2011, Beaumont et al. 2013, Thompson et al. 2016
14	Formicinae	<i>Anoplolepis</i>	PT IM	Absent	0.2 (0.21)	Good	Proteaceae, Rutaceae, Rhamnaceae	<i>Phylica, Mimetes, Leucospermum, Serruria, Spatalla, Coleonema</i>	Bond & Slingsby 1984, Bond & Stock 1989, Yeaton & Bond 1991, Christian 2001, French & Major 2001
15	Formicinae	<i>Brachymyrmex</i>	NA NT (PT*)	Absent	0.08 (0)	Poor	Passifloraceae, Polygalaceae	<i>Turnera, Polygala, Piriqeta</i>	Stuble et al. 2010, Salazar-Rojas et al. 2012

16	Formicinae	<i>Camponotus</i>	WW	Absent	0.15 (0.18)	Good	Aracea, Aristolochiaceae, Bromeliaceae, Capparaceae, Caricaceae, Celastraceae, Cyperaceae, Elaeocarpaceae, Euphorbiaceae, Fabaceae, Gesneriaceae, Melanthiaceae, Papaveraceae, Piperaceae, Ranunculaceae, Rhamnaceae, Urticaceae, Zingiberaceae	<i>Acacia, Aechmea, Anthurium, Capparis, Carex, Carica, Cnidoscopus, Codonnathe, Croton, Cytisus, Euphorbia, Globba, Helleborus, Hexastylis, Jatropha, Manihot, Maytenus, Peperomia, Poikilospermum, Rhamnus, Ricinus, Sanguinaria, Tetratecca, Trillium</i>	Ohara & Higashi 1987, Anderson 1988a, Ganeshaiah & Shaanker 1988, Brew et al. 1989, Higashi et al. 1989, Bossard 1991, Espadaler & Gomez 1996, Orivel & Dejean 1999, Gomez et al. 2003, Ness 2004, Pfeiffer et al. 2004, Boulay et al. 2005, Boulay et al. 2006, Martins et al. 2006, Alcantara et al. 2007, Boulay et al. 2007a, Boulay et al. 2007b, Gove et al. 2007, Leal et al. 2007, Manzaneda et al. 2007, Rey & Manzaneda 2007, Ness & Morin 2008, Youngsteadt et al. 2008, Manzaneda & Alcantara 2009, Menzel & Bluthgen 2009, Youngsteadt et al. 2009, Warren et al. 2010, Almeida et al. 2013, Leal et al. 2013, Leroy et al. 2016, Tanaka & Suzuki 2016
17	Formicinae	<i>Cataglyphis</i>	PA PT	Absent	0.3 (0.49)	Good	Ranunculaceae, Rhamnaceae	<i>Helleborus, Rhamnus</i>	Garrido et al. 2002, Gomez et al. 2003, Boulay et al. 2005, Boulay et al. 2007a, Manzaneda et al. 2007, Manzaneda & Alcantara 2009
18	Formicinae	<i>Formica</i>	NA PA IM	Absent	0.12 (0.19)	Poor	Amaryllidaceae, Aristolochiaceae, Asteraceae, Berberidaceae, Boraginaceae, Colchicaceae, Cyperaceae, Ericaceae, Euphorbiaceae, Fabaceae, Juncaceae, Melanthiaceae, Montiaceae, Orobanchaceae, Papaveraceae, Polygalaceae, Ranunculaceae, Rhamnaceae, Violaceae	<i>Allium, Anemone, Asarum, Carex, Centaurea , Chelidonium, Claytonia, Cordyalis, Corema, Corydalis, Cytisus, Delphinium, Dicentra, Disporum, Euphorbia, Helleborus, Hexastylis, Jeffersonia, Luzula, Melampyrum, Mercurialis, Mertensia, Polygala, Pulmonaria, Ranunculus, Rhamnus, Sanguinaria, Trillium, Uvularia, Viola</i>	Culver & Beattie 1978, Culver & Beattie 1980, Beattie & Culver 1981, Turnbull et al. 1983, Ohara & Higashi 1987, Oostermeijer 1989, Bossard 1991, Gibson 1993, Gorb & Gorb 1995, Gorb & Gorb 1999a, Gorb & Gorb 1999b, Wolff & Debussche 1999, Gorb & Gorb 2000, Gorb & Gorb 2001, Garrido et al. 2002, Oberrath & Bohning-Gaese 2002, Bale et al. 2003, Gomez & Oliveras 2003, Gomez et al. 2003, Peters et al. 2003, Ness 2004, Dostal 2005, Boulay et al. 2006, Jensen & Six 2006, Alcantara et al. 2007, Boulay et al. 2007a, Manzaneda et al. 2007, Rey & Manzaneda 2007, Heinken & Winkler 2009, Castro et al. 2010, Preiffer et al. 2010, Warren et al. 2010, Berg-Binder & Suarez 2012, Chlumsky et al. 2013, Hilley & Thiet 2015, Komatsu et al. 2015, Tanaka & Suzuki 2016, Tanaka & Tokuda 2016

19	Formicinae	<i>Lasius</i>	NA PA IM	Absent	0.03 (0.02)	Poor	Aristolochiaceae, Asteraceae, Caryophyllaceae, Colchicaceae, Cyperaceae, Dioscoreaceae, Ericaceae, Juncaceae, Melanthiaceae, Orobanchaceae, Papaveraceae, Plantaginaceae, Polygalaceae, Ranunculaceae, Violaceae	<i>Asarum, Borderea, Carex, Centaurea, Chelidonium, Corema, Corydalis, Helleborus, Hepatica, Luzula, Melampyrum, Maehringia, Polygala, Sanguinaria, Trillium, Uvularia, Veronica, Viola</i>	Culver & Beattie 1978, Culver & Beattie 1980, Beattie & Culver 1981, Ohara & Higashi 1987, Higashi et al. 1989, Oostermeijer 1989, Gibson 1993, Ohkawara & Higashi 1994, Ohkawara et al. 1997, Gorb & Gorb 1999a, Garrido et al. 2002, Oberrath & Bohning-Gaese 2002, Gomez & Oliveras 2003, Peters et al. 2003, Boulay et al. 2005, Jensen & Six 2006, Boulay et al. 2007a, Manzaneda et al. 2007, Rey & Manzaneda 2007, Casazza et al. 2008, Ness & Morin 2008, Prinzing et al. 2008, Servigne & Detrain 2008, Manzaneda & Alcantara 2009, Castro et al. 2010, Garcia et al. 2012, Reinfenrath et al. 2012, Hilley & Thiet 2015, Tanaka & Suzuki 2016, Tanaka & Tokuda 2016
20	Formicinae	<i>Lepisiota</i>	PT PA IM	Absent	0.05 (0)	Poor	Rhamnaceae	Phyllica	French & Major 2001
21	Formicinae	<i>Melophorus</i>	AU	Present (common)	0.03 (0.04)	Poor	Euphorbiaceae, Fabaceae, Rhamnaceae	<i>Adriana, Acacia, Trymalium</i>	Gove et al. 2007, Parr et al. 2007, Beaumont et al. 2009, Majer et al. 2011, Pascov et al. 2015
22	Formicinae	<i>Notoncus</i>	AU	Absent	0.04 (0.03)	Poor	Elaeocarpaceae, Fabaceae	<i>Acacia, Bossiaea, Tetratheca</i>	Brew et al. 1989, Hughes & Westoby 1994, Gove et al. 2007
23	Formicinae	<i>Oecophylla</i>	PT IMAU	Absent	0.02 (0.03)	Poor	Fabaceae, Zingiberaceae	<i>Acacia, Globba</i>	Andersen & Morrison 1998, Pfeiffer et al. 2004, Gove et al. 2007
24	Formicinae	<i>Opisthopsis</i>	AU	Absent	0.001 (0)	Poor	Fabaceae	<i>Acacia</i>	Gove et al. 2007
25	Formicinae	<i>Paratrechina</i>	NA NT PT IM	Absent	0.05 (0.04)	Poor	Berberidaceae, Cyperaceae, Ericaceae, Fabaceae, Juncaceae, Melanthiaceae, Papaveraceae, Passifloraceae, Polygalaceae, Proteaceae, Violaceae, Zingiberaceae	<i>Acacia, Bossiaea, Carex, Cordyalis, Epigaea, Epimedium, Globba, Grevillea, Luzula, Piriqueta, Polygala, Trillium, Viola</i>	Beattie & Culver 1981, Clay 1983, Hughes & Westoby 1995, Hughes & Westoby 1996, Andersen & Morrison 1998, Auld & Denham 1999, Pfeiffer et al. 2004, Gove et al. 2007, Parr et al. 2007, Stuble et al. 2010, Takahashi & Itino 2012, Tanaka & Suzuki 2016, Tanaka & Tokuda 2016, Zhu et al. 2017
26	Formicinae	<i>Polyrhachis</i>	PT AU IM	Absent	0.01 (0.01)	Poor	Fabaceae, Zingiberaceae	<i>Acacia, Globba</i>	Pfeiffer et al. 2004, Gove et al. 2007
27	Formicinae	<i>Prenolepis</i>	NT PA IM	Absent	Insufficient data	Poor	Aristolochiaceae	<i>Hexastylis</i>	Warren et al. 2010
28	Formicinae	<i>Prolasius</i>	AU	Absent	0.004 (0.001)	Poor	Elaeocarpaceae, Fabaceae	<i>Acacia, Tetratheca</i>	Brew et al. 1989, Gove et al. 2007
29	Formicinae	<i>Stigmacros</i>	AU	Absent	Insufficient data	Poor	Euphorbiaceae	<i>Adriana</i>	Beaumont et al. 2009

30	Myrmicinae	<i>Acromyrmex</i>	NT NA	Absent	0.3 (0.31)	Good	Anacardiaceae, Capparaceae, Clusiaceae, Euphorbiaceae, Fabaceae, Phytolaccaceae, Poaceae	<i>Acacia, Capparis, Clusia, Copaifera, Croton, Jatropha, Mabea, Ricinus, Schinus, Seguieira, Urochloa</i>	Morrone et al. 2000, Passos & Oliveira 2002, Peternelli et al. 2003, Varela & Perera 2003, Zelikova & Breed 2008, Pikart et al. 2010, Dominguez-Haydar & Armbrrecht 2011, Leal et al. 2013
31	Myrmicinae	<i>Aphaenogaster</i>	AU PA NA	Present (common)	0.25 (0.22)	Good	Aristolochiaceae, Berberidaceae, Boraginaceae, Burseraceae, Colchicaceae, Cyperaceae, Ericaceae, Euphorbiaceae, Fabaceae, Juncaceae, Marantaceae, Melanthiaceae, Orobanchaceae, Papaveraceae, Ranunculaceae, Rhamnaceae, Solanaceae, Violaceae	<i>Acacia, Anchusa, Anemone, Asarum, Calathea, Carex, Chelidonium, Commiphora, Corema, Corydalis, Cytisus, Datura, Dillwynia, Disporum, Euphorbia, Helleborus, Hexastylis, Jeffersonia, Luzula, Melampyrum, Rhamnus, Sanguinaria, Trillium, Viola</i>	Handel 1976, Culver & Beattie 1978, Beattie & Culver 1981, Ohara & Higashi 1987, Andersen 1988b, Higashi et al. 1989, Bossard 1991, Westoby et al. 1991, Hughes & Westboy 1992b, Gibson 1993, Le Corff & Horvitz 1995, Espadaler & Gomez 1996, Espadaler & Gomez 1997, Ohkawara et al. 1997, Gomez & Espadaler 1998, Morales & Heithaus 1998, Böhning-Gaese et al. 1999, Quilichini & Debussche 2000, Bono & Heithaus 2002, Garrido et al. 2002, Bale et al. 2003, Gomez & Oliveras 2003, Gomez et al. 2003, Ruhren & Handel 2003, Ness 2004, Boulay et al. 2005, Heithaus et al. 2005, Ness & Bressmer 2005, Boulay et al. 2006, Alcantara et al. 2007, Boulay et al. 2007a, Boulay et al. 2007b, Gove et al. 2007, Manzaneda et al. 2007, Ness & Morin 2008, Bas et al. 2009, Manzaneda & Alcantara 2009, Warren et al. 2010, Canner & Spence 2011, Berg-Binder & Suarez 2012, Canner et al. 2012, Canner et al. 2012, Rodriguez-Cabal et al. 2012, Takahashi & Itino 2012, Prior et al. 2014, Hilley & Thiet 2015, Tanaka & Suzuki 2016, Thompson et al. 2016
32	Myrmicinae	<i>Atta</i>	NA NT	Present (common)	0.19 (0.19)	Poor	Euphorbiaceae, Sapindaceae	<i>Ricinus, Croton, Mabea, Cupania</i>	Passos & Ferreira 1996, Guimaraes & Cogni 2002, Peternelli et al. 2003, Martins et al. 2006, Peternelli et al. 2008, Peternelli et al. 2009
33	Myrmicinae	<i>Carebara</i>	NA NT PT AU IM	Absent	0.03 (0)	Poor	Euphorbiaceae	<i>Ricinus</i>	Martins et al. 2006

34	Myrmicinae	<i>Crematogaster</i>	WW	Absent	0.04 (0.06)	Poor	Apocynaceae, Capparaceae, Caricaceae, Caryophyllaceae, Colchicaceae, Cyperaceae, Elaeocarpaceae, Ericaceae, Euphorbiaceae, Fabaceae, Gesneriaceae, Myrtaceae, Papaveraceae, Passifloraceae, Phytolaccaceae, Polygalaceae, Primulaceae, Proteaceae, Ranunculaceae, Rhamnaceae, Rutaceae, Urticaceae, Zingiberaceae	<i>Acacia</i> , <i>Aeschynanthus</i> , <i>Bossiaea</i> , <i>Capparis</i> , <i>Carex</i> , <i>Carica</i> , <i>Cnidoscopus</i> , <i>Coleonema</i> , <i>Corema</i> , <i>Croton</i> , <i>Cyclamen</i> , <i>Cytisus</i> , <i>Darwinia</i> , <i>Dischidia</i> , <i>Globba</i> , <i>Helleborus</i> , <i>Jatropha</i> , <i>Leucospermum</i> , <i>Liparia</i> , <i>Moehringia</i> , <i>Phyllica</i> , <i>Piriqueta</i> , <i>Poikilospermum</i> , <i>Polygala</i> , <i>Rhamnus</i> , <i>Sanguinaria</i> , <i>Segueira</i> , <i>Tetradthea</i> , <i>Uvularia</i>	Beattie & Culver 1981, Brew et al. 1989, Hughes & Westoby 1997, Hughes & Westoby 1998, Bossard 1991, Affre et al. 1995, French & Major 2001, Kaufmann et al. 2001, Garrido et al. 2002, Gomez & Oliveras 2003, Gomez et al. 2003, Ness 2004, Pfeiffer et al. 2004, Boulay et al. 2007a, Gove et al. 2007, Leal et al. 2007, Manzaneda et al. 2007, Rey & Manzaneda 2007, Casazza et al. 2008, Auld 2009, Menzel & Bluthgen 2009, Stuble et al. 2010, Dominguez-Haydar & Armbrrecht 2011, Almeida et al. 2013, Leal et al. 2013, Hilley & Thiet 2015, Tanaka & Suzuki 2016, Tanaka & Tokuda 2016
35	Myrmicinae	<i>Cyphomyrmex</i>	NA NT	Absent	0.004 (0)	Poor	Euphorbiaceae, Passifloraceae, Polygalaceae	<i>Cnidoscopus</i> , <i>Croton</i> , <i>Piriqueta</i> , <i>Polygala</i>	Leal et al. 2007, Stuble et al. 2010
36	Myrmicinae	<i>Leptothorax</i>	NA PT	Absent	0.02 (0.02)	Poor	Papaveraceae, Rhamnaceae, Violaceae	<i>Chelidonium</i> , <i>Rhamnus</i> , <i>Sanguinaria</i> , <i>Viola</i>	Culver & Beattie 1978, Gorb & Gorb 1999a, Oberrath & Bohning-Gaese 2002, Gomez et al. 2003, Peters et al. 2003, Ness & Morin 2008
37	Myrmicinae	<i>Lophomyrmex</i>	IM	Absent	0.04 (0)	Poor	Zingiberaceae	<i>Globba</i>	Pfeiffer et al. 2004
38	Myrmicinae	<i>Meranoplus</i>	AU IM PT	Present (rare)	0.02 (0.02)	Poor	Fabaceae, Proteaceae	<i>Acacia</i> , <i>Dillwynia</i> , <i>Leucospermum</i> , <i>Mimetes</i> , <i>Serruria</i> , <i>Spatalla</i>	Hughes & Westoby 1992a, Andersen & Morrison 1998, Christian 2001, Gove et al. 2007
39	Myrmicinae	<i>Messor</i>	PA PT IM	Present (common)	0.06 (0)	Poor	Euphorbiaceae, Rhamnaceae	<i>Euphorbia</i> , <i>Rhamnus</i>	Espadaler & Gomez 1997, Gomez et al. 2003, Gomez & Oliveras 2003
40	Myrmicinae	<i>Monomorium</i>	WW	Present (rare)	0.03 (0.03)	Poor	Cyperaceae, Ericaceae, Euphorbiaceae, Fabaceae, Passifloraceae, Polygalaceae	<i>Acacia</i> , <i>Adriana</i> , <i>Carex</i> , <i>Croton</i> , <i>Dillwynia</i> , <i>Epigaea</i> , <i>Piriqueta</i> , <i>Polygala</i>	Clay 1983, Ganeshiah & Shaanker 1988, Hughes & Westoby 1992a, Andersen & Morrison 1998, Gove et al. 2007, Parr et al. 2007, Beaumont et al. 2009, Stuble et al. 2010, Tanaka & Suzuki 2016

41	Myrmicinae	<i>Myrmica</i>	IM NA NT PA	Absent	0.3 (0.24)	Good	Aristolochiaceae, Asparagaceae, Asteraceae, Berberidaceae, Colchicaceae, Cyperaceae, Ericaceae, Euphorbiaceae, Fabaceae, Juncaceae, Liliaceae, Melanthiaceae, Orobanchaceae, Papaveraceae, Plantaginaceae, Polygalaceae, Primulaceae, Ranunculaceae, Rhamnaceae, Violaceae	<i>Asarum, Carex, Centaurea, Chelidonium, Corema, Corydalis, Delphinium, Epimedium, Erythronium, Euphorbia, Helleborus, Hepatica, Luzula, Melampyrum, Polygala, Primula, Rhamnus, Sanguinaria, Scilla, Trillium, Ulex, Uvularia, Veronica, Viola</i>	Culver & Beattie 1978, Culver & Beattie 1980, Beattie & Culver 1981, Turnbull et al. 1983, Kjellson 1985, Ohara & Higashi 1987, Gunther & Lanza 1989, Higashi et al. 1989, Gibson 1993, Ohkawara & Higashi 1994, Valverde & Silvertown 1995, Mark & Olesen 1996, Ohkawara et al. 1996, Ohkawara et al. 1997, Gorb & Gorb 1999a, Garrido et al. 2002, Oberrath & Bohning-Gaese 2002, Gomez et al. 2003, Peters et al. 2003, Boulay et al. 2005, Dostal 2005, Fischer et al. 2005, Gammans et al. 2005, Jensen & Six 2006, Boulay et al. 2007a, Fokuhl et al. 2007, Delatte & Chabrierie 2008, Ness & Morin 2008, Prinzing et al. 2008, Servigne & Detrain 2008, Castro et al. 2010, Berg-Binder & Suarez 2012, Reinfenrath et al. 2012, Prior et al. 2014, Bologna & Detrain 2015, Hilley & Thiet 2015, Zhu et al. 2017
42	Myrmicinae	<i>Ocymyrmex</i>	PT	Present (common)	Insufficient data	Poor	Rhamnaceae	<i>Phyllica</i>	Witt & Giliomee 2004
43	Myrmicinae	<i>Pheidole</i>	WW	Present (common)	0.25 (0.21)	Poor	Amaranthaceae, Annonaceae, Bursaraceae, Cactaceae, Capparaceae, Caricaceae, Caryophyllaceae, Celastraceae, Cyperaceae, Dioscoreaceae, Euphorbiaceae, Fabaceae, Marantaceae, Myrtaceae, Passifloraceae, Phytolaccaceae, Poaceae, Polygalaceae, Proteaceae, Ranunculaceae, Rhamnaceae, Rutaceae, Sapindaceae, Zingiberaceae	<i>Acacia, Adriana, Bertya, Borderea, Bossiaea, Calathea, Capparis, Carex, Carica, Cnidoscolus, Coleonema, Commiphora, Croton, Cytisus, Darwinia, Dillwynia, Dodonaea, Euphorbia, Genista, Globba, Hardenbergia, Helleborus, Jatropha, Leucospermum, Liparia, Manihot, Maytenus, Mimetes, Moehringia, Phyllica, Piriqueta, Polygala, Pultenaea, Renalmia, Rhamnus, Ricinus, Sclerolaena, Segueira, Serruria, Spatalla, Tacinga, Turnera, Urochloa, Xylopia</i>	Davidson & Morton 1981, Bond & Slingsby 1984, Ganeshiaiah & Shaanker 1988, Hughes & Westoby 1999, Hughes & Westoby 2000, Westoby et al. 1991, Yeaton & Bond 1991, Hughes & Westoby 1992a, Hughes & Westoby 1992b, Harrington & Driver 1995, Ireland & Andrew 1995, Le Corff & Horvitz 1995, Espadaler & Gomez 1996, Passos & Ferreira 1996, Espadaler & Gomez 1997, Andersen & Morrison 1998, Morrone et al. 2000, Christian 2001, French & Major 2001, Garrido et al. 2002, Gomez & Oliveras 2003, Gomez et al. 2003, Pfeiffer et al. 2004, Scott & Gross 2004, Boulay et al. 2005, Martins et al. 2006, Alcantara et al. 2007, Boulay et al. 2007a, Gove et al. 2007, Leal et al. 2007, Manzaneda et al. 2007, Parr et al. 2007, Rey & Manzaneda 2007, Zhou et al. 2007, Casazza et al. 2008, Ohnishi et al. 2008, Zelikova & Breed 2008, Auld 2009, Beaumont et al. 2009, Garcia-Robledo & Kiprewicz 2009, Lomov et al. 2009, Manzaneda & Alcantara 2009, Rowles & O'Dowd 2009, Christianini & Oliveira 2010, Stuble et al. 2010, Aranda-Rickert & Fracchia 2011, Dominguez-Haydar & Armbrrecht 2011, Lobo et al. 2011, Rickert & Fracchia 2010, Aranda-Rickert & Fracchia 2012, Garcia et al. 2012, Salazar-Rojas et al. 2012, Almeida et al. 2013, Beaumont et al. 2013, Leal et al. 2013, Ohnishi et al. 2013, Tanaka & Suzuki 2016, Tanaka & Tokuda 2016, Thompson et al. 2016

44	Myrmicinae	<i>Pheidologeton</i>	NA NT IM PT	Absent	0.05 (0)	Poor	Zingiberaceae	<i>Globba</i>	Pfeiffer et al. 2004
45	Myrmicinae	<i>Pogomyrmex</i>	NA NT	Present (common)	0.49 (0.22)	Poor	Asteraceae, Euphorbiaceae, Papaveraceae, Solanaceae	<i>Cirsium, Datura, Dendromecon, Jatropha,</i>	Carney et al. 2003, Ness & Bressmer 2005, Alba-Lynn & Henk 2010, Aranda-Rickert & Fracchia 2011, Rickert & Fracchia 2010, Aranda-Rickert & Fracchia 2012
46	Myrmicinae	<i>Pristomyrmex</i>	PT AU IM	Absent	0.004 (0)	Poor	Cyperaceae	<i>Carex</i>	Tanaka & Tokuda 2016
47	Myrmicinae	<i>Sericomyrmex</i>	NT	Present (common)	0.05 (0)	Poor	Piperaceae	<i>Peperomia</i>	Youngsteadt et al. 2009
48	Myrmicinae	<i>Solenopsis</i>	WW	Present (rare)	0.1 (0.1)	Poor	Capparaceae, Caricaceae, Euphorbiaceae, Fabaceae, Iridaceae, Marantaceae, Melanthiaceae, Papaveraceae, Passifloraceae, Phytolaccaceae, Polygalaceae, Violaceae	<i>Acacia, Calathea, Capparis, Carica, Cnidioscolus, Croton, Iris, Jatropha, Piriqueta, Polygala, Ricinus, Sanguinaria, Segueira, Stillingia, Trillium, Turnera, Viola</i>	Horvitz 1981, Horvitz & Schemske 1986, Zettler et al. 2001, Bale et al. 2003, Ness 2004, Martins et al. 2006, Gove et al. 2007, Stuble et al. 2010, Dominguez-Haydar & Armbrrecht 2011, Salazar-Rojas et al. 2012, Almeida et al. 2013, Cumberland & Kirkman 2013, Leal et al. 2013
49	Myrmicinae	<i>Stenamma</i>	NA PA IM	Present (common)	0.004 (0)	Poor	Violaceae	<i>Viola</i>	Culver & Beattie 1978
50	Myrmicinae	<i>Temnothorax</i>	NA PA PT IM	Present (rare)	Insufficient data	Poor	Aristolochiaceae, Papaveraceae, Plantaginaceae, Ranunculaceae, Violaceae	<i>Asarum, Chelidonium, Cordyalis, Hepatica, Veronica, Viola</i>	Reinfeinrath et al. 2012, Zhu et al. 2017
51	Myrmicinae	<i>Tetramorium</i>	WW	Present (rare)	0.06 (0.1)	Poor	Asteraceae, Berberidaceae, Boraginaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Hamamelidaceae, Juncaceae, Lamiaceae, Papaveraceae, Polygalaceae, Proteaceae, Ranunculaceae, Rhamnaceae, Rutaceae, Santalaceae, Violaceae	<i>Acacia, Adriana, Anchusa, Bertya, Bossiaea, Carex, Chelidonium, Cirsium, Coleonema, Cordyalis, Dillwynia, Epimedium, Euphorbia, Hardenbergia, Helleborus, Lamium, Leucospermum, Liparia, Luzula, Matudaea, Mimetes, Phylica, Polygala, Prunella, Rhamnus, Senecio, Serruria, Spatalla, Taraxacum, Thesium, Viola</i>	Oostermeijer 1989, Hughes & Westoby 2001, Westoby et al. 1991, Espadaler & Gomez 1996, Andersen & Morrison 1998, Quilichini & Debussche 2000, Christian 2001, French & Major 2001, Garrido et al. 2002, Gomez & Oliveras 2003, Gomez et al. 2003, Scott & Gross 2004, Witt & Giliomee 2004, Dostal 2005, Alcantara et al. 2007, Gove et al. 2007, Manzaneda et al. 2007, Parr et al. 2007, Ohnishi et al. 2008, Beaumont et al. 2009, Lomov et al. 2009, Lomov et al. 2009, Ohnishi & Suzuki 2009, Alba-Lynn & Henk 2010, Fokuhl et al. 2012, Ohnishi et al. 2013, Suetsugu 2015, Tanaka et al. 2015, Tanaka & Tokuda 2016, Zhu et al. 2017

52	Myrmicinae	<i>Trachymyrmex</i>	NA NT	Present (rare)	0.01 (0.001)	Poor	Caricaceae, Celastraceae, Euphorbiaceae, Marantaceae, Annonaceae, Marantaceae	<i>Calathea, Carica, Cnidoscolus, Maytenus, Calathea, Xylopia</i>	Le Corff & Horvitz 1995, Leal et al. 2007, Almeida et al. 2013
53	Myrmicinae	<i>Wasmannia</i>	NT (AU*)	Present (common)	0.02 (0)	Poor	Marantaceae	<i>Calathea, Xylopia</i>	Horvitz & Schemske 1986, Christianini & Oliveira 2010
54	Ponerinae	<i>Bothroponera</i>	PT IM	Absent	0.004 (0.004)	Poor	Fabaceae	<i>Acacia</i>	Andersen & Morrison 1998, Gove et al. 2007,
55	Ponerinae	<i>Brachyponera</i>	PT IMAU (NA*)	Present (rare)	0.01 (0)	Poor	Euphorbiaceae, Fabaceae	<i>Acacia, Euphorbia</i>	Parr et al. 2007, Ohnishi et al. 2008
56	Ponerinae	<i>Diacamma</i>	AU IM	Absent	0.04 (0)	Poor	Zingiberaceae	<i>Globba</i>	Pfeiffer et al. 2004
57	Ponerinae	<i>Dinoponera</i>	NT	Absent	0.16 (0)	Okay	Annonaceae, Euphorbiaceae	<i>Croton, Jatropha, Xylopia</i>	Christianini & Oliveira 2010, Leal et al. 2013
58	Ponerinae	<i>Hypoponera</i>	WW	Absent	0.01 (0)	Poor	Marantaceae	<i>Calathea</i>	Le Corff & Horvitz 1995,
59	Ponerinae	<i>Leptogenys</i>	NT NA PT IMAU	Present (rare)	0.02 (0.01)	Poor	Marantaceae, Zingiberaceae	<i>Calathea, Globba</i>	Horvitz 1981, Pfeiffer et al. 2004
60	Ponerinae	<i>Neoponera</i>	NA NT	Absent	Insufficient data	Poor	Bromeliaceae	<i>Aechmea</i>	Leroy et al. 2016
61	Ponerinae	<i>Odontomachus</i>	NA NT PT IMAU	Absent	0.07 (0.06)	Poor	Annonaceae, Araceae, Bromeliaceae, Capparaceae, Caricaceae, Celastraceae, Clusiaceae, Euphorbiaceae, Fabaceae, Gesneriaceae, Marantaceae, Phytolaccaceae, Zingiberaceae	<i>Acacia, Calathea, Xylopia, Anthurium, Aechmea, Capparis, Carica, Maytenus, Clusia, Cnidoscolus, Codonanthe, Croton, Globba, Manihot, Renealmia, Ricinus, Sequoiaria</i>	Horvitz & Beattie 1980, Horvitz 1981, Le Corff & Horvitz 1995, Orivel et al. 1998, Marini-Filho 1999, Passos & Oliveira 2002, Passos & Oliveira 2002, Martins et al. 2006, Leal et al. 2007, Parr et al. 2007, Zhou et al. 2007, Zelikova & Breed 2008, Garcia-Robledo & Kiprewicz 2009, Christianini & Oliveira 2010, Dominguez-Haydar & Armbrrecht 2011, Almeida et al. 2013
62	Ponerinae	<i>Odontoponera</i>	IM	Present (rare)	0.14 (0.0)	Poor	Zingiberaceae	<i>Globba</i>	Pfeiffer et al. 2004
63	Ponerinae	<i>Pachycandyla</i>	NA NT IM	Present (rare)	0.20 (0.18)	Good	Annonaceae, Araceae, Aristolochiaceae, Bromeliaceae, Caricaceae, Clusiaceae, Euphorbiaceae, Fabaceae, Gesneriaceae, Marantaceae, Piperaceae, Zingiberaceae	<i>Anthurium, Asarum, Aechmea, Carica, Clusia, Ricinus, Acacia, Codonanthe, Calathea, Globba, Peperomia, Renealmia, Xylopia</i>	Horvitz & Beattie 1980, Horvitz 1981, Horvitz & Schemske 1986, Le Corff & Horvitz 1995, Orivel et al. 1998, Orivel & Dejean 1999, French & Major 2001, Passos & Oliveira 2002, Martins et al. 2006, Zhou et al. 2007, Zelikova & Breed 2008, Garcia-Robledo & Kiprewicz 2009, Christianini & Oliveira 2010, Rodriguez-Cabal et al. 2012, Almeida et al. 2013