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Assessing risk to native ecosystems: using exotic ants as a model

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A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy in Biological Sciences
The University of Auckland, 2019

Abstract

Invasive species are widely recognised as drivers of ecological change. As such, understanding the processes that facilitate invasion success, and the potential mechanisms and magnitude of impact, are critical for effective biosecurity management. Predicting risk associated with exotic species, so that management actions can be justified, is a major challenge faced by pest managers.

In this thesis, I present a conceptual framework for assessing ecological risk in natural ecosystems developed from natural hazard (earthquake) risk assessment frameworks, and apply this using exotic ants as a model. To assess the vulnerability of different ecosystems to exotic ant invasion, I replicated a sampling study conducted across the Auckland region in 2004, allowing a temporal aspect to be included in the analyses. I found open-canopy ecosystems, consisting of short stature vegetation, to be most vulnerable to exotic ant invasion with several exotic ant species found to be ubiquitous throughout. In comparison, the same species were generally limited to the edges of closed-canopy forest ecosystems, even after a 10-year period.

I then focused on dietary impact-related research within open-canopy ecosystems. I assessed competition between native and invasive ants using isotopic niche overlap and co-occurrence patterns, as well as employing DNA metabarcoding, to understand what invertebrate taxa are most at risk via predation. Furthermore, I investigated the potential disruption of floral visitor networks by exotic ants through conducting surveys across the flowering plant community and using focal observations to determine whether ants may be competitively excluding important pollinators. I found trophic position varied between native and invasive ant species; although two invasive species represented the two lowest trophic positions, most other species reflected trophic positions similar to predators. There was evidence of competitive exclusion between a native and invasive ant species, as revealed through high isotope niche overlap and negative co-occurrence patterns. Exotic ants were found to be common floral visitors, although this varied spatially. The presence of exotic ants on flowers was associated with a significant, negative response by important insect pollinators.

Overall, my research greatly contributes to the ecological risk assessment posed by exotic species in native ecosystems and contributes to the impact-based research of invasive ants.

Acknowledgements

Contents

Chapter 1 — General introduction

1. 1 Biological Invasions

1.1.1 Overview

Contemporary biological invasions are occurring at an unprecedented rate, and are now considered a leading cause of global biodiversity loss (Maxwell, Fuller, Brooks, & Watson, 2016). Human-mediated introductions of species outside of their native ranges, either intentionally or unintentionally, alter the interactions and functioning of species and ecosystems, occasionally causing major impacts (Mack, Simberloff, Lonsdale, Evans, & Clout, 2000). Although the prevention of future arrivals is a fundamental goal for biosecurity practitioners, risk assessment must account for species that bypass border controls, with necessary actions taken based on perceived threats across various sectors. Prioritisation of management for exotic species is often based on their observed invasion impacts elsewhere; however, drawing parallels from previous examples of invasion for the same, or closely related species, is often difficult as invasion success and impact is context dependent (Moyle & Marchetti, 2006). Determining the characteristics of invasion success and subsequent impact is therefore dependent on not only the species, but also the recipient environment (Fitzgerald, Tobler, & Winemiller, 2016). Due to limited prior knowledge and resources, pest managers must often make decisions based on immediate threat, sometimes leaving exotic species to establish and spread where little information exists.

My research aims to develop an ecological risk assessment framework for pest managers, using exotic ants as a model system. Despite the ~29 exotic ant species considered established within New Zealand (*cf.* 11 native ants), little attention has been given to their pest status and the mechanisms in which they may impact the ecosystems in which they occur. By understanding the environments most likely at risk of invasion, and the potential consequences of ant invasion to native biodiversity and ecosystems, this thesis identifies ecological risk associated with invasive ant species, which may be used to justify future monitoring and/or management actions.

1.1.2 Definitions

The field of invasion biology is afflicted with debate regarding the language and definitions utilised by scientists and practitioners (i.e. Larson, 2005; Valéry, Fritz, & Lefeuvre, 2013).

Indeed, consensus surrounding the definition of the term *invasive species* has still not been achieved, with inconsistencies pertaining to whether invasion biologists include a component of harmful impact within the definition (Ricciardi & Cohen, 2007; Young & Larson, 2011). Despite several proposals for defined terminology relating invasion biology concepts (e.g. Falk-Petersen, Bøhn, & Sandlund, 2006; Richardson, Pyšek, & Carlton, 2010), dialogue regarding the ambiguity and disagreement around definitions, continues to persist (Courchamp et al., 2017).

For this thesis, I adopt the definitions following Falk-Petersen et al., (2006), and refer to introduced species as either 'exotic' or 'invasive'. I use the term 'exotic' for species that have been introduced outside of their native ranges, which may or may not have established, and 'invasive' for species that have established outside of their native ranges and are expanding their range (i.e. exhibiting spread). Therefore, the use of the term invasive does not imply impact *per se* but rather infers a potential for impact that is inherently perception-based and thus difficult to define.

1.2 Biosecurity

1.2.1 Invasive species

Increasing globalisation and trade route expansion has facilitated the movement of species outside their native ranges (Banks, Paini, Bayliss, & Hodda, 2015). Although the majority of species that establish in new environments are considered benign, a small subset may proliferate and spread across landscapes (Williamson & Fitter, 1996). These invasive species can negatively impact the economic, social and environmental sectors with potentially devastating consequences.

Invasive species are causative agents of extensive ecological impacts and are widely considered significant drivers of global biodiversity declines (Courchamp et al., 2017; Mack et al., 2000; Maxwell et al., 2016; Daniel Simberloff et al., 2013). Invasive species threaten native biodiversity and ecosystem functioning via various mechanisms such as competition, hybridisation, predation and disease transmission (Blackburn et al., 2014). Biological invasions cause extensive ecological impacts within the terrestrial and marine environments, including alteration of nutrient cycles, ecosystem service disruption, changes in community structure and species extinctions (Bellard, Cassey, & Blackburn, 2016; Walsh, Carpenter, & Zanden, 2016). However, because invasiveness is not consistent between invasion scenarios, predicting the

potential impacts of invasive species is difficult. For instance, a species may have different invasion success at different sites, which suggests that site-specific biotic and abiotic characteristics play an important role in determining a species persistence within a novel environment, as well as their mechanisms and magnitude of impact (Miller, Kneitel, & Burns, 2002; Schoener & Spiller, 1995).

1.2.2 Risk assessment

Due to the wide-ranging effects of invasive species, predicting, understanding, and managing the risk and impacts of biological invasions are central to invasion biology. The term 'biosecurity' relates to the system of preventing and managing risks from harmful organisms such as pests and diseases (Ministry for Primary Industries, 2016). Preventative biosecurity practices can and do fail under some circumstances, therefore biosecurity measures must encompass both the pre-border systems and post-border risk mitigation. At the national level, biosecurity measures are the responsibility of government agencies. However, international conventions may set standards to which partnership nations must adhere to, or face substantial fines and immediate disruption of trade. In addition, many species are already established but have not yet reached the spread stage of invasion. This represents 'invasion debt', and risk of established species spreading is an important component to consider in future pest management strategies (Rouget et al., 2016). Risk assessment is used to form frameworks and policies to manage risks associated with both potential pathways of arrival and post-border invasion debt (McGeoch et al., 2016; Ward, Stanley, Toft, Forgie, & Harris, 2008).

Risk assessments evaluate the potential risk associated with an exotic species through either quantitative, semi-quantitative or qualitative methods; they are widely employed across sectors and used as pest management prioritisation tools. The unique characteristics associated with both the arriving species and the recipient environment influence invasion outcome (Lockwood, Cassey, & Blackburn, 2005; Theoharides & Dukes, 2007; Williamson & Fitter, 1996). However, this context-dependent spread and impact outcome makes predicting the risk associated with newly established species difficult. Thus, risk profiles are not easily transferred between invasion scenarios. Although analytical capabilities for assessing invasive species risk are often well developed, especially in terms of control/eradication costs and forecasted economic losses, the risk of potential ecological impacts is more difficult to predict and quantify (Ricciardi, Hoopes, Marchetti, & Lockwood, 2013).

1.3 Invasive ants

1.3.1 Ants as successful invaders

Globally, ants are a widespread fauna, consisting of over 15,000 described species, and range across all continents excluding Antarctica (Béhague et al., 2018). They constitute a significant proportion of terrestrial biomass and represent a dominant fauna in most terrestrial ecosystems (Hölldobler & Wilson, 1990). Aside from one of their characteristic traits, eusociality, the success of ants can be largely attributed to niche divergence leading to ecological diversity and the evolution of distinct functional roles (Hölldobler & Wilson, 1990). A direct result of such diversity is the substantial role they play in ecological interactions, shaping the communities and ecosystems in which they occur (Folgarait, 1998).

The same traits that led ants to be one of the most dominant taxa on the planet have also facilitated their success as invasive species. However, of the ~ 240 species reported as established outside of their native range, only a subset are considered 'invasive' (Bertelsmeier, Ollier, Liebhold, & Keller, 2017). For many other exotic ant species, the term 'tramp' is commonly applied. This term relates to species whose expansion into new regions has been facilitated through accidental human-mediated movement and that are generally restricted to occurring in human-modified habitats within their extended ranges (McGlynn, 1999; Passera, 1994). Due to the potential harmful impacts associated with invasive ants, determining the predictive traits of invasion success has been subject to extensive research (Holway, Lach, Suarez, Tsutsui, & Case, 2002; Lester, 2005; Ward, Stanley, Toft, Forgie, & Harris, 2008), and indeed predicting risk is a cornerstone of invasion ecology (Pheloung, Williams, & Halloy, 1999; Pyšek & Richardson, 2008; Rejmanek & Richardson, 1996; Sutherland, 2004).

Various multispecies comparative studies have assessed common traits among species to understand factors that facilitate invasion for ants (Harris & Barker, 2007; Holway et al., 2002a; Lester, 2005; McGlynn, 1999; Sutherland, 2004; Ward et al., 2008). Despite invasion success and impact clearly being context dependent, there are some general characteristics that are likely to provide an invasive advantage (Rabitsch, 2011). For instance, unicoloniality (i.e. polygynous, having multiple queens), coupled with decreased intraspecific aggression appears frequently across 'highly invasive' species. Indeed, unicoloniality is thought to be a driving factor in the success of *Monomorium pharanosis*, *Linepithema humile, Wasmannia auropunctata* and *Pheidole megacephala* as invaders, facilitating their numerical dominance

(Hölldobler & Wilson, 1977; Holway et al., 2002a). Together, unicoloniality and the absence of intraspecific aggression promote the formation of large 'supercolonies' for some species, such as the Argentine ant (*L. humile*) (Tsutsui, Suarez, Holway, & Case, 2000), whose colonies can span areas up to 6000 km within their invaded range (Giraud, Pedersen, & Keller, 2002). Although these traits may directly translate to increased negative impact, a suite of other general traits found to promote ant species' success at various stages of the invasion process can be helpful in identifying invasion and impact potential (Lester, 2005; McGlynn, 1999).

1.3.2 Ecological impacts of invasive ants

Invasive ants can be agents of significant environmental and ecological change (Holway et al., 2002a). The mechanisms of impact are highly variable and species dependent, occuring via both direct and indirect interactions with recipient biodiversity and ecosystems. Despite the > 200 ant species established outside of their native ranges, two species, the Argentine ant (*L. humile*), and red imported fire ant (*Solenopsis invicta*), account for the majority of empirical research on invasive ant impacts and are considered model organisms in invasion biology (Pyšek et al., 2008).

Among the research investigating the ecological impacts of invasive ants, their influence on native arthropod communities has received substantial focus. Specifically, the response of native ant communities to invasion has been widely documented, albeit for only a subset of ants considered 'highly invasive' (Breton, Chazeau, & Jourdan, 2003; Hoffmann, Andersen, & Hill, 1999; Holway, 1999; Human & Gordon, 1996; Inoue et al., 2015; Masse et al., 2017; Porter & Savignano, 1990; Rowles & O'Dowd, 2009; Sanders, Barton, & Gordon, 2001; Zina, Branco, & Franco, 2017). Local ant communities are largely impacted via predatory and competitive behaviour of invasive ants, which are usually numerically and behaviourally dominant, leading to the displacement of their native counterparts (Holway, 1999; Human & Gordon, 1996; Inoue et al., 2015; Masse et al., 2017; Rowles & O'Dowd, 2009; Sanders et al., 2001; Zina et al., 2017). Multiple studies have demonstrated that the epigeic native ant community suffers in response to Argentine ant invasions, and have reported community richness and/or abundance to be lower at invaded sites when compared to uninvaded sites (Cooling, Sim, & Lester, 2015; Holway, 1999; Human & Gordon, 1996; Inoue et al., 2015; Rowles & O'Dowd, 2009; Suarez, Bolger, & Case, 1998). In most cases, invasive ants do not fulfil the functional role of the native ants they displace, such as seed dispersal and harvesting, leading to loss of ecosystem function (Carney, Byerley, & Holway, 2003; Christian, 2001;

Devenish, Gómez, Bridle, & Sumner, 2018). Many other studies have assessed effects of invasive ants on the wider invertebrate community, with different taxa exhibiting both/either positive and negative responses to ant invasion (Cole, Medeiros, Loope, & Zuehlke, 1992; Cooling, Sim, & Lester, 2015; Stanley & Ward, 2012; Walters, 2006). The wider implications are the indirect effects associated with invertebrate community change, which can also alter ecosystem functioning (Lavelle et al., 2006; Stanley & Ward, 2012). For instance, invasive ants have a high potential to disrupt pollination services via antagonistic interactions with floral visitors and may impact plant and pollinator communities (Lach, 2007, 2008; Sidhu & Wilson Rankin, 2016).

Invasive ants may also impact vertebrate taxa at the population-level, especially ground-dwelling and ground-nesting species, through highly aggressive behaviour and by placing significant predation pressure on eggs and young (Thawley & Langkildeb, 2016). For instance, some seabird species are at risk from yellow crazy ants (*Anoplolepis gracilipes*), which cause fewer active burrows and poorer chick condition within invaded habitats (Plentovich, Russell, & Fejeran, 2018). The red imported fire ant (*Solenopsis invicta*) causes avian nest mortality (Drees, 1994; Mueller, Dabbert, Demarais, & Forbes, 1999), and is associated with reductions in the abundance and richness of herpetofauna communities (Allen, Birge, Slater, & Wiggers, 2017). In contrast, despite reports of Argentine ants as nest predators, evidence suggests a negligible impact on the nesting success of birds (Boieiro et al., 2018; Suarez, Yeh, & Case, 2005). Once again, this highlights the variability in the magnitude of impact for different invasive ant species.

1.3.3 Economic impacts and invasive ant management

The economic cost associated with invasive ants can be substantial. In the United States, the red imported fire ant is estimated to cost US$6 billion annually in damage, repair and medical costs (Drees & Lard, 2006), and in Australia, the predicted annual costs exceed AU$1.65 billion (Wylie & Janssen-May, 2017). Cost-benefit analyses support investment in the control and containment of invasive ants. For example, an investment of US$40 million allocated to the management of the little fire ant (*Wasmannia auropunctata*) in Hawai'i is projected to save US$6 billion over 35 years through a reduction of control costs and the economic damages associated with this species (Lee, Motoki, Vanderwoude, Nakamoto, & Leung, 2015).

The high monetary and ecological cost of invasive ants has justified management programmes ranging from control to eradication (Hoffmann, Luque, Bellard, Holmes, & Donlan, 2016). In their recent review, Hoffmann et al. (2016) assessed 316 ant eradication campaigns, finding that eradication attempts usually targeted small infestation areas considered 'site-specific', with 144 of these considered successful. They found factors most likely to predict eradication success were the active ingredient, attractant type, and method of deployment. Whilst there has been an increase in publications relating to ant management in recent years (Boser et al., 2014; Hoffmann, 2015; Inoue et al., 2015), to progress invasive ant management, effort must be made to continually disseminate the results of successful and unsuccessful management in the literature through open-access publications and databases.

1.3.4 Exotic ants in New Zealand: a case study

The exotic ant fauna of New Zealand comprises of at least 29 species, represented by six subfamilies (Don, 2007). In contrast, there are only 11 native ant species, all of which are endemic. The majority of exotic species are Australian in origin, reflecting their 'tramp' status, linked through established trade between the two countries (Don, 2007). Taxonomic ambiguity persists for several established species and the basic biology for many is poorly understood. The potential for new introductions is high, and ants are frequently intercepted at the border (Ward, Beggs, Clout, Harris, & O'Connor, 2006). Mitigating the risk of ant introductions to New Zealand requires pre- and post-border measures; however, as ants are considered 'hitchhikers', biosecurity measures must consider the pathways of highest risk, rather than associations with commodities (Rabitsch, 2011). In response to the risk posed by exotic ants, the Ministry for Primary Industries, which governs biosecurity at a national level, developed the National Invasive Ant Surveillance Programme (NIAS). The programme primarily aims to detect newly established species at high-risk sites around New Zealand (i.e. ports and transitional facilities), as well as identify changes in the distribution of ants already established (Craddock & Mattson, 2014).

Despite the reported ~29 species persisting within the New Zealand environment, published literature investigating ecological impacts exists for only *L. humile* and *Monomorium sydneyense* (Stanley et al., 2013; Stanley & Ward, 2012; Stringer & Lester, 2008). Stanley & Ward (2012) found that Argentine ants altered the invertebrate and decomposer communities at sites they invaded, suggesting a potential 'bottom-up' impact, which could have implications at the wider ecosystem scale. Furthermore, detailed surveys across various ecosystems in New

Zealand showed that *L. humile* abundances negatively correlated with ant species richness (Cooling et al., 2015; Ward & Harris, 2005), which mirrors a global trend associated with *L. humile* invasion (Holway, 1998; Oliveras, Bas Lay, Casellas, & Gómez, 2005). Stringer & Lester (2008) examined the response of the ant community (comprising both native and exotic species) to *M. sydneyense* within a human-modified habitat around Tauranga Port. Using a comparison between invaded and non-invaded areas, their findings suggest that this species is able to coexist with—and thus have minimal effect to—the resident ant community.

The overwhelming body of international research detailing the negative ecological impacts of *L. humile* within its invaded range justifies its status as a pest species throughout New Zealand (e.g. Blancafort & Gómez, 2005; Carpintero, Reyes-López, & Arias de Reyna, 2005; Devenish et al., 2018; Hanna et al., 2015; Holway, 1999; Lach, 2007; LeVan, Hung, McCann, Ludka, & Holway, 2014; Menke, Ward, & Holway, 2018; Park, Hosoishi, & Ogata, 2014; Rowles & O'Dowd, 2009). There is evidence that *L. humile* populations may be decreasing in New Zealand (Cooling, Hartley, Sim, & Lester, 2012), or are at least transient in their local distribution (Stanley & Ward, 2012). However, continued management of this species is likely to persist as a priority, particularly in light of recent evidence that this species threatens the New Zealand apicultural industry (Sébastien et al., 2015).

Currently, only two ant species established in New Zealand are included under regional council pest management strategies within New Zealand. For instance, the Argentine ant has been the subject of eradication programmes on islands within the Hauraki Gulf (Ward, Anderson, & Barron, 2016). Additionally, Darwin's ant (*Doleromyrma darwiniana*), a species native to Australia, is listed as a pest species in pest management strategies throughout New Zealand. This species' invasion status is unique in that New Zealand is the only area it inhabits outside of its native range (Don, 2007). Despite no evidence of ecological or economic impact for Darwin's ant, due to the perceived similarity to Argentine ants, this species is included in surveillance programmes and has been categorised as a high risk species. Risk profiles for ants present (or likely to arrive) in New Zealand were determined via a scorecard developed by Ward et al. (2008); however, this method of risk assessment has little weighting on impact. Rather, it focuses more on the likelihood of arrival and establishment for different species. The relative absence of knowledge on the impacts of established exotic ants in New Zealand presents a major knowledge gap for pest managers, necessitating an impact assessment to determine if additional ant species should be included as priority pests requiring surveillance or management.

1.4 Research aims and thesis layout

This thesis investigates ecological risk assessment by developing a conceptual risk assessment framework and applying its components to exotic ants that have spread into natural ecosystems. Exotic ants established in New Zealand represent an ideal case study to investigate and apply risk assessment concepts. Very little research has focused on how the occurrence of exotic ants influences native biodiversity and ecosystems despite the capacity for some ant species to cause negative impacts.

Therefore, the overarching aim of this thesis was to review the literature on risk assessment and develop a framework for assessing ecological risk and then apply components of this framework to exotic ants as a model taxon. My first objective was the development of a risk assessment framework that integrates both the exotic species and the recipient environment, thereby providing a systematic method to formulate evidence-based management decisions for pest species. My second objective was to determine the habitat types that are most vulnerable to exotic ant invasion and investigate the mechanisms by which exotic ants cause impacts.

This thesis is presented as a set of self-contained chapters that may be read independently, intended for publication. As such, there is some repetition, but where possible this has been minimised.

The thesis is structured as follows;

Chapter 2 presents a novel, conceptual framework for assessing the ecological risk to natural ecosystems by exotic species. This framework is presented in five modules, based on current disaster risk frameworks (Reese, King, Bell, & Schmidt, 2007; Schmidt et al., 2011), and emphasises the importance of integrating the characteristics of both the species and the recipient ecosystems for risk prediction. The framework is specifically designed for use by pest managers, who often have limited information and resources available, to enable justifiable pest management decisions. Although a case study on the Argentine ant is presented to illustrate the framework, the framework is adaptable and has a wide application to those working on invasive species management worldwide. Once the framework was conceived, it

was used to develop research ideas and provide context for research directions for the following chapters.

Chapter 3 begins to apply the risk framework presented in Chapter 2, by investigating the invasibility of different ecosystems by exotic ants. I test whether a key ecosystem trait (canopy type) influences the invasion of exotic ants by replicating an ant occurrence survey in different ecosystems (tall vs low stature vegetation) 10 years after initial sampling. The results of the initial study in 2004 are compared to my results from 2014, with the resulting spatial and temporal trends explored within the context of invasion biology.

Chapter 4 continues applying the risk framework presented in Chapter 2 by investigating the potential dietary impact of invasive ants. Using stable isotope and molecular (metabarcoding) techniques, the isotopic signatures of ants, and the DNA amplified from gut contents, I infer dietary breadth and predict risk associated with feeding habits through competition with native ant species and predation of the arthropod community within natural ecosystems.

Chapter 5 continues with the investigation of impact by invasive ants, with a focus on impact through the disruption of pollination. I construct floral visitor networks to determine which species of ants commonly exploit floral resources, and conduct focal observations on natural and manipulated artificial flowers to test whether ant presence alters floral visitor behaviour.

Chapter 6 provides an overall discussion and synthesises the findings in the context of the risk assessment framework, highlighting management implications (the final module of the framework). Although the most direct application for this is for the Auckland Council, the results may be useful for other regional councils, as the risk framework has broad applications for ecological risk assessment under various invasion scenarios.

Chapter 2 — Conceptual risk framework: integrating ecological risk of introduced species with recipient communities

Abstract

Global changes, such as increasing trade and climate change, are predicted to facilitate the introduction, establishment and spread of species into new environments, leading to negative impacts on local biodiversity. There is an increasing need to evaluate risk before a species arrives (pre-border), but also the risk to specific natural ecosystems once a species has established (post-border). The use of trait-based risk assessment is well established when assessing ecological risk, but a major shortfall is that it does not consider the recipient environment, which will influence the likelihood of establishment and the magnitude of the impact of an introduced species. Here I outline a step-wise framework to provide a basis for assessing the ecological risk of introduced species in order to facilitate justifiable management decisions. It consists of five modules, which have been adapted from generalised natural disaster (earthquake) risk assessments. The framework integrates information based on both the species and the (potential) recipient ecosystems, utilising existing tools to guide pest managers through the step-wise process. The framework enables the prediction of high-risk species and the identification of those ecosystems most vulnerable to invasion and facilitates understanding of the potential mechanisms and magnitudes of pest impacts. Social and cultural values can be incorporated to effectively inform overall management and restoration decisions. This conceptual framework integrates species and ecosystem information to produce a more comprehensive system for ecological risk assessment. The framework can be applied to different invasion scenarios to evaluate the risks and impacts of 'risk' species.

2.1 Introduction

2.1.1 Invasive species

Biological invasions are considered to be one of the major threats to biodiversity (Maxwell et al., 2016). Introduced species have the potential to impact the economic, social and environmental sectors, which means biosecurity prevention measures are of key importance for policy and management. For this reason, invasion ecology has been a rapidly growing area of research, with findings affecting policy and legislation at both the local and the global scale (Genovesi, Carboneras, Vilà, & Walton, 2015; Rayment, 2006).

2.1.2 Risk assessment

Risk assessments are important prioritisation tools employed in biosecurity and pest management to provide a measure (qualitative or quantitative) of risk associated with the arrival of species to a region outside of their natural range (Fuentealba, Alfaro, & Bauce, 2013; Kolar & Lodge, 2002; Pheloung et al., 1999). Although the likelihood of a species establishing outside its native range is generally low (Williamson & Fitter, 1996), historical introductions have demonstrated the devastating effects introduced species can have in their new range (Brockerhoff et al., 2010; Harper & Bunbury, 2015; Kenis et al., 2009). The risk of invasion impacts has driven the development of risk assessment before an introduced species arrives (pre-border), enabling the identification and prioritisation of high-risk species and pathways so that preventive measures can be used to reduce the risk of introduction and strategies can be developed to manage the species if it does arrive (Leung et al., 2012; Moles, Gruber, & Bonser, 2007).

A high level of uncertainty is associated with the various stages of the invasion pathway (arriving, establishing, spreading). The ability to confidently predict and quantify the risk of a species and the potential magnitude of impact is often difficult due to the lack of a standard metric and existing baseline data (Leung et al., 2012). In some cases, the species may be unstudied in its native range and not introduced elsewhere. Lag phases may also make it difficult to detect impact until the species is widespread across a landscape, leading to some management decisions (such as eradication) becoming unachievable (Aikio, Duncan, & Hulme, 2010; Crooks, 2005). In addition, the introduction of a species to a new ecosystem may lead to unique interactions between the species and the recipient biodiversity that can be difficult to predict *a priori* (Green et al., 2011; O'Dowd, Green, & Lake, 2003).

Difficulties are particularly apparent when assessing ecological risk, especially if knowledge and existing risk models do not easily transfer between ecosystems. For this reason, risk assessments for predicting invasion and impacts in natural ecosystems are frequently based on expert opinion. As a result, species are often classified as having a low risk when information on invasion and impact is lacking (i.e. there are no comparative data for previous impact in a similar ecological context). This can lead to underestimation of risks associated with introductions. In contrast, productive systems (e.g. crops, horticulture) are far more similar around the globe, often with the same few plant species dominating the ecosystem (e.g. *Pinus radiata* forestry, corn (*Zea mays*) fields), making the impacts of specific introduced species easier to predict. Predictions of invasion and impacts can be even more difficult in regions where natural ecosystems contain high endemism and unique species interactions.

2.1.3. Trait-based risk assessment

Previous research on—and risk assessments of—invasive species have favoured evaluating the biological traits of a species as a predictive method to understand potential risk relating to establishment and impact (Grabowska & Przybylski, 2015; Lester, 2005; Marchetti, Moyle, & Levine, 2004; Pheloung et al., 1999; Rejmanek & Richardson, 1996; Sutherland, 2004; van Kleunen, Weber, & Fischer, 2010). This concept of 'risky traits' has been employed to model risk associated with different weed species, although only a small number of these models are widely applied (Pheloung et al., 1999; Weber & Gut, 2004). Of these models, the most commonly used is the Australian Weed Risk Assessment (AWRA), a score-based model for identifying plants that should be banned for importation based on their risk of becoming invasive (Pheloung et al., 1999). The AWRA has been adapted for use in several countries (Crosti, Cascone, & Cipollaro, 2010; Daehler, Denslow, Ansari, & Kuo, 2004; Daehler & Carino, 2000; Gordon, Onderdonk, Fox, & Stocker, 2008; Kato, Hata, Yamamoto, & Yoshioka, 2006; Krivanek & Pysek, 2006). As a result of its widespread application, the AWRA provides a relatively rare example of a pre-border risk assessment model that has been frequently tested and subsequently improved. However, risk assessments that primarily assess traits that may promote the establishment and spread often omit impact, or only assess impact very broadly, such as classifying the likelihood of impacting production or natural ecosystems (Ward et al., 2008). Since there is no fine-scale differentiation among different ecosystems types, or accountability for uncertainty (Sheppard, Burns, & Stanley, 2016), the validity of risk assessments has been questioned (Hulme, 2012).

The use of trait-based impact assessment is similar to that used in other sectors, such as the insurance industry, which often uses a form of 'class approach', whereby risk is assessed for classes or types of interactions rather than for each unique individual. Applying the insurance industry approach to assessing the risk of introduced species based on classes (e.g. plant families or insect functional groups) becomes difficult in natural ecosystems where each interaction between the introduced species and native species is likely to be novel and untested. This major gap prohibits managers from making predictions on a finer scale, particularly for types of endemic natural ecosystems, nor does it enable land managers to make post-border biosecurity decisions on a small scale (such as where surveillance and management should be targeted).

2.1.4. The need for integrative risk assessment

The approach of integrating both species and recipient community information to assess ecological risk allows for a more comprehensive level of risk assessment. While this method is widely used for assessing risk to productive landscapes (where risk is usually quantified in terms of potential economic loss), ecological risk assessment generally relies on the trait-based approach. By considering both the introduced species and the ecosystem, uncertainty relating to the likelihood of establishment, spread and impact can be better accounted for.

The need to evaluate invasion risk will undoubtedly increase. Global changes (e.g. increasing trade and climate change) are predicted to facilitate the spread of introduced species, and species' ranges may change in response (Chen, Hill, Ohlemueller, Roy, & Thomas, 2013; Rahel & Olden, 2008). A formalised risk assessment framework that includes both pre- and post-border risk assessment and predicts ecological threats posed by introduced species offers a practical tool for managers to prioritise introduced species management. I present a new framework (Fig. 1) for assessing the risk posed to ecosystems by introduced species, which I have adapted from generalised natural disaster frameworks (Reese et al., 2007; Schmidt et al., 2011). After examining risk frameworks from other disciplines (e.g. insurance, financial, population health), I found that a natural disaster framework aligned best with introduced species risk assessments due to the integration of the hazard and asset characteristics, where unpredictable frequency and scale of event can be accounted for to predict the magnitude of impact and guide management and restoration decisions. Consisting of five modules (Fig. 1), the framework integrates the hazard (introduced species instead of a natural disaster) with the assets (natural ecosystems instead of building infrastructure) to determine which ecosystems

are particularly vulnerable and exposed (ecological impacts instead of infrastructure damage), based on various characteristics of species and ecosystems. The framework utilises existing ecological assessment tools, and incorporates them into a step-wise system to facilitate management prioritisation and response, factoring in social perceptions of values and available resources.

2.2 The risk framework

2.2.1 Hazard: introduced species

Know your species

Identification and parameterisation of the hazard—in this case, the introduced species—is the first step in risk assessment. The hazard species may arrive into a region via a number of pathways (Hulme, 2009), including intentionally introduced species (such as biological control agents) and unintentionally introduced species. However, pathway analysis is considered a different process and is not covered within this framework.

Basic information required to assess risk includes correct taxonomic identification, information on current and potential distributions, and ecological function. For some species, this is difficult because they are considered cryptogenic, with little or no information on their distribution elsewhere, or even of their native range (Cowie, 2001). In these cases, the use of molecular diagnostic tools may be useful to identify the species and the source population (Avery, Fonseca, Campagne, & Lockwood, 2013). Although accurate identification is often necessary to understand the initial pathways of the species and prevent reintroduction, comparison to a closely related or functionally similar species may be appropriate for assessing risk in terms of potential impact (the insurance industry 'class' approach).

Once a hazard species has been identified, there are two key aspects that have an extremely important role in further assessment: species biology/traits, and distribution.

Species biology/traits

Evaluating the potential of an organism to impact a new environment is an important part of risk assessment. Functional traits are considered to be the underlying biotic component of how species affect ecosystem processes (Cadotte, Carscadden, & Mirotchnick, 2011; Gagic et al., 2015; Hillebrand & Matthiessen, 2009) and can be used to understand the role of organisms in ecosystems. More recently, trait-based research on invasive species, particularly plants, has

a

| HAZARD — Earthquake | ASSETS — Infrastructure |

↓

EXPOSED ASSETS
Vulnerability

Exposure risk + priority/value

↓

DAMAGE STATE
Impact

(Magnitude)

↓ *Prioritisation of resources*

MANAGEMENT AND RESTORATION
Decision considers,
• Magnitude of impact
• Social/cultural values
• Resource availability

b

| HAZARD — Introduced species | ASSETS — Ecosystems |

Traits
SDMs
Scorecards

Ecosystem
prioritisation
(e.g. zonation)

↓

EXPOSED ASSETS
High value ecosystem
vulnerable to invasion
Exposure risk + priority/value

↓

DAMAGE STATE
Impact
(Mechanism and extent of
environmental change)

↓ *Prioritisation of resources*

MANAGEMENT AND RESTORATION
Decision considers,
• Value of ecosystems
• Impact
• Social/cultural perspectives
• Resource availability

Figure 1 – (a) A generalised framework for assessing risk of natural disasters (e.g. earthquakes), based on exposed assets and predicted extent of impact, and (b) an adapted risk framework applied to introduced species (HAZARD). Prioritisation of ecosystems (ASSETS) can be conducted through various methods, such as Zonation, a publically available spatial conservation planning tool (Moilanen et al., 2005). Using this information, priority ecosystems most at risk of exposure to the introduced species can be identified (EXPOSED ASSETS), allowing predictions of the potential mechanisms of impact (i.e. competition, predation, ecosystem service disruption) to determine the possible magnitude of impact (DAMAGE STATE). This then informs MANAGEMENT AND RESTORATION decisions, which factor in ecosystem value, potential impact, social-cultural perspective and resource availability. **Definitions:** Species distribution models (SDMs): used to predict a species' distribution across a landscape pre- or post- establishment; scorecards: a methodology that allocates values to risk, allowing prioritisation of species based on rank; Zonation: spatial priority software used to support decision-making relating to spatial conservation planning.

focused on identifying functional characteristics and may provide a better basis for more accurately predicting invasion success (Funk, Standish, Stock, & Valladares, 2015; Gallagher, Randall, & Leishman, 2015; te Beest, Esler, & Richardson, 2015).

Species distribution

Accurate risk assessment requires information on a species' current and potential range. Baseline information on the species' distribution elsewhere (native and invaded range) can be used to model the fundamental niche in the recipient environment. These models, commonly referred to as species distribution models (SDMs) and ecological niche models (EDMs), provide a valuable tool for making species distribution predictions. They have been widely used to investigate the predicted expansion of invasive species' ranges (Peterson, 2003; Peterson & Scachetti-Pereira, 2004; Villemant et al., 2011; Ward, 2007), and are being increasingly used to investigate invasive species' potential range shifts under various climate change scenarios (Hill, Axford, & Hoffmann, 2014; Sheppard, Burns, & Stanley, 2014; Tonini, Divino, Lasinio, Hochmair, & Scheffrahn, 2014; West et al., 2015).

Distribution patterns may also be used to determine whether the surrounding ecosystem types may promote or impede the establishment of the species based on trends in their presence/absence, or their density. For instance, many invasive species exhibit 'edge effects', whereby they occur in much higher densities at the periphery of an ecosystem, declining in abundance towards the interior. Information on present distribution as well as the ability to forecast the potential expansion in distribution will also enable managers to establish surveillance programmes, allowing for a more rapid response if the introduced species is newly detected in an area (Stanley & Bassett, 2015).

2.2.2 Assets: ecosystems

Ecosystem-based risk assessment

The next module of the framework identifies and characterises the ecosystems (assets) for the region/country for which risk is being predicted. The framework is suitable for use at both smaller regional scales and larger geographical scales; however, high- to medium-level resolution of data on the geographic distribution of different ecosystem types within the region is required for informed decision-making. For primary production ecosystems, authorities often hold distribution maps for particular agricultural and horticultural land-use types (e.g. specific crop maps for a region). Increasingly, natural ecosystems are being spatially mapped at the

regional or country scale, with most regions already having some type of ecosystem classification system (Singers & Rogers, 2014; Williams, Wiser, Clarkson, & Stanley, 2007). Once the geographic distribution of ecosystems is known, they can be prioritised based on their conservation value. Although this is inherently value-driven, with 'value' depending on the user's perspective, prioritisation does allow for the consideration of ecological factors (e.g. the presence of threatened species) and, in some cases, social, cultural and economic factors.

Several tools already exist for ecosystem prioritisation, which can help to identify sites that may be considered high-value conservation sites and therefore could be integrated into the framework. For example, the publically available software Zonation can be used for spatial conservation planning, facilitating the identification of areas important for retaining habitat quality and connectivity for multiple species (Moilanen, 2007; Moilanen et al., 2005; Moilanen, Wintle, Elith, & Burgman, 2006). Zonation has been used in conservation planning (Di Minin et al., 2013; Wan, Wang, Han, & Yu, 2014) as well as for assessing whether existing protected areas are capturing the true landscapes of conservation importance for different taxa (Dunn, Buchanan, Stein, Whittingham, & McGowan, 2016; Klorvuttimontara, McClean, & Hill, 2011; Tognelli, Abba, Bender, & Seitz, 2011). Therefore, this type of tool can be utilised to determine an ecosystem's value, enabling managers to rank different ecosystem types and prioritise them for pest species' exclusion and ecosystem enhancement.

Ranking ecosystems based on attributes can help to identify high-value conservation sites. At a global scale, this is demonstrated through the International Union for Conservation of Nature's *Red List of Ecosystems*, which has been developed to assess the conservation status of various ecosystem types, ranking them from 'Critically Endangered' to 'Least Concern' (IUCN, 2016). This provides a global standard that can be used to inform legislators and policymakers regarding the future use and management of these ecosystems. Such an approach is also used at more local scales, where ranking ecosystems based on conservation values provide a way for managers to prioritise sites for active management under resource constraints (e.g. the Natural Heritage Management System, developed by the New Zealand Department of Conservation).

Factors considered during the prioritisation stage may include ecosystem type, presence of threatened species, cost of long-term management, and where the greatest conservation gains can be made in order to identify 'high value' sites. The approach of using ecosystem-based risk assessment allows for conservation at a wider scale, ensuring important species interactions

and ecological processes can be considered (Keith, 2015). However, incorporating the social and cultural values of sites is more difficult in many regions and is likely to require expert opinion and local indigenous traditional knowledge. While these values should be considered when ranking ecosystems, they are more strongly incorporated in the final decision-making process (see module 5: Management and restoration).

2.2.3 Exposed assets: estimating vulnerability

Vulnerability is a function of risk of exposure to the hazard (introduced species) and the priority or value of the asset (ecosystem). Vulnerability assessment can be based on qualitative, semi-quantitative or quantitative data through various forms of risk analysis. Tools that already exist for vulnerability assessment include score-based semi-quantitative systems that help to predict various likelihoods relating to the invasion process (D'hondt et al., 2015; Nentwig, Kühnel, & Bacher, 2010; Ward et al., 2008). In some cases, it may be possible to apply or adapt pre-existing tools for the introduced species as a way to assess vulnerability.

Assessing the vulnerability of an ecosystem to a potential introduced species can be considered both temporally and spatially. At the broadest scale, the framework requires characterising key ecosystem traits—such as vegetation type, canopy cover percentage and available resources—to assess the suitability of the habitat for the introduced species. By identifying key ecosystem characteristics that may facilitate or impede the establishment of the hazard species, managers can identify and prioritise sites of high risk. Determining what characteristics are important predictors in a species' ability to thrive within an ecosystem requires an understanding of the biological community within the ecosystem and a trait-based prediction of how the introduced species may interact with specific ecosystems. This allows predictions, based on the introduced species' traits, of how it may interact within the environment. For instance, broad-scale ecosystem traits such as open versus closed canopy may be useful initial characteristics to include in site-specific assessment.

Integrating the information gained through the species and ecosystem modules allows for finer-scale predictions of vulnerable priority ecosystems. This is achieved by coupling the information on an introduced species' potential distribution using SDMs with the priority ranking of the ecosystem allocated after assessing biodiversity/ecosystem occurrence. Integrating information in a step-wise approach—from satellite land cover information geographic information system (GIS), SDMs, and field-collected data on habitat preferences, occurrence and density—a fine spatial resolution of ecosystem types most vulnerable to

invasion can be identified. This step-wise integrated approach provides a useful tool for predicting and prioritising sites for biosecurity surveillance (if not management). This is particularly useful as a predictive tool for managers in scenarios that involve assessing the risk of species that have not yet been introduced, because the information can be used for decision-making in the rapid-response stages of incursion in order to concentrate resources in areas of highest risk.

2.2.4 Damage state: impact

Mechanism and extent of environmental change

Once the vulnerability of ecosystems has been estimated, an attempt at predicting impact must be made, including both the mechanism(s) and effects (*sensu* Parker et al. 1999) by which species may impact ecosystems, and the magnitude of those impacts. Although the ideal approach is to understand the mechanisms of impact and their magnitude for all introduced species, this is not possible because the effects of each potential invasion will often be context dependent. Furthermore, often research cannot be carried out robustly (i.e. lack of replication) or ethically on new incursions, because species in the earlier stages of invasion frequently have low impact (Hulme, 2009; Mack et al., 2000). Despite this, predictions can be made based on (1) expert opinion, (2) impact in other contexts, and (3) sister taxa / similar functional groups (the insurance industry 'class' approach).

The use of expert opinion is particularly useful when information is lacking and time constraints limit the ability to collect empirical data. Introduced species are managed and monitored in various areas worldwide using expert opinion, although the information is frequently not published in peer-reviewed journals. Although the methodology selected in eliciting expert information can introduce biases, there are robust ways of extracting and applying robust expert knowledge available in the literature (Drescher et al., 2013; Martin et al., 2012). The use of expert opinion in assessing the distribution, impact and management of pests has been recently demonstrated for the feral pigs (*Sus scrofa*) in Argentina (Ballari, Cuevas, Cirignoli, & Valenzuela, 2015). In this case, eliciting knowledge from managers working in protected areas has provided a comprehensive understanding of feral pigs on a wider scale, enabling management recommendations for high-value conservation sites at a national level (Ballari et al., 2015).

Understanding the potential mechanisms of impact by comparison with other contexts (i.e. when the introduced species has already established outside of its native range, and documented accounts of interactions and impact are available) provides a useful basis for predicting potential risk elsewhere. The mechanisms by which introduced species may impact a new environment provide a basis for assessing risk, which facilitates further predictions of potential magnitude. For instance, Traveset and Richardson (2006) outline the potential mechanisms through which invasive species may disrupt plant reproductive mutualisms. Understanding the role of the introduced species in its native environment, and using the information available on the mechanisms of impact, can help to identify the potential role the introduced species may play in the new environment. For example, the density of colonies (abundance *sensu* Parker et al. 1999) and foraging success (effect *sensu* Parker et al. 1999) of the Asian paper wasp were used as proxies for ecological impact by Ward and Morgan (2014), thus demonstrating differential impacts across landscapes (range *sensu* Parker et al. 1999), with the greatest ecological impacts restricted to relatively localised areas.

Where there is no example of the species already having been introduced outside its native range, adopting the insurance industry class approach by applying predictions of impact to a sister taxon or functionally similar species with a known invasion history can be useful to provide a link between a species' characteristics and the likelihood of ecosystem invasion (te Beest et al., 2015). While this is useful to guide predictions, in some cases introduced species can change when they are introduced into a new environment and do not have the same biotic pressures as in their native range. This has been seen in the *Vespula* wasp invasions in New Zealand and Hawai'i, which both lack native social wasps, with *Vespula* reaching high densities in ecosystems with an abundance of sugar resources (Beggs et al., 2011). It is widely accepted that the impact of a species will be greatest when it is introduced into an environment where it performs a completely novel function (Andersen, Adams, Hope, & Powell, 2004).

The magnitude of impact can be broadly determined through the available literature. Kumschick et al. (2012) and Blackburn et al. (2014) have both proposed structured frameworks to classify environmental impact based on mechanisms of impact and their associated outcomes in terms of environmental change. These frameworks provide a straightforward tool to predict the potential consequences of an introduced species through the assessment of available data. The classification system can be applied at different spatial and temporal scales, allowing pest managers to reassess species as required. The classification scheme identifies species in terms of their highest impact, so prioritisation can also be allocated to species depending on the

magnitude of their predicted impact. Therefore, the system proposed by Blackburn et al. (2014) provides a sound tool for pest managers to use when predicting the potential magnitude of impact, at least until further tools are developed.

More specific tools are also available for predicting ecological impact. For example, Todd et al. (2015) have developed a framework for pre-release risk assessment of biological control agents (BCAs), which could easily be adapted to assess non-BCA invertebrates. This tool uses five criteria: (1) direct and indirect hazards posed by the agent, (2) likelihood of exposure to the hazards, (3) ecological impacts that may result from that exposure, (4) species' anthropocentric value, and (5) testability.

2.2.5 Management and restoration

The final step of the framework is to determine the appropriate response to the introduced species. Management decisions will need to consider the potential magnitude of impact caused by the hazard species, future ecosystem vulnerability under climate and land-use change, the social and cultural values of the general public, and the available resources (Stanley & Bassett, 2015; Sheppard et al., 2016). Consultation with experts, stakeholders and the wider public is important to incorporate different values, as perception of ecological risk can vary between the public and experts (Estévez, Anderson, Pizarro, & Burgman, 2015; Slimak & Dietz, 2006). There is an increasing body of research focused on public perception of risk associated with pest management strategies (Bremner & Park, 2007; Gamble, Payne, & Small, 2010; McNeil, Cotnoir, Leroux, Laprade, & Schwartz, 2010; Nimmo, Miller, & Adams, 2007; Russell, 2014), because opposition from public groups can have drastic impacts on potential management (Bertolino & Genovesi, 2003). Incorporating cultural and social values into the co-management or decision-making process is critical not only to ensure all stakeholders are represented, but also because alternative perspectives often provide valuable information on how ecosystems work. Greater inclusion of indigenous perspectives in decision-making and management creates better outcomes for indigenous people and the environment. This has been demonstrated through the collaborative environmental management programmes involving the Dhimurru Aboriginal Corporation in the Northern Territory of Australia (Hoffmann et al., 2012). For instance, despite ant eradications being incredibly difficult (Hoffmann et al., 2016), there has been a highly successful site-specific eradication programme of the yellow crazy ant (*Anoplolepis gracilipes*) as a result of a multi-agency collaborative programme led by the Dhimurru Aboriginal Corporation (Hoffmann et al., 2012).

Following the outlined step-wise approach to risk assessment allows management objectives for improving biodiversity outcomes to be decided. These can be broadly categorised into: direct control (eradication or reduction of population to functionally absent), ongoing management (e.g. reduce below a damage threshold), or no response. A further decision needs to be made on whether additional active restoration of the environment is required to achieve the overall ecological goals. Decisions should include cost-benefit analyses to ensure overall transparency for final justifications. Cost-benefit analyses can be used at the prioritisation stage to ensure the benefits of implementing management strategies outweigh the costs (Stanley & Bassett, 2015). Validation of the decisions should be sought through monitoring programmes, which will ultimately inform improvements to risk assessment via an adaptive management process (O'Donnell & Hoare, 2012). This is particularly important because pest management programmes do not necessarily achieve restoration objectives. For instance, the management of feral deer in native New Zealand forest has been found to have limited restoration success due to continued high browsing of a few preferred species even when deer are at low densities (Coomes, Allen, Forsyth, & Lee, 2003).

It should be noted that, where possible, data obtained through pre- and post- management monitoring should be published or made available for the overall transparency and improvement of future pest management. While in some cases publishing work as peer-reviewed documents is not possible, integrated databases available online are recommended. This will help to disseminate information, promoting the future improvement of risk assessment and strengthening future risk predictions. A recent example of this is the Global Eradication and Response Database (GERDA), which aims to summarise incursion response and eradication programmes from around the world, but has also been useful for retrospectively determining the key elements that influence success and failure in eradication programmes (Tobin et al., 2014).

2.2.6 Case study: The Argentine ant, *Linepithema humile*, in New Zealand

The hazard species, *Linepithema humile*, known commonly as the Argentine ant, is a species of ant native to South America, which was first detected in Auckland in 1990 (Green, 1990). This species has established in the North and South Islands as well as several offshore islands. Species attributes, particularly unicoloniality and reduced intraspecific aggression, coupled with budding dispersal, facilitate the formation of extensive 'supercolonies' across hundreds, and in some cases thousands, of kilometres (Moffett, 2012). Climate modelling indicates a

large proportion of New Zealand to be unsuitable for Argentine ants due to both temperature tolerances and habitat preferences, largely restricting its distribution to the northern and coastal regions of the North Island, and pockets towards the north of the South Island (see Ward et al. 2010 and references therein). Within invaded areas, Argentine ant distribution appears to be limited by abiotic factors; in arid regions, water availability limits Argentine ants (Holway, Suarez, & Case, 2002; Menke & Holway, 2006). In New Zealand, Argentine ant distribution appears to be limited based on ecosystem characteristics. For instance, despite long-term persistence in surrounding open-canopy ecosystems, Argentine ants rarely penetrate into closed-canopy native forest habitats (Ward & Harris, 2005). Scrub and other short-stature, open canopy habitats in the North Island appear to be the most exposed assets to Argentine invasion within New Zealand.

Predation and competition are the major mechanisms through which Argentine ants cause impact to native biodiversity. The invasion of Argentine ants has been associated with significant changes to the richness and composition of the epigeic invertebrate community via predation, which has implications for wider ecosystem functioning (Cooling et al., 2015; Holway, 1999; Human & Gordon, 1997; Inoue et al., 2015; Rowles & O'Dowd, 2009; Stanley & Ward, 2012). Furthermore, competition for carbohydrate resources may negatively impact pollinator and plant communities (Hanna et al., 2015; Lach, 2007, 2008). Based on the classification presented in Blackburn et al. (2014), the impact of Argentine ants is likely to be "massive", to the extent of causing local extinction and irreversible changes to the community.

The application of this framework to Argentine ants as a case study has identified the potential extent of ecological risk as being highest in open-canopy, short-stature ecosystems in the North Island; however, pest managers with significant ecological areas under their administration (i.e. rare ecosystems, habitats for threatened native species) outside of high-risk sites, should consider monitoring for Argentine ants as a preventative measure. As Argentine ants reproduce via budding, whereby a subset of the colony including queens, workers and brood establish new nest sites (Heller & Gordon, 2006), containment and prevention of spread are relatively easy compared to species that reproduce during nuptial flights, as long as human-mediated dispersal is minimised. Site-specific control can, therefore, aim to eradicate or control Argentine ant populations. My framework demonstrates that pest managers with habitats identified as exposed assets should consider Argentine ants a serious threat and justifies their inclusion in pest management strategies, eradication, control and/or containment efforts. In this case study, ecological impacts of Argentine ants were already demonstrated outside of New

Zealand; however, the framework holds value when little information is available, providing a guide to evaluate potential risks to inform decision making.

2.3 Conclusions

Pest managers are often faced with having to make and justify decisions based on limited information. To strengthen the overall success of current and future pest management strategies, decisions should be transparent and defendable. I believe the proposed framework, adapted from a framework assessing the risk of natural disasters, offers a solid basis for assessing ecological risk, at both the broad and the fine spatial scales. By integrating information on the introduced species and the local environment, a finer scale of risk can be predicted, guiding pest management actions to a more focused area. The framework can be used for assessing risk for either (a) 'unwanted' species that have not yet been introduced or established, or (b) already established introduced species.

Predicting the potential risk of an introduced species is easier with increasing information, particularly if the species has already established outside of its native range. The framework has the ability to predict risk more accurately with increasing available data, and it can be used to guide pest managers in making predictions when information is lacking. However, I believe that comparisons among closely related species may be instructive, particularly when little information is available and managers are under pressure to prioritise and make decisions. While any form of risk assessment is plagued by uncertainty, and species introduced outside of their native ranges can exhibit vast differences in behaviour in different contexts, my framework allows pest managers to integrate available information and tools to better inform management decisions.

Chapter 3 — Habitat filters limit the invasibility of natural ecosystems by exotic ants

Abstract

Exotic species have the ability to negatively impact on the recipient biodiversity and ecosystems in which they invade. However, they must first go through a series of abiotic and biotic filters that limit their ability to establish and spread, which ultimately influences their distribution across different habitats. Through understanding which habitats are most vulnerable to invasion, pest managers can prioritise their surveillance areas to focus on those most at risk. I assessed invasion by ants within open- and closed-canopy ecosystems, using a replicated sample design based on data collected in 2004, which I then repeated 10 years later. I predicted that closed-canopy ecosystems would be more resilient than open-canopy ecosystems to invasion as a result of unfavourable abiotic factors that limit ant establishment and spread, even after a 10-year period. In both sampling years, open-canopy ecosystems were more susceptible to invasion by exotic ants, with ants found throughout these habitats. Conversely, in closed-canopy ecosystems, exotic ants were concentrated to the forest edges. Although there was invasion of exotic ants species further into open-canopy ecosystems over the 10-year period, spread did not penetrate into closed-canopy ecosystems suggesting that the variation of abiotic factors between habitat types may be important in determining exotic ant occurrence and spread. My findings have important implications for pest surveillance and management, and highlight the need for future work on the mechanisms of impact of exotic ants in these ecosystems.

3.1 Introduction

Understanding the processes and mechanisms that facilitate the invasion of species is a fundamental question in invasion biology (Hierro, Villarreal, Eren, Graham, & Callaway, 2006; Skurski, Rew, & Maxwell, 2014; Tsutsui et al., 2000). Complex interactions between ecosystem invasibility and species invasiveness may facilitate or impede the establishment of exotic species (Hunt, Galatowitsch, & McIntosh, 2017; Lewis et al., 2017). Therefore, classifying ecosystem types based on key attributes and vulnerability to invasion can help to identify invasion risk.

The attributes of an ecosystem can influence exotic species' invasion potential and distribution via abiotic factors (e.g., microclimate) and biotic factors (e.g., mediation of biotic resistance) (Byers 2002; Pyšek et al. 2010). Abiotic factors are fundamental to a species' ability to colonise and establish outside of its native range, whereas biotic factors are more important at a local scale (McGill, 2010; Theoharides & Dukes, 2007; Zefferman et al., 2015). Thus, the invasibility of an ecosystem is determined through the interplay of abiotic and biotic factors. One of the direct benefits of understanding the invasibility of species into various ecosystems is the ability for pest managers to prioritise surveillance of exotic species based on habitat.

Over 240 species of ants (Hymenoptera: Formicidae) have been transported outside of their native ranges and many are considered highly invasive (Bertelsmeier et al., 2017). For most ant species, human-mediated dispersal facilitates the first stage of their invasion, and key life history and behavioural traits associated with invasion success include polygyny and superior competitive ability (Lester, 2005; McGlynn, 1999; Rabitsch, 2011). However, determinants of invasion success for ants varies between invasion contexts and invasion success will be mediated through numerous factors at a finer spatial scale, relating to the environmental and community characteristics. For instance, Argentine ant (*Linepithema humile*) distribution in California is strongly determined by environmental factors, in particular, soil moisture (Menke & Holway, 2006). Strong preferences for moist habitat has limited Argentine ants to riparian edges of Californian ecosystems, where conditions favour this species, and distinct differences are apparent between edge and interior of local habitat (Holway, 2005). Furthermore, temporal patterns in the establishment and spread of invasive ants are rarely documented, due to the lack of long-term data sets.

Lag times between the establishment and spread of exotic species may occur, making predictions difficult since determinants of lags are context dependent (Crooks, 2005). Once established, species may proliferate and maintain numerical dominance, or in some cases, exhibit boom-bust trends; where after an initial period of rapid population growth, a significant decline is apparent (Simberloff & Gibbons, 2004). Understanding temporal dynamics, and how they relate to particular environmental characteristics, can help to identify what facilitates the spread of particular exotic species, which can enhance pest management programmes.

The aim of this study was to understand the invasibility of different ecosystem types by exotic ants, using data collection with a 10-year time interval between sampling. Specifically, I was interested in i) how exotic species invade habitat fragments broadly classified as having open vegetation canopies versus closed vegetation canopies, and ii) how this varied over a 10-year period. The study was conducted in New Zealand, which has a relatively depauperate native ant fauna (11 species), but with almost three times as many established exotic species (Don, 2007). Because many of the established exotic ants in New Zealand have little or no record of being introduced elsewhere, the potential impact of these species on native biodiversity and ecosystems is poorly understood. Determining the ecosystems most at risk to invasion by exotic ants is, therefore, an important step that facilitates the future prioritisation of surveillance areas by pest managers.

I first predicted that 'open-canopy' ecosystems, (i.e., short stature vegetation without a continuous tree canopy) would facilitate the establishment of exotic species, with exotic ant species occurrence generally ubiquitous from the edge to the interior of the ecosystem. In contrast, I predicted that 'closed-canopy' ecosystems, (i.e., native forest with an established tree canopy), would impede the establishment of exotic ants although an 'edge effect' would occur, with exotic ant occurrence clustering at the edges of these habitats. Lastly, I predicted that 'lag effects' would be observed in open-canopy ecosystems, whereby species that appeared only at the edges in 2004, would be observed to penetrate into the interior of open-canopy ecosystems, but would remain at the edge in closed-canopy habitats.

3.2 Methods

3.2.1 Study sites

Twelve sites were selected in the wider Auckland region (Fig. 1), to represent natural ecosystems that were broadly categorised as open- (short stature vegetation without a continuous tree canopy) and closed-canopy (native forest with an established tree canopy). Sampling occurred at six closed- and six open-canopy sites (Table 1).

3.2.2 Sampling

Sampling occurred during the austral summer between December 2004 and February 2005 and was repeated 10 years later between December 2014 and March 2015 (herein referred to as the 2004 and 2014 sampling years). All sampling was conducted in fine weather (low wind, no precipitation) between 1000h and 1500h. At each site, a transect of up to 200 m was established, running from the edge of the habitat (-20 m and 0 m) into the interior (Table 1). Transect length was determined by the topography at each site. Lure stations were positioned at 20 m intervals along the transect.

Food preferences vary within and between ant species (Hooper-bùi, Appel, & Rust, 2002; Nyamukondiwa & Addison, 2014) so lure stations consisted of different food types to maximise their attractiveness to different ant species. Five lure types were used: 1 tsp peanut butter, 1 tsp tuna (in spring water), 5 g non-toxic Xstinguish^{TM} (protein + lipid + carbohydrate; Bait Technology Ltd, New Zealand), 5 ml soybean oil (in cotton wool), and 5 ml sugar-water (1:4 ratio, in cotton wool). Each lure type was placed in a separate 35 ml plastic vial, which were then positioned in a circular arrangement (15 cm diameter) with the lids removed. Lure stations were left unattended for 1 - 2 h before collection. To offset limitations associated with lure sampling, additional hand collection of ants via direct visual surveying of the area surrounding lure stations was conducted at each station for 3 min when lures were being collected. A description of the immediate microhabitat and environment was recorded at each lure station including vegetation type, canopy cover (%) and litter cover (%). All specimens were kept in 75% ethanol and later identified to species level using the Don (2007) taxonomic key for New Zealand ant species.
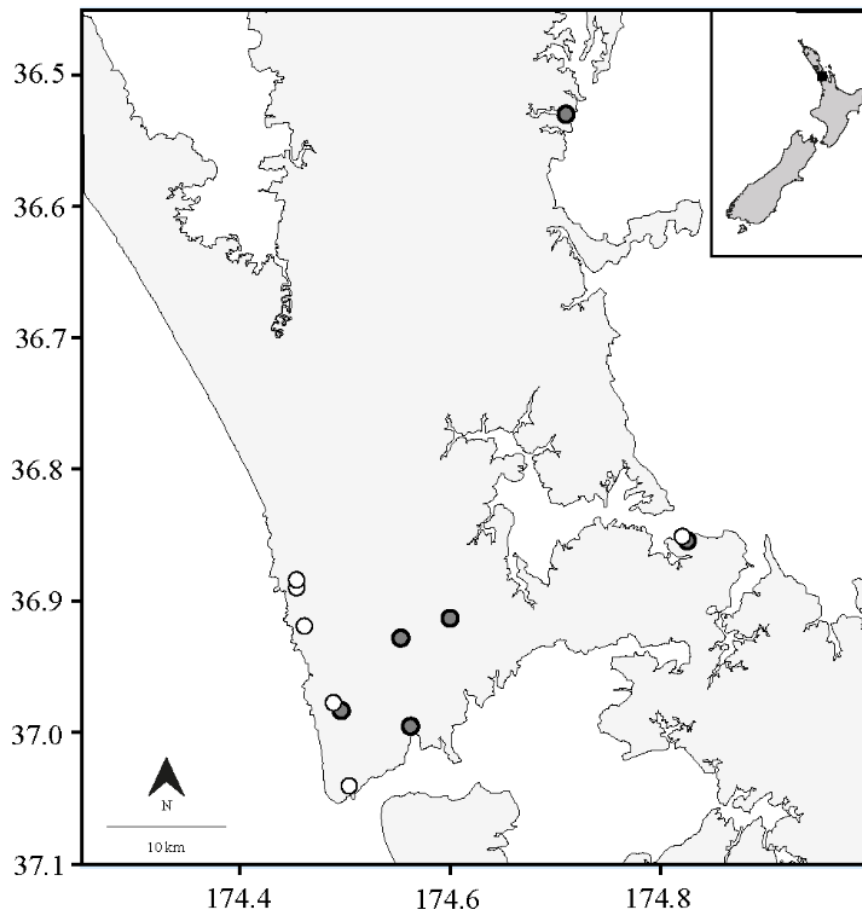
Figure 1 – Map of sampling sites across the greater Auckland region in New Zealand. Insert indicates sampling region within New Zealand. White dots indicate open-canopy ecosystem sampling sites and grey dots indicate closed-canopy ecosystem sampling sites

Table 1. Sampling sites from the greater Auckland area, including information on canopy classification, ecosystem vegetation characterisation (Wardle, 2002) and the distance each ecosystem was sampled from the edge.

Site	Canopy type	Ecosystem vegetation characterisastion	Distance Sampled*
Bastion Point	Closed	Pōhutukawa-broadleaved forest	140m
Huia	Closed	Kauri-podocarp -broadleaved forest	180m
Hunua	Closed	Mixed conifer-broadleaf forest	140m
Karakare	Closed	Pōhutukawa-broadleaved forest	180m
Oratia	Closed	Kauri-podocarp -broadleaved forest	140m
Wenderholm	Closed	Pōhutukawa-broadleaved forest	160m
Anawhata	Open	Coastal scrub	200m
Bastion Point	Open	Restoration planting/scrub	140m
Karakare	Open	Coastal scrub	140m
Te Henga	Open	Sand dunes	160m
Te Henga	Open	Wetland	160m
Whatipu	Open	Wetland	100m

* Distance varies because topography influenced the ability to sample consistently between sites.

3.2.3 Statistical analyses

All analyses were conducted in R version 3.4.2 (R Core Team, 2017). Significance was assessed at the $P < 0.05$ level.

Species occurrence

Ant species occurrence (i.e., presence) was used in all analyses rather than species abundance (i.e., number of individuals collected), due to biases related to the variation of recruitment strategies among species (Holway, 1999).

To examine the differences in exotic and native ant species occurrence, I performed logistic regression using generalised linear mixed effect models with a Poisson distribution using the

'lme4' package (Bates et al. 2015). Two models were used with exotic ant species and native ant species occurrences at lure stations as the response variables. For both models, the explanatory variables were distance from the edge, ecosystem type (open-canopy versus closed-canopy), and year, with site as a random effect.

Ecosystem type was found to be correlated with canopy cover and litter cover using the polyserial function in the 'polycor' package (Fox, 2016), the two latter variables were omitted from models unless data for open- and closed-canopy ecosystems were being analysed separately. Model reduction was conducted until only significant variables remained, with summary statistics reported if the variables were retained in the final model.

The models found that for exotic ant species, there was a significant interaction between the ecosystem type and distance from edge ($z=3.0$, $P=0.001$). Several species were frequently found in lure stations at -20, 0, and 20 m for closed-canopy ecosystems, but did not occur further into the interior of the site. For this reason, I analysed exotic ant data for open- and closed-canopy ecosystems separately.

Ant community composition

To assess the variation in ant communities between open- and closed-canopy ecosystems, I performed: i) classical multidimensional scaling ordination (CMDS, also known as principal co-ordinates analysis), ii) permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), iii) permutational analysis of multivariate dispersions (PERMDISP; Anderson 2006) and iv) similarity percentage (SIMPER) analysis.

As I was most interested in assessing the presence of an edge effect, I initially grouped data into two categories: 'edge', which consisted of ant sampling data at the -20 m, 0 m and 20 m sampling distances, and 'interior', which consisted of ant sampling from 40 m onwards. The distance determined for 'edge' was based on other research finding the most pronounced effects of edge on ants to be within the first 25 m (Ivanov & Keiper, 2010; Ward & Harris, 2005). I visually assessed differences in ant community composition based on ecosystem type, sampling year and sampling location using CMDS with the Bray-Curtis dissimilarity. Before testing differences, I made sampling distance a fixed factor based on distance from habitat edge, with three levels (edge: -20 – 20 m; middle: 60 - 100 m; interior: 120 – 200 m), which allowed us to analyse variation at a finer-scale. PERMANOVA was used to test if ant

community composition differed between open- and closed-canopy ecosystems, using the 'adonis' function of the 'vegan' package based on 999 permutations (Oksanen et al., 2018). I included an interaction term between ecosystem type (open- or closed-canopy), sampling distance and year, as well as canopy height, canopy cover, and litter cover as fixed effects, with site as a random effect. There was a significant interaction between ecosystem type and sampling distance so I used pairwise comparisons between different sampling groups for open- and closed-canopy ecosystems using the 'pairwise adonis' package (Martinez Arbizu, 2017).

To assess the differences in the variability (i.e. dispersion) of ant communities between open- and closed-canopy ecosystems I applied PERMDISP analyses using the 'betadisper' function of 'vegan', then I used the 'permutest' function to compare the mean dispersion between groups based on 999 permutations. Finally, I used SIMPER analysis to identify the species contributing most to the differences in ant community composition.

3.3 Results

In total, I captured 21 ant species from 17 genera (13 exotic and 8 endemic species), with 20 species captured in 2004 (13 exotic, 7 endemic), and 16 species captured in 2014 (9 exotic, 7 endemic) (Table 2).

Ant species occurrence in open-canopy versus closed-canopy ecosystems

I found more exotic ant species compared to native ant species for both open-canopy ecosystems (t=4.24, df=11, P=0.001) and closed-canopy ecosystems (t=2.25, df=11, P=0.046); though overall species diversity was low, which is reflective of the New Zealand ant fauna (Fig. 2). In open-canopy ecosystems, I found 3.8±0.7 exotic species per site in 2004 compared to 2.8±0.4 in 2014. I found a similar number of exotic species per site in closed-canopy ecosystems with 3.5±0.8 in 2004 and 2.5±06 in 2014. In the open-canopy sites, I found 1.8±0.5 native ant species in 2004 and 1.0±0.3 in 2014. In closed-canopy sites I found 2.7±0.2 native ant species in 2004 and 1.3±0.5 in 2014.

In closed-canopy ecosystems there was a significant negative relationship between exotic ant occurrence and distance from edge (z= -3.72, P<0.001), as the number of ant species was higher around the edge (Fig. 3a, 3c). In open-canopy ecosystems, the occurrence of exotic ant species at lure stations did not correlate with distance from edge (z=0.372, P=0.710) (Fig. 3b, 3d), year (z=-0.085, P=0.387), percentage litter cover (z=0.426, P=0.670), or percentage

canopy cover (z=-1.311, P=0.190). Neither percentage canopy cover, nor percentage litter cover were significant predictors of exotic ant occurrence in closed-canopy ecosystems. For native ant species, there were no differences between ecosystem type and species occurrence at lure stations with distance from edge (z=1.96, P=0.050) (Fig. 3).

Ordination revealed differences in ant community composition when comparing between open- and closed-canopy ecosystems, sampling year, and sampling location (Fig. 4). Differences in ordination space were revealed when comparing the ant communities at the edge and interior of closed-canopy ecosystems, providing evidence of an 'edge effect'. In open-canopy ecosystems, ant communities at the edge and interior were comparably more similar in ordination space.

When comparing ant community composition between open- and closed-canopy ecosystems, PERMANOVA analysis revealed a significant interaction between ecosystem type and distance from edge ($F_{3,136}$ = 2.0, P = 0.022), as well as a significant difference in percentage canopy cover ($F_{1,136}$ = 5.0, P = 0.004).

Further, pairwise tests showed a clear 'edge effect' for closed-canopy ecosystems, with the ant community at the outside edge significantly different compared to the communities further into the ecosystem (Table 3). However, no significant differences were found in ant community composition between any of the distance groups for open-canopy ecosystems. PERMDISP revealed no differences in dispersion within ant communities between open- and closed-canopy ecosystem, so whilst the ant communities in open- and closed-canopy ecosystems were compositionally distinct, they had similar dispersion.

SIMPER analysis revealed differences in ant community composition to be driven by five key species, of which four were exotic, with *Monomorium antarcticum* (native), *Nylanderia* sp., *Mayriella abstinens*, *Ochetellus glaber* and *Tetramorium grassii* contributing to more than 60% of the total difference when comparing open- with closed-canopy ecosystems.
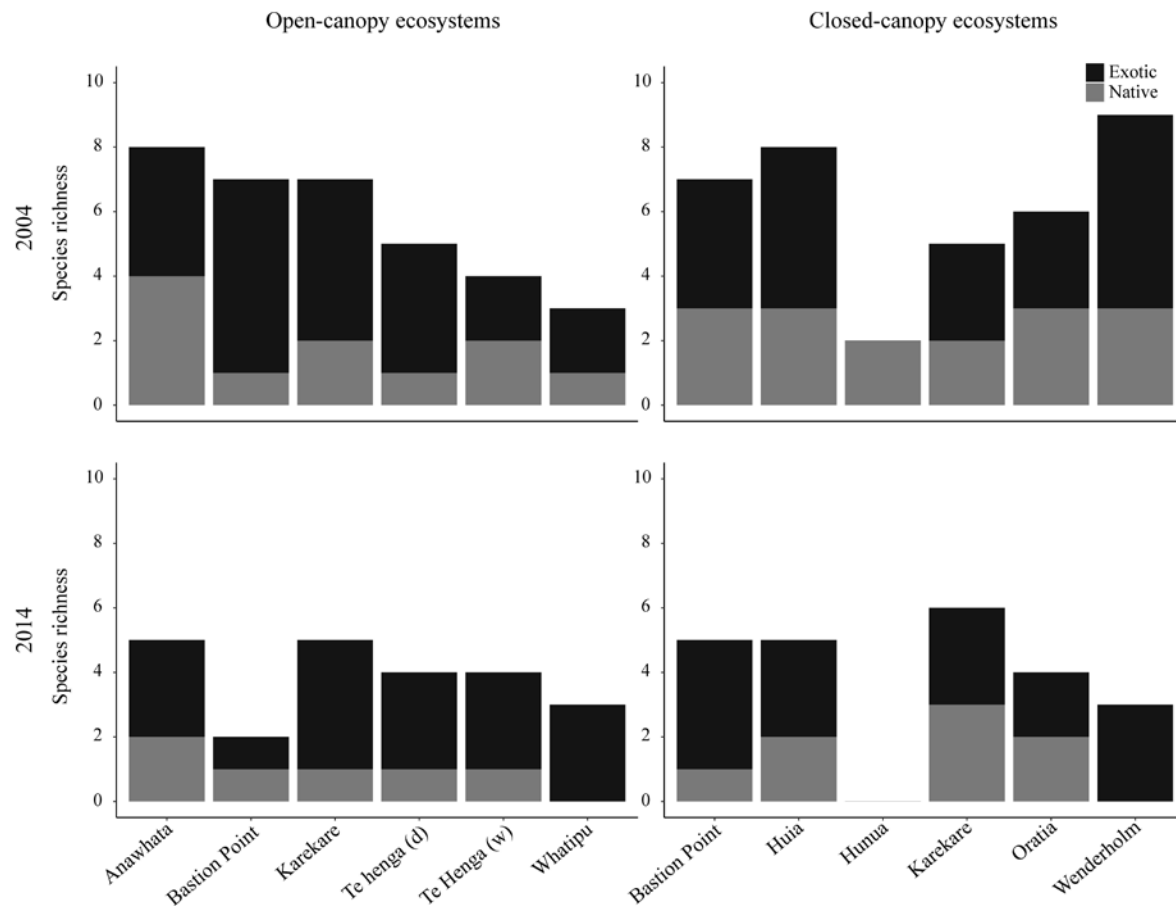
Figure 2 – Species richness of ants at the different study sites sampled. Each bar represents the species richness for a particular site, with the number of exotic and native ants shown through shading of bars. Te Henga (d) represents the sand dune site, Te Henga (w) the wetland site.

Table 2. Percentage of lure stations at which ant species were detected for open- and closed-canopy ecosystems for the 2004 and 2014 sampling years (n=59 lure stations for each ecosystem, for each year of sampling).

Species	Biostatus	% lure stations detected			
		Open-canopy		Closed-canopy	
		2004	2014	2004	2014
Amblyoponinae					
Amblyopone australis	Exotic	0	0	2	0
Dolichoderinae					
Iridomyrmex suchieri	Exotic	3	10	0	3
Linepithema humile	Exotic	2	0	0	2
Ochetellus glaber	Exotic	19	25	2	2
Technomyrmex jocosus	Exotic	12	0	10	0
Ectatomminae					
Rhytidoponera chalybaea	Exotic	2	2	0	0
Rhytidoponera metallica	Exotic	3	0	0	0
Formicinae					
Nylanderia sp.	Exotic	12	19	19	19
Prolasius advenus	Native	0	0	2	0
Heteroponerinae					
Heteroponera brouni	Native	2	2	10	0
Myrmicinae					
Huberia brounii	Native	0	0	0	2
Huberia striata	Native	3	5	2	2
Mayriella abstinens	Exotic	0	2	29	14
Monomorium antarcticum	Native	22	15	15	5
Monomorium antipodum	Native	7	0	12	5
Pheidole rugosula	Exotic	2	2	5	7
Solenopsis sp.	Exotic	0	0	0	5
Tetramorium grassii	Exotic	25	12	19	0
Ponerinae					
Austroponera castanea	Native	2	2	7	3
Austroponera castaneicolor	Native	0	0	2	0
Hypoponera eduardi	Exotic	2	0	0	0
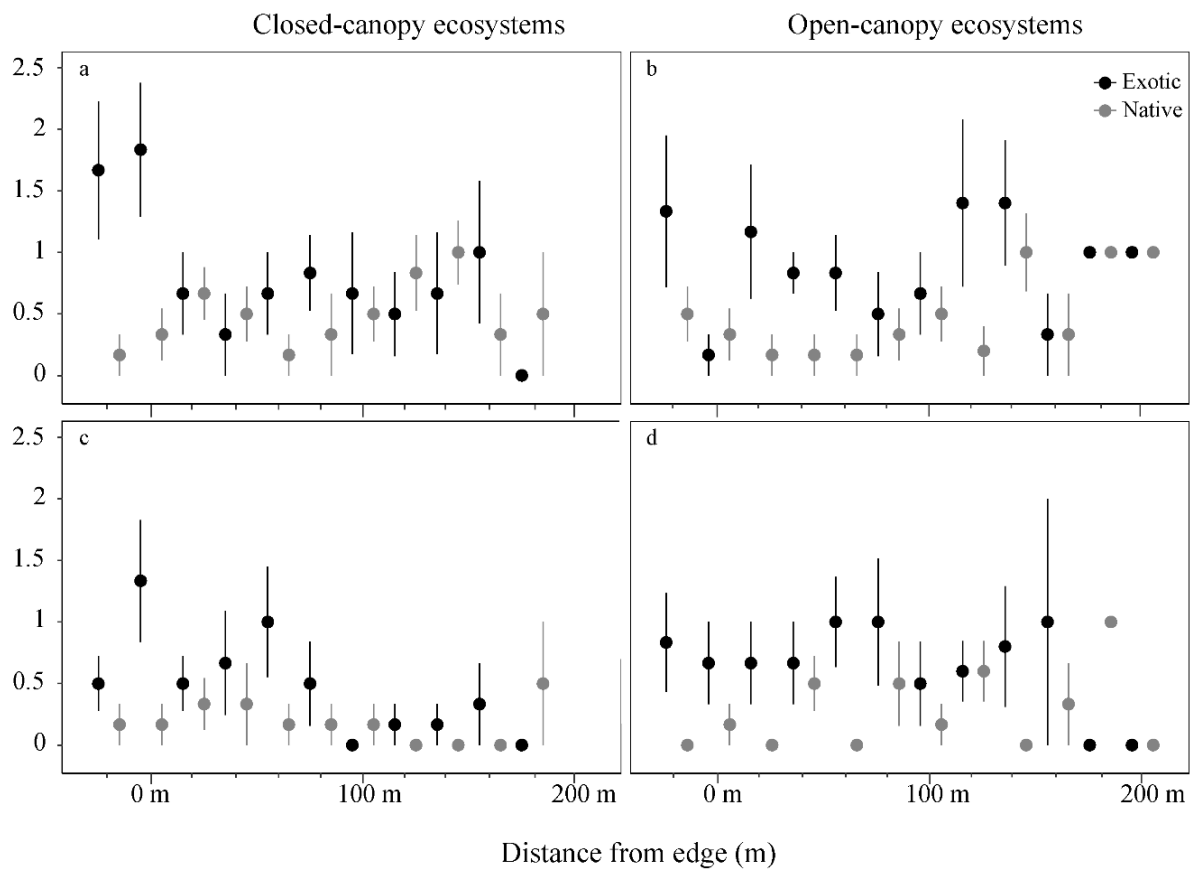
Figure 3 – Mean number of exotic and native ant species at lure stations along an edge (-20 m) to interior (200 m) transect at 20 m intervals in a) closed-canopy ecosystems in 2004, b) open-canopy ecosystems in 2014, c) closed-canopy ecosystems in 2004 and d) open-canopy ecosystems in 2014.
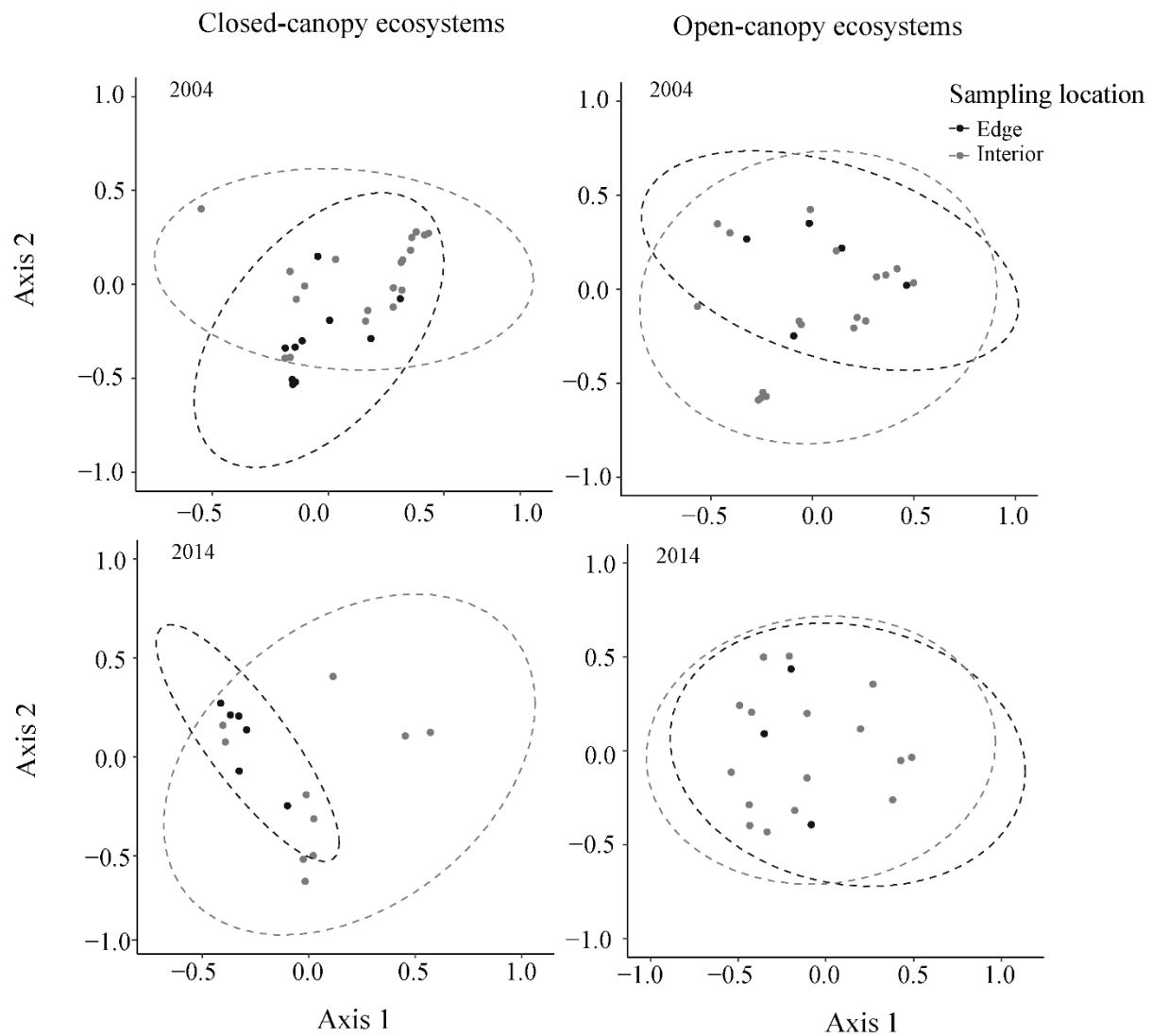
Figure 4 – CMDS ordinations of sites for different ecosystems types and sampling years. Shows ordination of open-canopy and closed-canopy ecosystems in 2004 and 2014 sampling years. Black dots indicate 'edge' sampling plots (-20 m, 0 m, 20 m) with grey dots indicating 'interior' sampling plots (40 m onwards). Ellipses indicate 95% confidence intervals.

Table 3. Results of pairwise tests among the distance groups for both open- and closed-canopy ecosystems. Tests were conducted using the 'pairwise adonis' function in R (Martinex Arbizu, 2017), which uses bonferroni adjusted *P* values. Pair names refer to the following sampling distances: edge (-20 m, 0 m, 20 m), middle (40 m, 100 m), interior (80 m - 200 m)

Pairs	Open-canopy	Closed-canopy
Edge v middle	*P*= 1.0	*P*= 0.027*
Edge v interior	*P*= 0.204	*P*= 0.003*
Middle v interior	*P*= 1.0	*P*= 0.891

* Denotes significance after bonferroni adjustments

Presence of lag/temporal differences

PERMANOVA analysis revealed significant differences in ant community composition between sampling years ($F_{1,136}$ = 5.20, *P* = 0.001). Variation in ant community composition between sampling years was driven by five key species, as revealed by SIMPER analysis, of which four were exotic, with *Nylanderia* sp., *M. antarcticum* (native), *T. grassii*, *O. glaber*, *M. abstinens* and *T. jocosus* contributing to more than 65% of the total difference.

In closed-canopy ecosystems, there were no exotic species in 2004 that were only detected at the edge (up to 40 m from edge), which then penetrated further into the habitat in 2014. Conversely, in two of the open-canopy ecosystems, there were exotic species that were only detected at the edges in 2004, which then penetrated much further into the ecosystem in 2014. The species that spread were *O. glaber*, *Nylanderia* sp. and *Iridomyrmex suchieri* in the Te Henga sand dune, and *O. glaber*, *Nylanderia* sp. and *T. grassii* in the Te Henga wetland site.

Exotic ant species occurrence at lure stations was lower in 2014 compared to 2004 (*z*=-2.24, *P*=0.025). This was largely driven by the differences in the occurrence of *T. grassii*, *M. abstinens*, and *Technomyrmex jocosus* as they were more frequently detected in 2004 than 2014 (Table 2).

There were fewer native species occurrences in 2014 (z=-3.17, P=0.002). This difference was mainly driven by *Heteroponera brouni* and *M. antarcticum* (Table 2).

3.4 Discussion

My study reveals habitat-associated variation in exotic ant distributions within natural ecosystems. In agreement with my predictions, I found distinct differences in species occurrences between open- and closed-canopy ecosystems. Generally, exotic species occurrences were uniform across transects in open-canopy ecosystems, and restricted to the edges for closed-canopy ecosystems. Similarly, I found corresponding patterns in the community composition between the two ecosystem types. Finally, I found temporal differences between 2004 and 2014, although there was no evidence of a lag for exotic species in closed-canopy ecosystems. Together, my results suggest that open-canopy ecosystems are more vulnerable to invasion by exotic ant species, conversely, closed-canopy ecosystems appear to impede ant spread from the surrounding area. These findings have implications for potential surveillance and management of pest ant species.

Exotic ant species dominated, in terms of species richness, in both open- and closed-canopy ecosystems for both sampling years. This is unsurprising given the low diversity of the New Zealand native ant fauna (Don, 2007). While I did not conduct abundance analyses due to biases related to obtaining colony size estimates, exotic ant species dominated lure stations in nearly all circumstances and were visually conspicuous within ecosystems compared to their native counterparts (A. Probert, pers. obs.). This is congruent with small colonies sizes and densities recorded for most New Zealand ants.

I found differences in both ant species occurrence and community composition between open- and closed-canopy sites. In open-canopy ecosystems, the occurrence of exotic ant species was generally ubiquitous across the sampled area, yet restricted to edges for closed-canopy ecosystems. This difference is likely explained, in part, by the environmental preferences of exotic ants. Temperature, and in particular soil temperature, is often an important factor associated with ant distribution, with species richness and colony size positively associated with increasing temperature (Kaspari, Ward, & Yuan, 2004; Kwon, 2016; Sanders, Lessard, Fitzpatrick, & Dunn, 2007; Warren & Chick, 2013). The majority of exotic ant species present in New Zealand originate from warmer regions (Lester, 2005; Don, 2007), and therefore, are

predicted to be more restricted by thermal tolerances relating to a 'temperature-humidity envelope' (Hölldobler & Wilson 1990). Open-canopy sites were associated with sparse canopy cover and presumably higher solar insolation and soil temperature, compared to closed-canopy sites (Davies-Colley, Payne, & Elswijk, 2000). Although soil moisture is an important factor driving distribution for some ant species, this is usually in water-stressed environments (Menke & Holway, 2006), and is therefore unlikely to be a limiting factor for exotic ants in Auckland, where average annual rainfall is ~1100mm (Chappell, 2013). Thus, the closed-canopy temperate forests in New Zealand may be restricting exotic ants through an abiotic, rather than a biotic filter. Future work should attempt to quantify microclimatic conditions between habitat types to determine if these abiotic factors are limiting exotic ant species at a micro-scale.

The microclimatic differences driving variation in exotic ant species occurrences and community compositions are likely to similarly drive the observed 'edge effect' at closed-canopy ecosystems. The strength and direction of a species' response to the presence of edges depend on its size and mobility, with smaller species generally more sensitive to edges at a finer spatial scale (Lidicker Jr, 1999; Vasconcelos & Bruna, 2012; Wiens, Clifford, & Gosz, 1985). The edge effect I report is stronger than other ant communities studies, which report the most pronounced effects to be within 25–50 m of the habitat interior (Ivanov & Keiper, 2010; Sobrinho & Schoereder, 2007). Significant differences were found when conducting contrasts between the edge and the middle and interior within closed-canopy ecosystems, and ordination plots indicated differences in the edge were maintained at the community level. Previous sampling has demonstrated that within Auckland forests, Argentine ants are limited to 20 m from the edge (Ward & Harris, 2005), suggesting these habitats are largely inhospitable to at least some exotic ants species.

I show temporal variation at the community-level for exotic ant species occurrence in closed-canopy ecosystems, and for native species overall. I found no evidence that exotic species occurring at the edge of the closed-canopy ecosystems in 2004 were invading towards the interior in 2014. This supports my prediction that distribution limitations are determined for exotic ants via the abiotic filter rather than a potential lag effect. In contrast, some invasive spread in open-canopy ecosystems, with *T. grassii*, *I. suchieri, O. glaber* and *Nylanderia* sp. expanding their distribution from the edge to the interior over the ten-year period. Species richness was lower in the 2014 sampling year for both ecosystem types, and there were fewer exotic species occurrences compared to 2004. This disparity may reflect different climatic

conditions between the sampling years as the 2014 summer was associated with below average rainfall and above average temperatures (NIWA, 2018). Some ant species exhibit a positive response to increasing rainfall (Heller, Sanders, Shors, & Gordon, 2008; Sanders, Moss, & Wagner, 2003), and this may explain the reduced diversity and occurrence, particularly for native ant species, which may be more sensitive to extreme climate variation. Alternatively, or in addition, these differences could be related to competitive interactions and nesting behaviours. For instance, competition by exotic ants has been found to drive variation in community composition (Lester, Abbott, Sarty, & Burns, 2009; Sanders, Gotelli, Heller & Gordon, 2003), and competitive advantages may lead to the suppression of other species that would occur in the absence of particular species (Holway, 2005). Furthermore, although many ant nests are maintained for many years, some species exhibit ephemeral nest sites, relocating nests regularly for various reasons (McGlynn, 2012).

The ant species driving differences between open- and closed-canopy sites and sampling years were, unsurprisingly, the species that represented the highest number of species occurrences overall. Several of these are generalists that dominate urban ant communities within New Zealand (Stringer, Stephens, Suckling, & Charles, 2009). *Mayriella abstinens* was almost exclusively found within closed-canopy ecosystems, whereas *O. glaber* was found throughout open-canopy ecosystems, but restricted to the edges of closed-canopy sites. The *Nylanderia* sp. followed a similar pattern to *O. glaber*, in open-canopy ecosystems; however, this species was common throughout the Bastion Point closed-canopy site. Considered a 'tramp' species and, by definition strongly associated with anthropogenic activity (Passera, 1994), it is possible the location of this site within a highly urbanised matrix facilitated the *Nylanderia* sp. occurrence. It is likely that its distribution within this site is facilitated through mechanisms of both increased microhabitat and food resources through rubbish dumping and human disturbance or propagule pressure.

This study demonstrates ecosystem type is an important factor in determining the distribution of exotic ants, highlighting open-canopy ecosystems as vulnerable to invasion. In contrast, closed-canopy ecosystems appear to impede exotic ant spread, constraining their distribution to the edges of habitats, even after a ten-year period. This suggests an abiotic filter is important in determining the distribution of exotic ants in natural ecosystems. Understanding ecosystems most at risk to potential invasion of species allows pest managers to narrow their field of surveillance and prioritise management. For exotic ants, I recommend increased focus towards

open-canopy ecosystems that are representative of some of the rarest terrestrial ecosystems within New Zealand (Williams et al., 2007). This steps away from the species-trait based method of risk assessment and towards a more integrated system, whereby ecosystem attributes can provide predictive traits for risk. Future work is required to determine the impact associated with exotic ants in New Zealand, as to date there is little understanding of how these species are interacting with native biodiversity and ecosystems.

Chapter 4 — Dietary-related impacts of invasive ants in natural ecosystems

Abstract

Understanding the mechanisms and the impacts of biological invasions is critical for accurate risk assessment to inform pest management programmes. Invasive species may alter the dietary-related interactions within the communities they invade, often out-competing their native counterparts. In New Zealand, invasive ants are common throughout non-forested, natural ecosystems, however, their dietary-related impacts are poorly understood. In this study, I used stable isotopes and metabarcoding of ant gut contents to identify the dietary niche of invasive ants. δ^{15}N and δ^{13}C values were obtained for 9 exotic and 4 native species of ant. Overall, ants fed mainly across two trophic levels. Two exotic ant species *Linepithema humile* and *Technomyrmex jocosus* were found to feed at a lower trophic level when compared to other ant species, consistent with trophic studies for these species elsewhere. The largest isotopic niche overlap was observed between the native *Monomorium antarcticum* and the invasive *Ochetellus glaber*, with analyses revealing this species had a significant negative co-occurrence pattern. Sequence data of ant gut content yielded practical data for only 71/171 samples, identifying 51 OTUs, 22 Orders and 34 families; primarily arthropod DNA. My results indicate that dietary competitive exclusion may affect native ant species distribution at the local scale and highlight that further development of molecular dietary assessment for ants is required.

4.1 Introduction

Ecological interactions between invasive species and native biodiversity occur both directly and indirectly, and in the most severe cases, may cause impacts at the ecosystem-wide scale (Penk et al., 2017). A fundamental way that invasive animals drive ecological change is via dietary-related interactions with their recipient community. Some dietary-related impacts may be clearly evident (e.g., direct predation and a corresponding reduction in prey population), and therefore relatively easy to measure. However, due to the complexities of both species interactions and food webs, impact is often difficult to discern. Furthermore, certain ecological traits of invasive species, such as habitat preferences, cryptic feeding behaviour, and/or feeding strategies, may add further uncertainty to conclusions related to dietary ecological interactions and effects (Cohen, 2015).

Although the feeding strategies of invasive species vary from specialised to opportunistic omnivores, discerning impact for the latter can be challenging considering the complexity of interactions associated with generalist species (Snyder & Evans, 2006). In some cases, the establishment and persistence of generalist species may be facilitated through their dietary flexibility, as food availability is less likely to be a limiting factor, thus increasing their niche opportunities within the invaded environment (Shea & Chesson, 2002; Tonella et al., 2018). Such a strategy enables populations to persist despite variations in food availability (Caut, Angulo, & Courchamp, 2008), and may broaden their potential impact through dietary-related ecological interactions within an ecosystem. For instance, exotic species feeding across multiple trophic levels will act as both consumers and potential competitors at a broader scale, making impact assessment more difficult than if they were dietary specialists (Snyder & Evans, 2006). Furthermore, this strategy may confer a competitive advantage, allowing invaders to outcompete, and potentially displace, native species (Snyder & Evans, 2006).

An opportunistic generalist diet is characteristic of many tramp and invasive ants (McGlynn, 1999; Rabitsch, 2011), thus, determining the diet of ants can be difficult due to broad feeding habits and foraging behaviours (Tillberg et al., 2006). Stable isotope analysis is a well-developed tool that has been used to assess trophic structure of highly invasive species and can be used to infer ant diet through carbon and nitrogen isotopic signatures (Menke et al., 2010; Roeder & Kaspari, 2017; Tillberg et al., 2007). Isotopes of nitrogen ($\delta^{15}N$) allow for estimates of trophic position as each trophic level is typically enriched with nitrogen 3-4%, meaning

primary consumers have lower $\delta^{15}N$ values than secondary and higher consumers (Post, 2002). Isotopes of carbon ($\delta^{13}C$) provide estimates for the base of a food web, where the $\delta^{13}C$ of a consumer is similar to its diet, allowing inference between the importance of C_3 and C_4 plants as food sources within ecosystems (Post, 2002). Whilst stable isotope analysis is a frequently employed method to understand impact through invasion for a variety of taxa, it is limited in its ability to accurately describe diet at the finer scale (Cucherousset et al., 2012; Karlson, Gorokhova, & Elmgren, 2015; Rakauskas et al., 2018; Roemer, Donlan, & Courchamp, 2002).

A solution to the problem of poor resolution related to trophic-level dietary analysis can be found through employing genetic-based approaches to identify the DNA of prey items and gut or faecal content (Nielsen, Clare, Hayden, Brett, & Kratina, 2018). The reduced costs associated with DNA barcoding and high-throughput sequencing methods have vastly increased this method for dietary analysis for many taxa, allowing identification for a wide dietary scope (Bohmann et al., 2011; Connell, O'Rorke, Jeffs, & Lavery, 2014; Gómez & Kolokotronis, 2017; Jedlicka, Vo, & Almeida, 2017). Advantages of this approach include the ability to more accurately describe the diet of omnivorous species, without conducting laborious observation periods, or sorting samples based on morphological identification (Barba et al., 2014; Harms-Tuohy, Schizas, & Appeldoorn, 2016; Robeson et al., 2017). This approach is becoming a standard practice for dietary analysis in some taxa (e.g., bats [Arrizabalaga-Escudero et al., 2018; Bohmann et al., 2011; Czenze et al., 2018; Razgour et al., 2011; Zeale, Butlin, Barker, Lees, & Jones, 2011]), with methods more well-developed for vertebrates than invertebrates. Although it is becoming more regularly used to investigate the diet of arthropods (Gomez-Polo et al., 2015; Lima et al., 2016; Paula et al., 2016), it has not yet been applied to ants, a group well-known for dietary-related impacts where they invade (Holway et al., 2002a).

In this study, I use both stable isotope and high-throughput sequencing methods to investigate the diet of invasive ants inhabiting short stature indigenous ecosystems, which are often highly invaded by ants (Chapter 3). Specifically, my aim was to determine whether dietary niches of dominant invasive and native ant species overlapped. In addition, I used co-occurrence patterns of invasive and native ant species to infer whether competitive exclusion may occur between species that share similar isotopic niches. I predicted that stable isotope signatures and DNA metabarcoding gut contents of invasive ant species would reveal a more generalist feeding strategy, compared to a more specialised, predatory feeding strategy for native ants.

4.2 Methods

4.2.1 Sampling

I conducted this study in the Auckland region of New Zealand from October 2016 to March 2017. I sampled ants for both stable isotope and metabarcoding analysis across seven sites ranging from coastal scrub to dune habitats. These sites are characterised by their open canopy (low stature vegetation), and are vulnerable to invasion by exotic ants, which dominate the local ant communities (Chapter 3).

4.2.2 Stable isotope analysis of ant diet

Stable isotope ratios of nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) were used to estimate trophic position and source of dietary carbon for ant species. To reduce spatial biases related to obtaining a reliable nitrogen baseline, sampling at each site was limited to areas < 500 m x 500 m (Woodcock et al., 2012). Ant sampling was conducted within the 500 m x 500 m area at each site (n =7) through direct visual searching to maximise the number of species, using an entomological aspirator. At each ant collection point, I recorded soil type based on the topsoil layer (i.e., organic, loam, sand, clay), collection location (i.e., ground, plant, coarse-woody debris), and vegetation cover (%) within a 2 m radius of the collected foraging ants.

To provide a relative comparison for stable isotope values from ant samples, within the same 500 m x 500 m area, I collected plant material and other arthropod species broadly classified by their known trophic level (e.g., predators and primary consumers). Ants and other arthropods were returned to the laboratory and stored at -20°C until preparation for analysis.

Sample preparation

I identified all ants to species level using the key in Don (2007). In preparation for stable isotope analysis, ants and other invertebrates were thawed, rinsed with distilled water to remove any debris, and then placed in a drying oven at 50°C for 48 hours. The gaster, petiole, and post petiole of each ant were removed to ensure that recently ingested food did not affect isotopic signatures (Tillberg, McCarthy, Dolezal, & Suarez, 2006). To avoid this issue for other arthropods, individuals were either starved for 72 hours prior to freezing, or their digestive tract was dissected out prior to processing. Plant material was rinsed in distilled water, placed in a drying oven for 48 hours, and then ground into a fine powder using a Mixer mill MM301

(Retsch, Haan Germany). Only ants collected in March 2017 were prepared for stable isotope analysis (*cf.* metabarcoding). This sampling period, towards the end of the Austral summer, provides isotopic information for colonies reflecting the temporal assimilation of isotopes for the previous months, when ant abundance, and potential impact peaks.

To obtain a sample of sufficient weight for stable isotope processing, I was required to pool several ants per each colony for most of the ant species. A pilot stable isotope analysis determined that the optimal sample weight required for the ant samples was 0.5-0.65 mg. I weighed samples using an analytical balance (UMX5, Mettler Toldedo, Switzerland, precise to 0.001) into 5 x 9 mm tin capsules (OEA labs, United Kingdom) that were then crushed using sterilized forceps and placed into individual wells in a 96-well plate.

All stable isotope analyses were carried out at the National Institute of Water and Atmospheric Research (NIWA) stable isotope laboratory in Wellington, New Zealand. Analyses were performed with a MAS200 autosampler connected to a Flash 2000 (Thermo-Fisher Scientific, Bremen, Germany) elemental analyser coupled with a Delta V Plus (Thermo-Fisher Scientific, Bremen, Germany) continuous flow, isotope ratio mass spectrometer.

Stable isotope ratios were expressed as delta values (δ) (permil units (‰)), which represents the ratio of heavy to light isotopes within a sample (R_{sample}), relative to the ratio in an international standard ($R_{standard}$) as: $\delta = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) \times 1000$. Trophic position (TP), was determined as: $TP = \lambda + \frac{\left(\delta^{15}N_{ant} - \delta^{15}N_{base} \right)}{\Delta N}$ where λ represents the basal food source trophic level (e.g., primary producer = 1), $\delta^{15}N_{ant}$ represents the individual sample, and $\delta^{15}N$ represents the nitrogen baseline obtained from vegetation (see below). ΔN was the standard enrichment per trophic level, which is typically 3.4‰ enrichment per trophic level (Post, 2002). To obtain the nitrogen baseline, I regressed the $\delta^{15}N$ values of plant material against site, and found that only the Piha site was significantly different from the other sites. Therefore, I applied corrections to ant and invertebrate samples from the Piha site using the formula: $\delta^{15}N_{corrected} = \delta^{15}N_{measured} + (\delta^{15}N_{sites} - \delta^{15}N_{piha})$, where $\delta^{15}N_{sites}$ was the mean nitrogen value for all sites excluding Piha, and $\delta^{15}N_{piha}$ was the mean nitrogen value for the Piha site (Pfeiffer, Mezger, & Dyckmans, 2014). $\delta^{15}N_{sites}$ was used as the nitrogen baseline in trophic positioning, and in all cases I analyse and report the corrected $\delta^{15}N$ values.

4.2.3 DNA analysis of ant diet

Sample preparation

I sampled ants for DNA gut content analysis at each site as per stable isotope sampling; however, sampling encompassed a wider area of the surrounding habitat and, for each colony, at least ten workers were randomly selected and placed in 95% ethanol, then stored at -80℃ at the laboratory.

Each sample consisted of ten workers from a colony that I surface sterilised in a sterile petri dish containing 5% bleach for 1 minute, and then rinsed in molecular-grade water three times (*sensu* Łukasik et al., 2017). I dissected out gut contents of each individual ant onto UV sterilised Kimwipes™ to: 1) reduce the amount of ant DNA in each sample; and 2) reduce potential PCR inhibition related to the crop structure that has been found for some ant species (Penn, Chapman, & Harwood, 2016). Between dissections for each colony, forceps were soaked for 15 minutes in 10% bleach, washed with sterile water, and then placed under UV for 15 minutes.

DNA from the Kimwipes™ with the gut contents of ants were extracted whole. DNA extraction was conducted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen Ltd, Crawley, UK), following the manufacturer's instructions for animal tissues. I selected this extraction kit as it has previously been employed in arthropod gut content research (Chapman, Schmidt, Welch, & Harwood, 2013; Penn & Harwood, 2016; Pons, 2006).

I initially tested several primer pairs to target the COI gene region to identify invertebrate prey, and the chloroplast gene rbcL to identify consumed plant matter (Table 1), modified to include Illumina adapter sequences. I selected the mlCOIinfF and HCO2198 primer pair as they universally amplify a 313bp region of the COI gene, and have successfully been used in DNA barcoding for various taxa. Despite the fact they would amplify ant DNA, this approach has been utilised in arthropod diet analysis previously, and advocated as a practical method to target arthropod-predator prey (Piñol, San Andrés, Clare, Mir, & Symondson, 2014). In addition, the LepF1 and MLepR1 primer pair are relatively general towards different insect species, targeting a 307bp region of the COI gene, with a low affinity towards Hymenoptera (Rougerie et al., 2011; Ward & Ramón-Laca, 2013). Therefore, this primer pair appeared to be ideal candidates to amplify non-ant DNA from the gut. The rbcL*a*F and rbcL*a*R target the rbcL gene

region of chloroplasts and have been widely used for plant barcoding (Hollingsworth, Graham, & Little, 2011; Kress & Erickson, 2007) and have been applied to studies investigating the plant-material diet in insects (Lima et al., 2016).

Amplification was initially performed in a 25µl reaction volume consisting of 5.5-7.5 µL nuclease-free water, 12.5 µL Kapa Hifi Polymerase, 1 µL of each primer, and 3-5µL DNA template. Every PCR cycle included a positive and negative control. The PCR cycles that resulted in optimum amplification for each primer pair were:

i) mlCOIinfF and HCO2198: 95°C for 3 minutes for initial denaturation, followed by 15 cycles of 95°C 30s, 63°C 20s (using "touchdown" PCR – decreasing 1°C every cycle), and 72°C 60s, then for 35 cycles of 95°C for 30s, 48°C for 20s, 72°C for 60s and 72°C at 10 min for the final extension.

ii) LepF1 and MLepR1: 95°C for 5 minutes for initial denaturation, followed by 10 cycles of 95°C 40s, 55°C 40s (using "touchdown" PCR – decreasing 0.5°C every cycle), and 72°C 60s, then for 45 cycles of 94°C for 40s, 50°C for 40s, 72°C for 60s and 72°C at 10 min for the final extension, and

iii) rbcL*a*F and rbcL*a*R: 95°C for 3 minutes for initial denaturation, followed by 15 cycles of 95°C 30s, 63°C 20s (using "touchdown" PCR – decreasing 1°C every cycle), and 72°C 60s, then for 35 cycles of 95°C for 30s, 48°C for 20s, 72°C for 60s and 72°C at 10 min for the final extension.

Due to poor amplification, 0.75 µL bovine serum album (BSA; MP Biomedicals, California, USA) was tested with a subset of samples using the mlCOIinfF/ HCO2198 and LepF1/MLepR1 primer pairs to determine whether PCR inhibition may be influencing PCR amplification success. In no cases did the addition of BSA improve amplification.

Both the LepF1/MLepR1 and rbcL*a*F /rbcL*a*R primer pairs failed to produce consistent amplicons, despite the positive control working, as indicated through gel electrophoresis. Therefore, the mlCOIinfF and HCO2198 primer pair was used for all sequencing with the final PCR performed in a 25µl reaction volume consisting of 5.5 µL nuclease-free water, 12.5 µL Kapa Hifi Polymerase, 1 µL of each primer, and 5µL DNA template, with the optimal PCR cycle for these primers. PCR products were examined by electrophoresis at 90V for 40 minutes in a 1.5% agarose gel in 0.5% TAE buffer with SYBR Safe, the marker used DNA ladder 1kb.

Table 1 Primer pairs tested (without Illumina adapter sequences)

Gene region	Primer name	Sequence (5'-3')	Annealing temp (°C)	Length (bp)	Reference
COI (animal)	mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC			(Leray et al., 2013)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	63 - 48	~313	(Folmer et al., 1994)
	LepF1	ATTCAACCAATCATAAAGATATTGG			(Hajibaba et al., 2006)
	MLepR1	CCTGTTCCAGCTCCATTTTC	55 - 50	~307	Hajibaba et al., 2006)
rbcL (plant)	rbcLa F	ATGTCACCACAAACAGAGACTAAAGC			(Levin et al., 2003)
	rbcLa R	CTTCTGCTACAAATAAGAATCGATCTC	55 - 50	~550	(Kress & Erickson, 2007)

PCR products were individually purified using ZR-96 DNA Clean-up kits (Zymo Research, USA), then quantified using Qubit double-stranded DNA (dsDNA) HS assay kit (Life Technologies, USA). The amplified material (*N*= 171) was then sent to Auckland Genomics, at the University of Auckland (Auckland, New Zealand) for sequencing on an Illumina MiSeq instrument using 2 x 300 bp chemistry. Before sequencing, the sequencing provider attached a unique combination of Nextera XT dual indices (Illumina Inc., USA) to the DNA from each sample, to allow for multiplex sequencing.

Bioinformatics methods

I quality filtered the sequence data and picked *de novo* operational taxonomic units (OTUs) using USEARCH v 7.0 (30). The first 26 bp were trimmed off the start of both the forward and reverse reads before using the fastq_mergepairs command to merge the reads. Any read with a quality score (Q score) of < 3 was truncated at the first position, and I set the minimum length of the merged read to 200 bp. Reads with > 1 expected error were discarded. Sequence data was dereplicated (-derep_fulllength), singletons were removed (-sortbysize), and then clustered into OTUs at 97% sequence similarity, using the UPARSE-OTU algorithm, which also removes chimeras (Edgar, 2013).

Taxonomic classification was conducted using BLAST (Altschul, Gish, Miller, & Myers, 1990) against the partially non-redundant NCBI nucleotide database (downloaded on 21/02/2018). Only one sequence match per OTU was allowed, and a minimum similarity threshold of 80% was used for the BLAST search. The resulting BLAST file was imported into Megan6 (Huson, Auch, Qi, & Schuster, 2007) to obtain taxonomic paths for each OTU. Bacterial and fungal sequences were removed from the dataset, as these were likely the result of unspecific primer binding. Additionally, all sequences matching Formicidae were also removed for each sample. This resulted in a large range of sequences per sample (1–3386 reads per sample, with from 1–8 OTUs per sample). To achieve a standard sequencing depth across all samples, I rarefied to 60 reads per sample. Although this is a very low rarefaction threshold, rarefaction curves indicate that this adequately sampled the diversity present (Fig. 1).
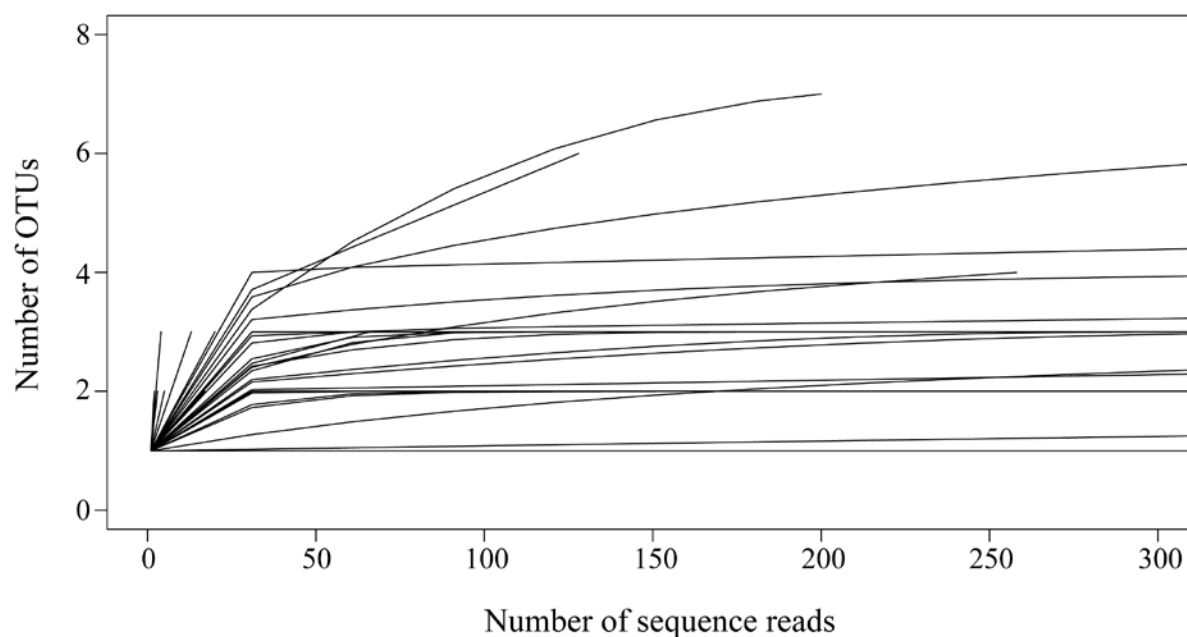
Figure 1 – OTU diversity for arthropods found in ant gut contents. Rarefaction curves indicate the expected OTU richness of ant guts based on 71 samples, given the number of sequence reads. Each individual line represents a sample of the gut contents of 10 worker ants from the same colony.

4.2.4 Statistical analyses

All analyses were performed in R version 3.4.2 (R Core Team 2015), I report significance at the *P* <0.05 level.

Stable isotope analysis of ant diet

To investigate differences for $\delta^{15}N$ and $\delta^{13}C$ between species I used two linear mixed-effect models using the 'lme4' package (Bates et al., 2015) in R, with $\delta^{15}N$ and $\delta^{13}C$ as the response variables. Top soil type (organic, loam, sand or clay), and collection locations (ground, plant or coarse-woody debris), were also included in the model as explanatory variables, and site was included as a random effect. I conducted model selection by comparing models using maximum likelihood tests until only significant variables remained. I then conducted pairwise comparisons between different ant species using the 'pairs' function in the 'emmeans' package (Lenth, 2018), using the 'fdr' *P*-adjustment correction (Benjamini & Hochberg, 1995).

I also compared niche width using a Bayesian approach that uses multivariate ellipse-based metrics using the 'SIBER' package (Jackson, Inger, Parnell, & Bearhop, 2011). This approach allows for comparisons between different species among and between different communities, even when sample sizes differ, by creating standard ellipse areas (SEAs), which are comparable to standard deviation in univariate cases (Jackson et al., 2011). SEA_C ($_C$ denotes that the SEA was corrected for small sample size), contains the core aspects of a population's niche and is less sensitive to outliers and small sample sizes. I calculated the differences between species' SEA_C using Bayesian inference (SEA_B), using 2 chains of 100 000 iterations, with a burn-in of 1000 and thinning of 10.

Co-occurrence patterns

To assess species associations, I used occurrence data at lure stations for the species sampled within open-canopy ecosystems (i.e., short-stature, without a continuous canopy) previously collected (Chapter 3). This dataset is largely representative of ant species I collected for dietary analysis, with the exception of *Linepithema humile*. There is strong evidence of *L. humile* suppressing other ant species where it occurs (Human & Gordon, 1996; Sanders et al., 2001; Suarez et al., 1998), and at the collection sites where *L. humile* was present, I only observed other species co-occurring with *L. humile* at the periphery of the invaded area, suggesting a possible negative associations between *L. humile* and other ant species at these sites.

I used a probabilistic model approach using the 'cooccur' package in R (Griffith, Veech, & Marsh, 2016). Of the total pair combinations (*N*=120), 81.6% had an expected co-occurrence of <1 so were removed from the analysis and 22 pairs were analysed (Veech, 2013). This analysis allows for comparison of the 'observed co-occurrence' to the 'expected co-occurrence', where the latter is the product of the two species' probability multiplied by the number of sampling sites, or lure stations in this case: $E(N_{1,2}) = P(1) \times P(2) \times N$ (Veech, 2013). Overall patterns of co-occurrence can then be tested between species pairs, calculating the probability that the observed co-occurrence is great than the expected frequency (a positive association), less than the expected frequency (a negative association), or random.

4.3 Results

4.3.1 Stable isotope analyses of ant diet

I processed 158 ant samples for stable isotope analysis, consisting of 13 species (9 exotic, 4 native) (Table 2). δ^{15}N values could only be obtained for 153 samples. Isotope values for ants encompassed a broad range; from -17.04‰ (*Nylanderia* sp.) to -28.59‰ (*Tetramorium grassii*) for δ^{13}C and 1.40‰ (*Iridomyrmex suchieri*) to 15.88‰ (*Mayriella abstinens*) for δ^{15}N. Overall, ants fed mainly across two trophic levels as primary and secondary consumers (Fig. 2).

δ^{13}C values were not affected by vegetation cover or soil type; however, δ^{13}C was affected by sampling location, with ants collected from plants (*df*= 144.86, *t ratio*= 2.88, *P*= 0.005) and the ground (*df*= 143.49, *t ratio*= 2.195, *P*= 0.03) associated with higher δ^{13}C values than ants collected from coarse woody debris. Estimated marginal means for δ^{13}C values were not found to significantly differ between any species pair.

δ^{15}N values were not affected by soil type or location, however, increasing vegetation cover was found to negatively affect δ^{15}N values. Estimated marginal means for δ^{15}N values were found to significantly differ between three species pairs, *Technomyrmex jocosus* and *T. grassii* (*df*= 155.72, *t ratio*= 3.49, *P*= 0.03), *T. jocosus* and *Austroponera castanea* (native sp.) (*df*= 155.34, *t ratio*= 3.26, *P*= 0.03), and *T. jocosus* and *Rhytidoponera chalybaea* (*df*= 155.72, *t ratio*= 3.49, *P*= 0.03); *T. jocosus* was found to have the lowest δ^{15}N values of all ant species.

Isotopic niche overlap between species

The isotopic niche, which combines δ^{13}C and δ^{15}N values and is measured by the standard ellipse area (SEAc) (Newsome, del Rio, Bearhop, & Phillips, 2007), overlapped between almost all ant species and the predator reference group; however, overlap with the predator reference group was low (≤10%) for *Linepithema humile* and *Monomorium smithii*, and absent for *Technomyrmex jocosus* (Table 3). In contrast, SEAc values differed clearly between the primary consumer reference groups and all ant species except *T. jocosus*; although the overlap between the primary consumer reference group and *T. jocosus* was low (3%). The largest overlap in isotope niche was observed between the invasive species *Ochetellus glaber* and the native *Monomorium antarcticum*. These two species had very similar niche breadth for δ^{15}N, although *M. antarcticum* had a wider δ^{13}C niche breadth (Fig. 3). A relatively large overlap

was also observed between *L. humile* and *Iridomymrex suchieri* (66%), and *Austroponera castanea* and the predator reference group (50%); all other overlaps were <50% (Table 3). *Rhytidoponera chalybaea* and *Austroponera castanea* had the largest and *L. humile* and *T. jocosus* had the smallest SEA_B (Fig. 4).

Co-occurrence patterns

Of the 22 species pairs analysed, random associations represented the majority (90.9%) of co-occurrences between ant species at lure stations. Two significant, negative associations were detected with the probabilistic modelling; between *O. glaber* and *T. jocosus* (both invasive species, see Chapter 3), and between *O. glaber* and the native species, *M. antarcticum.*

4.3.2 DNA analysis of ant diet

A total of 171 samples were sequenced; as expected, the majority of reads belonged to the host, with 99.7% of sequence reads corresponding to ant DNA. Once poor sequence reads (i.e. bacterial/fungal OTUs and unassigned OTUs) were removed, 71 samples yielded practical data (51 OTUs from 19,988 sequences). Among the 51 OTUs, 22 Orders and 34 families were identified (Table 4). There is relatively poor coverage of native New Zealand arthropods in reference databases (Holdaway et al., 2017); therefore, genus- and species-level resolution for many sequences produces misleading identifications and should, therefore, be interpreted with caution.

For instance, sequences for two moth genera *Oenochroma* and *Operophtera* (Lepidoptera: Geometridae) were reported in my samples, neither of which have ever been recorded in New Zealand (R. Hoare, pers. comm.). In contrast, sequences for the endemic dobsonfly *Archichauliodes diversus* (Insecta: Megaloptera: Chauliodinae) were identified in my samples due to sequence data being deposited from a New Zealand freshwater stream macroinvertebrate study (Dowle, Pochon, Banks, Shearer, & Woods, 2016). As this is the only species occurring within the genus *Archichauliodes,* I can be confident in the species-level identification for this particular OTU.

After rarefaction, data from 34 colonies across eight ant species allowed comparison; however, as more than half of these colonies were represented by *Nylanderia* sp., comparative species analyses were not feasible. The class Insecta was dominant in the diet across all ant species

(Fig. 5), with the proportionally more OTUs detected from the orders Coleoptera and Lepidoptera, although Trombiformes mites were also abundant (Fig. 6)

Table 2. Summary of information for ant species and the primary consumer and predator reference groups. N represents the number of colonies $\delta^{13}C$ and $\delta^{15}N$ (in brackets) for which values were obtained. Species in bold are native to New Zealand.

Taxa/Trophic reference group	N	Native/ Exotic	$\delta^{13}C$	$\delta^{15}N$
Dolichoderinae				
Iridomyrmex suchieri	29(28)	Exotic	-22.80±0.39	2.84±0.10
Linepithema humile	14	Exotic	-23.85±0.26	2.44±0.07
Ochetellus glaber	25	Exotic	-24.43±0.39	2.80±0.08
Technomyrmex jocosus	6	Exotic	-24.54±0.33	2.24±0.07
Ectatomminae				
Rhytidoponera chalybaea	5	Exotic	-22.67±0.66	3.12±0.35
Formicinae				
Nylanderia sp.	26(24)	Exotic	-22.76±0.56	2.83±0.08
Myrmicinae				
Huberia striata	1	Native	-25.90	2.73
Monomorium smithii	3	Native	-24.92±0.89	2.99±0.11
Monomorium antarcticum	20	Native	-24.30±0.52	2.80±0.09
Pheidole rugosula	8(6)	Exotic	-20.51±0.76	3.21±0.08
Solenopsis sp.	2(0)	Exotic	-26.30±0.65	-
Tetramorium grassii	16	Exotic	-24.82±0.56	3.11±0.19
Ponerinae				
Austroponera castanea	4	Native	-23.27±1.58	3.29±0.18
Trophic reference group				
Primary consumer	32		-26.55±0.43	1.45±0.12
Predator	42		-23.24±0.43	3.16±0.08
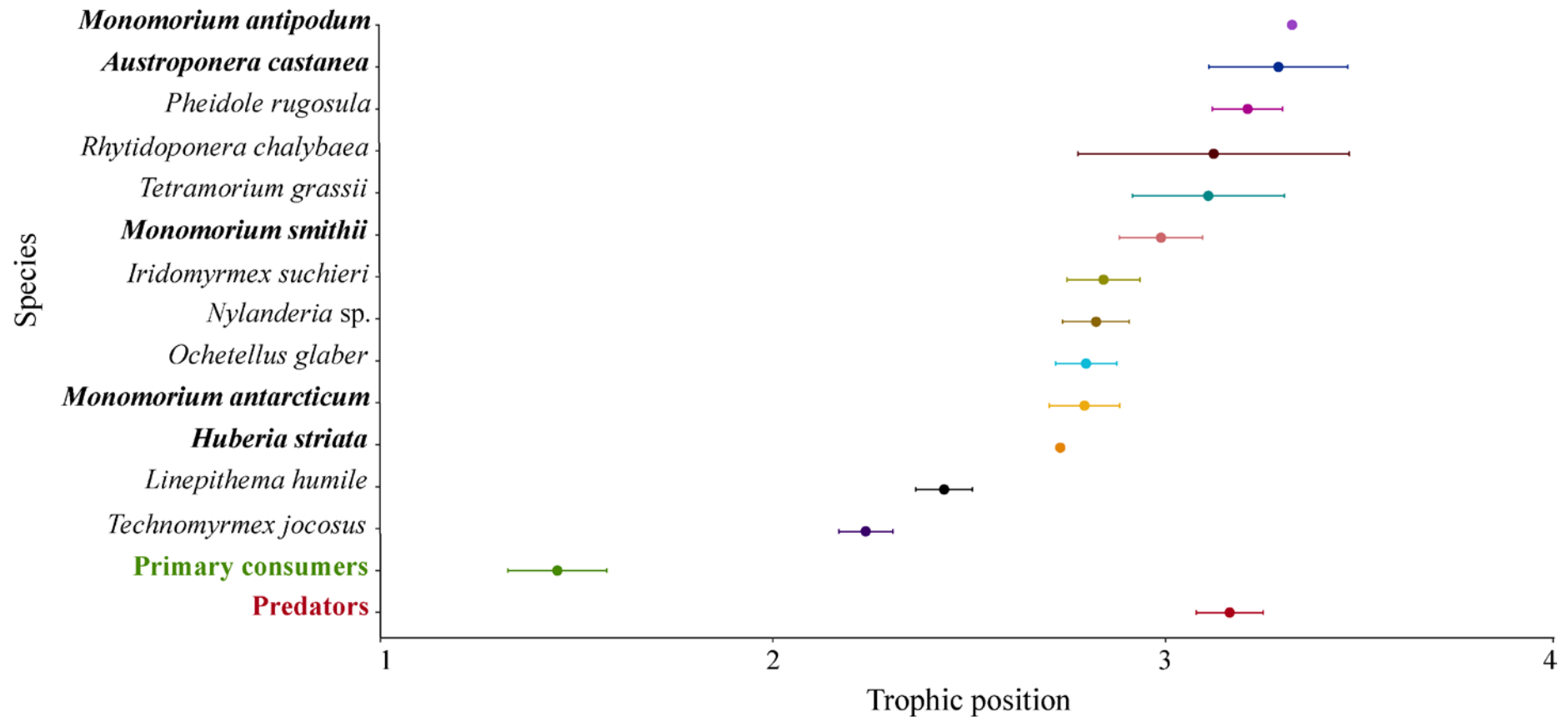
Figure 2 – Mean (±SE) trophic position of ants, primary consumers and predacious arthropods, sampled across open-canopy (short stature vegetation without a continuous tree canopy) sites in Auckland, New Zealand. Trophic position represents a continuous score, with lower numbers reflecting lower trophic position (i.e. primary consumers). Species in bold are native to New Zealand.

Table 3. Percentage of isotopic niche area overlap, as determined through standard ellipse area (SEAc), between different ant species. Species in bold are native to New Zealand

	Isuc	Lhum	Nyl	Ogla	Tgra	Tjos	Rcha	Prug	**Acas**	**Mant**	**Msmi**	Prim
Isuc												
Lhum	18											
Nyl	66	12										
Ogla	33	10	38									
Tgra	21	8	22	39								
Tjos	1	21	0	0	2							
Rcha	40	4	32	14	21	0						
Prug	21	0	17	4	4	0	17					
Acas	10	0	42	12	27	0	24	16				
Mant	39	9	43	82	28	0	18	7	11			
Msmi	3	0	1	19	10	0	4	3	14	16		
Prim	0	0	0	0	0	3	0	0	0	0	0	
Pred	34	2	29	31	37	0	44	22	50	33	10	0

Isuc: *Iridomyrmex suchieri*; Lhum: *Linepithema humile*; Nyl: *Nylanderia* sp; Ogla: *Ochtellus glaber*; Tgra: *Tetramorium grassii*; Tjos: *Technomyrmex jocosus*; Rcha: *Rhytidoponera chalybaea*; Prug: *Phediole rugosula*; **Acas: *Austoponera castanea*; Mant: *Monomorium antarcticum***; **Msmi: *Monomorium smithii***; Prim: Primary consumers; Pred: Predators. Native species are in bold.
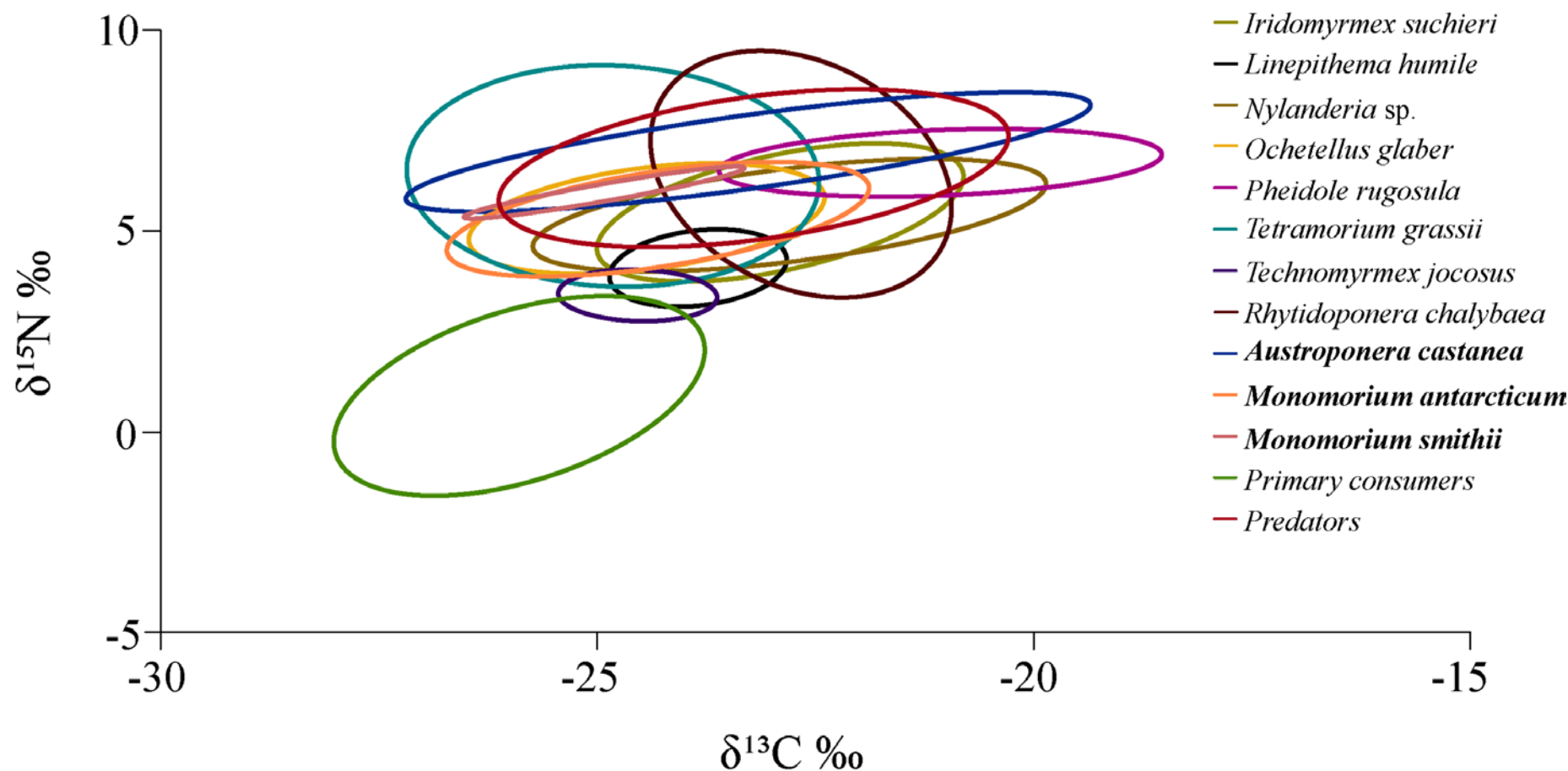
Figure 3 – Stable isotope bioplot illustrating the isotopic niche of different ant species and two functional groups (primary consumers, predators) in Auckland, New Zealand. Ellipses represent the isotopic niche width of 40% (SIBER default) corrected for small sample size (SEAc; Jackson et al 2011). Species in bold are native to New Zealand.
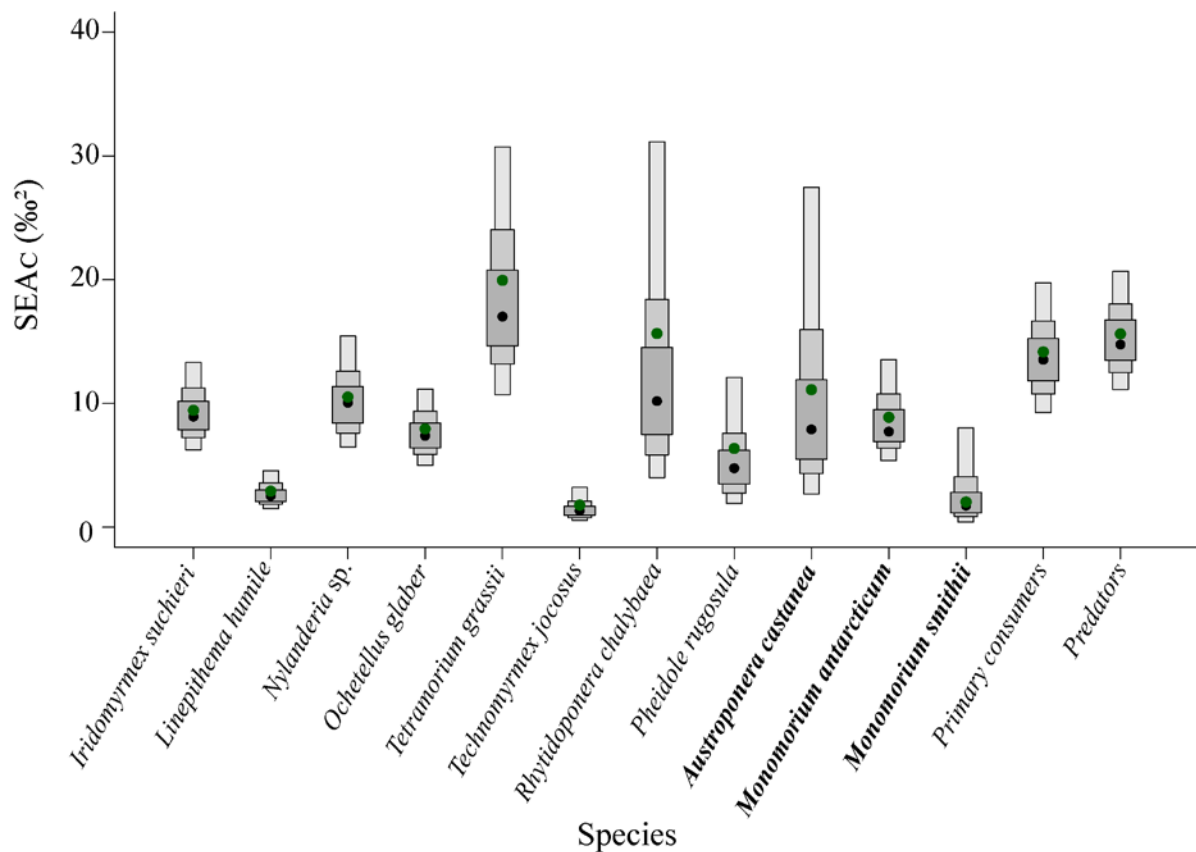
Figure 4 – Mean and variation in the isotopic niche for each ant species, primary consumer, and predator reference groups as measured by the standard ellipse area Bayesian estimations (SEAb). Mode (black dots) and probability of data distribution (50% dark grey boxes, 75% intermediate grey boxes, 95% light grey boxes). The mean standard ellipse area corrected for small sample size (SEAc) is also shown as green dots. Species in bold are native to New Zealand.

After rarefaction, data from 34 colonies across eight ant species allowed comparison; however, as more than half of these colonies were represented by *Nylanderia* sp., comparative species analyses were not feasible. The class Insecta was dominant in the diet across all ant species (Fig. 5), with the proportionally more OTUs detected from the orders Coleoptera and Lepidoptera, although Trombiformes mites were also abundant (Fig. 6).

Table 4. List of taxonomic ranks identified in gut contents for all samples sequenced (all ant species combined), as detected through amplification of the COI gene region. Family-level rank was included if present in New Zealand

Class	Order	Family
Arachnida	Araneae	Theridiidae
	Mesostigmata	Parasitidae
	Sarcoptiformes	Acaridae
		Brachypylina
		Ceratoppiidae
	Trombidiformes	Eupodidae
Chromadorea	Rhabditida	Steinernematidae
Clitellata	Haplotaxida	Lumbricidae
Collembola	Entomobryomorpha	Entomobryidae
		Isotomidae
	Symphypleona	Sminthuridae
Diplopoda	Helminthomorpha	Julidae
Gastropoda	Stylommatophora	Helicidae
Insecta	Archaeognatha	
	Coleoptera	Chrysomelidae
		Coccinellidae
		Scarabaeidae
		Staphylinidae
	Diptera	Agromyzidae
		Anthomyiidae
		Calliphoridae
		Cecidomyiidae
		Chironomidae
		Culicidae
		Muscidae
		Mydidae
		Sciaridae
		Tachinidae
	Hemiptera	Aphididae
	Hymenoptera	Apidae
		Braconidae
	Lepidoptera	Geometridae
		Tineidae
	Megaloptera	Corydalidae
	Orthoptera	Gryllidae
	Psocodea	
Malacostraca	Amphipoda	Talitridae
Protura	Sinentomata	
Reptilia	Squamata	

Figure 5 – Proportion of OTUs identified at Order level occurring within the gut contents of different ant species, after rarefying data, based on amplification of the COI gene region. Numbers in brackets after species names correspond to sample size, where each sample comes from an independent colony consisting of the gut contents for 10 individual worker ants.

Figure 6 – Proportion of OTUs identified at the Family level occurring within the gut contents of different ant species, after rarefying data, based on amplification of the COI gene region. Numbers in b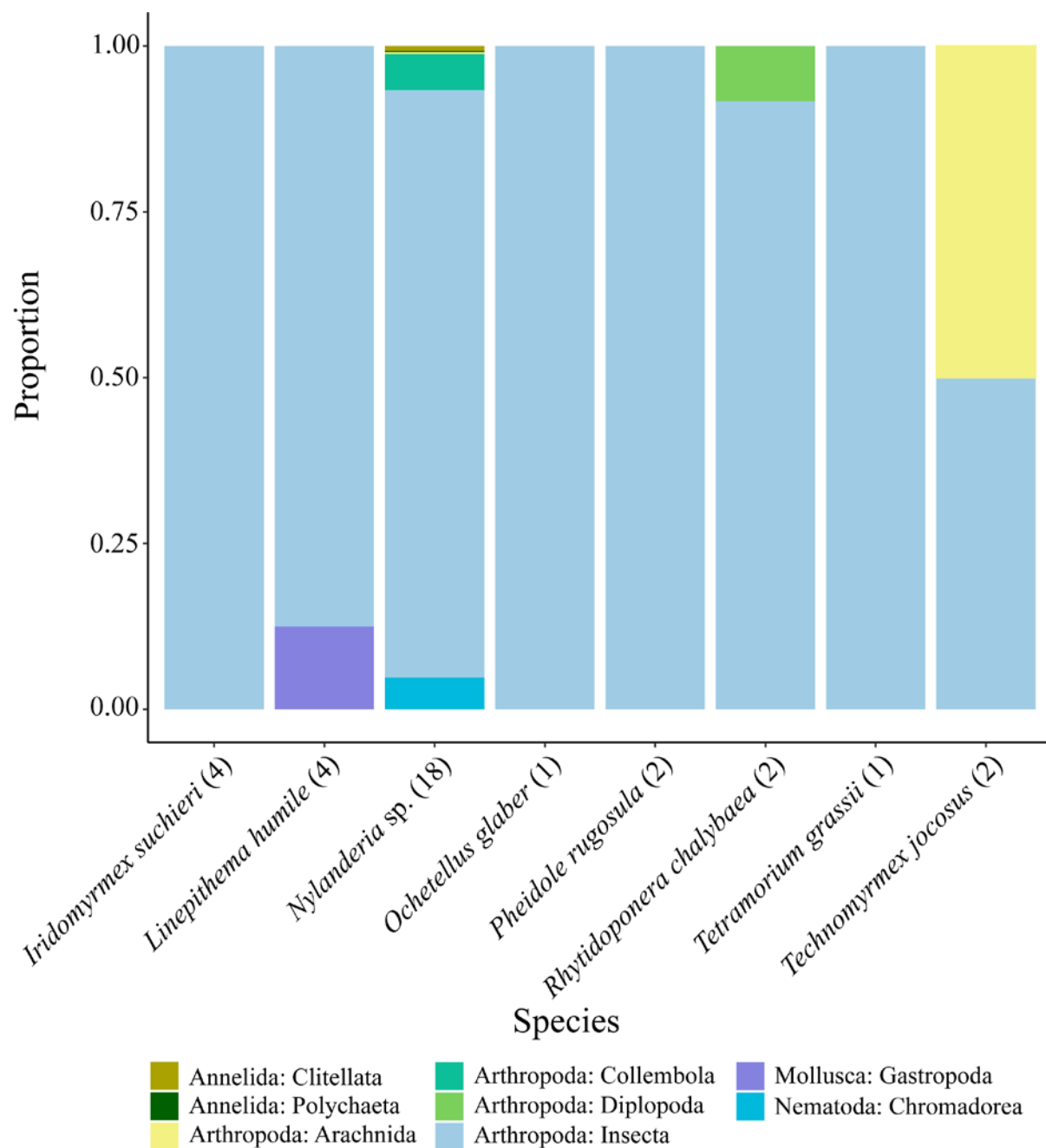rackets after species names correspond to sample size, where each sample comes from an independent colony consisting of the gut contents for 10 individual worker ants.
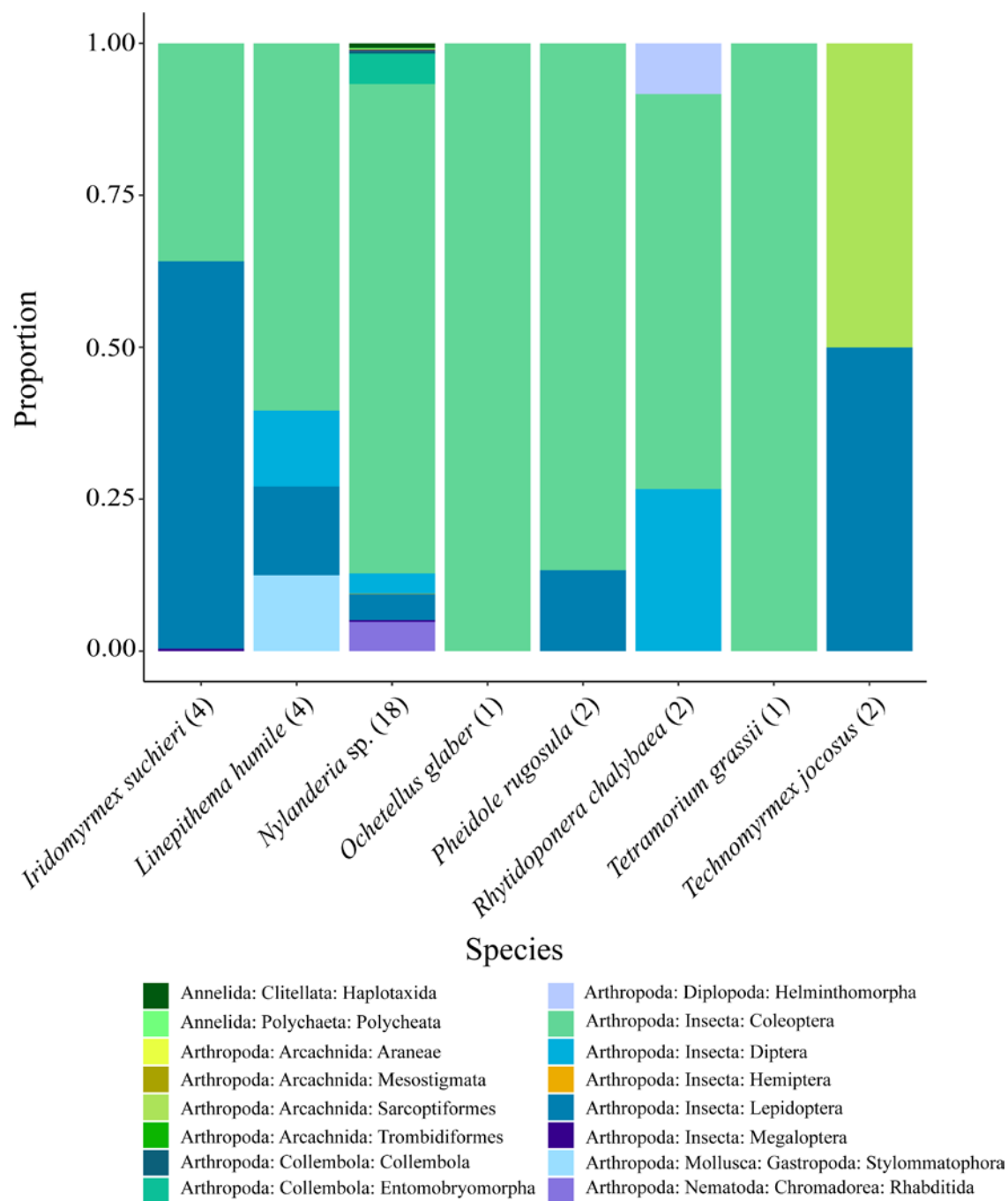
4.4 Discussion

4.4.1 Trophic breadth of invasive ants

I predicted that the dominant invasive ant species would exhibit a relatively broad dietary niche, as revealed through their isotopic signatures. My findings partially verified this, with both *Tetramorium grassii* and *Rhytidoponera chalybaea*, found to exhibit relatively broad isotopic niches and variation within trophic position. In contrast, other dominant species showed less variation, particularly within their nitrogen signatures. Results suggest *Linepithema humile* and *Technomyrmex jocosus* obtain nitrogen from lower trophic levels, and that these species are more reliant on plant-derived food resources than through scavenging or predation of other animal species. This finding is well supported in the literature, at least for *L. humile*, where outside of their native range, they exhibit a dietary shift from obtaining nitrogen from animal-sources to plant-sources (Tillberg et al., 2007). The utilisation of carbohydrate-rich resources has been implicated in invasion success of ants (Rowles & Silverman, 2009), thus finding *T. jocosus* exhibits a similar feeding habit to *L. humile*, may justify the inclusion of monitoring this species in pest surveillance programmes. Indeed, documentation of a seemingly benign species, occurring at low levels until honeydew availability significantly increased through the arrival of invasive scale insects, provides perhaps one of the most exceptional examples of ecological impact via an invasive ant species (Green et al., 2011). Since species in the genus *Technomyrmex* tend to be arboreal or sub arboreal (Heterick, 2009), the lower trophic position for *T. jocosus*, may reflect their habitat associations with vegetation, with plant-based food sources (nectar) being more accessible. This idea is supported by the finding of a negative relationship between delta N signatures and vegetation coverage, which is unsurprising given nitrogen is a limiting nutrient in plant-based diets (Mattson, 1980). Because many invasive ant species feed opportunistically (Holway et al., 2002a), colonies in close proximity to vegetated areas are more likely to have access to honeydew and plant nectar and exploit such resources.

4.4.2 Competition through invasion

The establishment of invasive species is often associated with the suppression of other ant species (Holway et al., 2002a). Whilst I found mainly random associations between ant species, a significant negative association between *Ochetellus glaber* and both *T. jocosus* and *Monomorium antarcticum* were revealed. Moreover, *Ochetellus glaber* and *M. antarcticum*

were also found to feed at the same trophic position and had an 82% overlap in their isotopic niches. This dietary overlap, together with the reduced likelihood of co-occurrence, suggest competitive exclusion for the same dietary niche. *Monomorium antarcticum* represents New Zealand's most ubiquitous native ant species (although likely represents a species complex, see: Dann, 2008; Wang & Lester, 2004), occurring across a range of habitats in both the North and South Islands, as well as many offshore islands. This species has been demonstrated to exhibit aggressive behaviour towards other ant species, and in some contexts may be able to suppress small colonies of *L. humile* (Sagata & Lester, 2009). Although *M. antarcticum* may suppress small invading colonies (potentially influencing the earlier stages of invasion), any competitive edge is expected to diminish if the *L. humile* colony can increase beyond a threshold (Sagata & Lester, 2009)**.** It is possible the negative association between *M. antarcticum* and *O. glaber* reflects a similar competitive exclusion of the former species by the latter, which relative to *M. antarcticum,* consists of vastly more worker ants foraging outside of the nest (A. Probert, pers. obs.).

Interestingly, *O. glaber* and *T. jocosus* were found to have zero overlap in isotopic niche. Compared to *T. jocosus,* the diet of *O. glaber* was enriched with nitrogen, even though *O. glaber* is a dominant floral visitor, commonly feeding on nectar (Chapter 5). The data I used to obtain association patterns were largely representative of the exotic ant communities occurring within the ecosystems in which I sampled, with the exception of *L. humile*, which was absent in the occurrence data I used. However, *L. humile* has been widely documented to suppress other ant species in the areas it invades (Holway, 2005; Inoue et al., 2015; Stringer et al., 2009), and for this study, was found to only co-occur with other ant species at the very periphery of its invaded habitat (A. Probert, pers. obs).

Three of the most dominant species occurring within open-canopy, short stature, indigenous ecosystems, *Iridomyrmex suchieri*, *Ochetellus glaber* and *Nylanderia* sp. (Chapter 3), were found to feed at the same trophic position, with 66% overlap in the isotopic niche of *I. suchieri* and *Nylanderia* sp. However, I found no evidence of negative associations between any of these species, even though they co-occurred at some sites. Although competition for similar niches may be expected to influence co-existence patterns at the sites they occurred, it is possible that habitat complexity facilitates the co-occurrence of exotic ant species utilising similar dietary niches (Sarty, Abbott, & Lester, 2006). In contrast to the invasive species, the

trophic position for three of the five native species overlapped with the predacious reference group, suggesting native ant species may be more reliant on animal-based sources of nitrogen than invasive ants. Whether this finding is due to high competition for nectar-based resources by invasive ants, or simply dietary preferences, requires further investigation, but lends support the idea that carbohydrate availability can support invasive ant dominance in some ecosystems (O'Dowd et al., 2003; Tillberg et al., 2007).

4.4.3 DNA analysis of ant diet

I took the approach advocated by (Piñol et al., 2014), to obtain prey sequence data without the use of blocking probes for invertebrate prey. Once non-target DNA was filtered, I was left with 19,000 sequence reads. The majority of ant colonies sampled (58%, n=100/171) failed to amplify anything other than ant and non-target DNA (i.e. bacteria, fungi). There are several possible explanations for this; it is possible that the ant guts did not contain amplifiable material because either it was absent or highly degraded. Although other studies have found evidence of PCR inhibition related to the crop structure of ants (Penn, Chapman, & Harwood, 2016), I attempted to reduce this by dissecting out the gut contents from the digestive tract. In some cases, dissections may have still contained parts of the digestive tract; however, in pilot samples there was no improvement in amplification even with the addition of BSA. Whilst I selected primers intended to amplify a broad range of taxa as evidenced through the literature (Brandon-Mong et al., 2015), primers will inherently preferentially bind to sites introducing a bias that is difficult to overcome without adding increasingly specific primer pairs. This highlights the uncertainty in identifying the actual diet of consumers. Nevertheless, metabarcoding provides an invaluable tool to rapidly assess diet within a snapshot of time.

An additional problem associated with the metabarcoding was the low comparable sample sizes after rarefying data. Despite this, the sequence data revealed novel information of the diets for several invasive ant species and provided taxa-specific information unavailable through stable isotope analysis alone. Coleoptera was the dominant food source for six of the eight species (n=28 samples), with five OTUs identified from four families of Coleoptera. Lepidoptera was the second most dominant taxa, comprising a high proportion of sequence reads for the diet of *I. suchieri* and *T. jocosus* colonies. Whilst overall diversity within colonies was low, this was not unexpected due to the foraging behaviour of ants. Although it is not possible to distinguish between food sources acquired via predation or scavenging, identifying the arthropods most

likely to be consumed by exotic ants is an important first step to assess the ecological risk of species occurring in natural ecosystems.

4.4.4 Conclusions

A common characteristic for many invasive ant species is their generalist feeding strategy, which is posited to explain, at least in part, the establishment success of species outside of their native ranges (Holway et al., 2002a). I used two different methods to investigate the diet of ants commonly occurring within natural ecosystems to understand the potential impact of dietary-related ecological interactions associated with exotic ants. Stable isotope analyses revealed broader-scale variation in the isotopic niche of exotic ant species compared to native ants, whilst metabarcoding identified some of the invertebrates at risk of predation impacts from invasive ants. The use of metabarcoding to infer fine-scale variation in diet provides a potential tool for future dietary analyses, although it proved difficult to obtain sufficient data to compare the diet of native and exotic species. Overall, these findings contribute to our knowledge of impact of invasive ants in indigenous New Zealand ecosystems. The native ant species *M. antarcticum* appears to be most at risk from *O. glaber*; and *T. jocosus* appears to have a key trait (carbohydrate exploitation) associated with highly invasive ant species, which may have potential implications for surveillance and management.

Chapter 5 — Exotic ants as floral visitors and their effect on floral visitor behaviour

Abstract

Invasive ants frequently exploit floral resources, such as nectar, and exhibit antagonistic behaviour towards other floral visitors, including those providing pollination services. Thus, the presence of ants on flowers comes with an associated risk to floral-visitation, with potential implications for plant and pollinator fitness. I investigated this risk to floral visitors within open-canopy, short-stature vegetation ecosystems, vulnerable to invasion by ants, by observing the use of floral resources by ants and other floral visitors during the Austral spring and summer. Two exotic ant species (*Ochetellus glaber* and *Nylanderia* sp.) were found to dominate ant floral visitation to both exotic and native plants within these ecosystems, and their presence on flowers was associated with fewer visits by honeybees and native bees. Focal observations on harakeke (*Phormium tenax*) flowers indicated neither honey bees nor native bees rejected flowers more often when exotic ants were present; however, the duration of native bee visits were 1.4 times longer when ants were absent. Furthermore, in an artificial inflorescence experiment, honey bees (*Apis mellifera*) were found to be five times more likely to accept inflorescences, and spend 65% longer on inflorescences, when ants were excluded from inflorescences. This study shows that exotic ants can influence the visitation behaviour of important pollinators, although future work is required to determine if this translates into negative impacts on plant fitness.

5.1 Introduction

Biological invasions can alter the ecological interactions among native biodiversity, threatening native communities and ecosystem functioning (Kumschick et al., 2015). Newly established species may disrupt ecological processes and/or introduce novel interactions within their recipient ecosystems (Tylianakis, Didham, Bascompte, & Wardle, 2008). Interactions between native and invasive biota may be direct, or indirect, resulting in impacts that are difficult to observe and measure, particularly given the constraints associated with the time scales of most research studies (Simberloff et al., 2013). Consequently, despite a wide recognition of the importance in understanding the impacts of invasive species to ecosystem processes, predicting potential risk associated with established exotic species can be challenging. Invasive species have been highlighted as a considerable threat to important ecological processes facilitated by mutualisms, such as seed dispersal and pollination, by altering interaction networks (Traveset & Richardson, 2014; Vanbergen, Espíndola, & Aizen, 2018). From a risk management perspective, it is thus necessary to consider the potential threat of invasive species to ecosystem processes in at-risk ecosystems rather than biodiversity measures alone.

Invasive ants have a high capacity to alter ecosystem processes, due to their dominant role in most terrestrial ecosystems, influence on ecosystem functioning, and engagement in numerous interactions within their environment (Hölldobler & Wilson, 1990). A key trait associated with the success of invasive ants is their exploitation of carbohydrate-rich resources, such as honeydew and nectar (Holway et al., 2002a). Although ants are often common floral visitors that consume nectar resources, they are generally considered poor pollinators due to a suite of behavioural, morphological and physiological adaptations (Rostás & Tautz, 2011). For instance, ant foraging behaviour does not lend itself to effective out-crossing due to their tendency to forage on the same individual plant (Rostás, Bollmann, Saville, & Riedel, 2018). Thus, ants are often considered classic nectar thieves, exploiting plant resources, without providing pollination services (Lach, 2005). Furthermore, invasive ants often display aggressive behaviour towards other floral visitors, which may ultimately impact on both plant and native and exotic floral visitor fitness (Blancafort & Gómez, 2005; Hansen & Müller, 2009; Lach, 2007; Sidhu & Wilson Rankin, 2016); however, the majority of empirical evidence

comes from studies focusing on the Argentine ant (*Linepithema humile*), and only a few other ant species.

The New Zealand ant fauna provides an anomaly in terms of diversity, wherein only 11 native species are present (Don, 2007). In contrast, ~29 species of exotic ants have established in New Zealand (Don, 2007), and are commonly found in urban, or open-canopy, short-stature ecosystems (Chapter 3; Stringer, Stephens, Suckling, & Charles, 2009; Ward & Harris, 2005). Although ants are observed as floral visitors in New Zealand ecosystems (Iwasaki, 2017; Van Noort, 2016), species-level, or indeed native/exotic classifications of ants as floral visitors are uncommon and no studies have focused on the effect of ants on floral-visitation. Therefore, whether exotic ants pose risks to plant and their potential pollinators through competitive mechanisms for floral resources is unknown.

A prerequisite for assessing risk to pollination interactions is to characterise plant-visitation networks, and determine if exotic ants alter visitor patterns and behaviour. In this study, I investigated the influence of exotic ants on floral visitation patterns of both native and exotic arthropods within natural ecosystems. Specifically, I was interested in identifying the potential risk associated with exotic ants to floral visitation, by determining which ant species are common floral visitors in native ecosystems identified as most susceptible to their invasion (Chapter 3). In addition, I wanted to quantify the difference in floral visitation patterns by exotic honey bees (*Apis mellifera*) and native bees (*Leioproctus, Hylaeus* and *Lassioglossum* spp.) when ants were present versus absent. To address these research aims, I conducted observations 1) of floral visitation across the plant community, 2) on a focal plant species, harakeke (*Phormium tenax*), and 3) by experimentally manipulating artificial inflorescences to either allow or exclude ants from accessing the 'flowers'. First, I predicted that fewer visits would be recorded on flowers when ants were present, when compared with flowers when ants were absent. Second, I predicted that observable differences in honey bee and native bee behaviour would be apparent through visitation rate and visit duration when comparing flowers and inflorescences with ants present versus ants absent, wherein visitors would avoid flowers when ants were present.

5.2 Methods

5.2.1 Study sites

For data collection during the Austral summer of 2015/2016, I initially set up one 200 m transect at each of three sites (Anawhata: $36^0$55'34.885''S, $174^0$27'31.974''E, Karekare: $36^0$59'12.049''S, $174^0$28'45.277''E, and Whatipu: $37^0$02'24.889''S, 174^0 30'24.585''E) on the west coast of Auckland, New Zealand. The sites are characterised as natural ecosystems with open-canopy, short-stature vegetation, occurring in transitional zones of coastal scrub to native forest, which have been identified as having a high risk of exotic ant establishment (Chapter 3). Common plant species varied between sites, with dominant native plant species including mānuka (*Leptospermum scoparium*), harakeke (*Phormium tenax*), pōhutukawa (*Metrosideros excelsa*) and karo (*Pittosporum crassifolium*). Common exotic species included myrtle-leaf milk wort (*Polygala myrtifolia) and yellow lupin (Lupinus arboreus*), which has been planted extensively on the west coast to prevent erosion and stabilise sand dune habitats. The regional climate is temperate, with annual means of rainfall and temperature of approximately 1231 mm and 15℃, respectively (Chappell, 2013).

For data collection during the Austral spring/summer of 2016/2017, a 50 m x 200 m plot was constructed at the Karekare site. This sole focus on Karekare in the second season was to take advantage of the higher number of plant-visitor interactions observed in 2015/16 at this site compared to Whatipu and Anawhata and the prevalence of flowering plants.

5.2.2 Ant community sampling

To determine the ant communities at the three sites, I sampled for ants using lures on a monthly basis between October 2015 and March 2016. Sampling commenced between 10:00 and 15:00 during fine weather. During each sampling period, I set up lure stations at 20 m along the transect, resulting in 11 lure stations each month per site. Each lure station consisted of five lure types ensuring that all food types (lipid, protein, carbohydrate) were available for ants with different food preferences: 1 tsp peanut butter, 1 tsp tuna (in spring water), 5 g non-toxic Xstinguish™ (protein + lipid + carbohydrate; Bait Technology Ltd, New Zealand), 5 ml soybean oil (in cotton wool), and 5 ml sugar-water (1:4 ratio, in cotton wool). Each lure was placed in separate 35 ml plastic vials, positioned in a circular arrangement (15 cm diameter) with the lids removed. Lure stations were left for 1 h before collection. Additional hand

collection of ants via direct visual surveying was conducted at each station for 3 min when lures were being collected.

Sampling of the ant community at the Karekare site between October 2016 and March 2017 was conducted using the same lure station and direct visual searching methodology as the previous year; however, lure stations were set up at 25 m intervals across the plot (50 m x 200 m), resulting in a total of 27 lure stations each month for the duration of the 6 month sampling period.

5.2.3 Floral visitation: community scale

To assess the floral visitation patterns at Anawhata, Karekare and Whatipu, all accessible flowering plants 2 m either side of the transect were tagged with a unique code. Observations of floral-visitor interactions on inflorescences of each plant were conducted between 09:00 and 17:00 h, only during periods of fine weather, with low wind. I conducted sampling by first collecting flying invertebrates visiting flowers via sweep netting or directly with a vial, over a 60 second period. After this, ants and other crawling invertebrates were counted and a subset collected off flowers using an entomological aspirator or directly with a vial, for later identification. An exception was made for thrips (Insecta: Thysanoptera), which I did not collect, as they occurred at high densities, and accurate recording and collection of all individuals would have required me to destroy flowers. All collected invertebrates were placed in labelled vials containing 70% ethanol. For easily identifiable species (i.e. honey bees), collections were not always made, and in the case where floral visitors evaded collection, I noted them to the lowest possible taxonomic rank, or group (e.g. native bee, *Vespula* wasp).

Floral visitation patterns at the Karekare plot during the 2016/2017 sampling period used the same approach as the previous year, although the number of inflorescences per plant species was controlled for by only sampling a subset for each observation period. Removal of one weed species, myrtle-leaf milk wort (*P. myrtifolia*) early in the season (accidentally by council staff), meant that some individual plants were removed from the study before the end of their flowering period.

5.2.4 Floral observations: focal species (harakeke)

To determine the effect of exotic ants on floral-visitors at the individual flower scale, I conducted focal observations on harakeke (*P. tenax*). Harakeke occurs throughout New

Zealand from sea level to ~1300 m in altitude, although it is most common in coastal, wetland and riparian areas. An herbaceous plant, with robust leaves that form from fan-like bases, it produces inflorescences that are up to 5 m tall. Each inflorescence consists of a group of pendcules, which carry clusters of between one and five tubular flowers between 25–50 mm long, typical of a bird-pollinated species (Becerra & Lloyd, 1992). The flowers are capable of producing high quantities of nectar; a four year study on Tiritiri Matangi Island in the Hauraki Gulf of Auckland, New Zealand, reported averages of between 135 to 166 µl per flower, with sugar concentrations of between 15.8–20.0% (Craig & Stewart, 1988). Despite harakeke being considered predominately bird pollinated (Craig & Stewart, 1988), frequent visitors include a range of native bee species (Donovan, 2007), and exotic ant species (A. Probert, pers. obs), making it an ideal candidate species assess the effect of ants on floral visitor patterns.

I selected flowers at roughly eyesight level for all observations from inflorescences that either had i) exotic ants present and foraging on the plant and within the focal flower or ii) exotic ants completely absent from the entire inflorescence. I conducted observations at Karekare over the 2016/2017 Austral summer consisting of a 10 minute focal period during which non-ant floral visitation rate and duration were recorded. A total of 137 observations were conducted on 137 individual harakeke flowers (n=91 ants absent, n=46 ants absent). Prior to each observation, I assessed ant traffic by counting the number of ants travelling up or down the peduncle stalk for 30 seconds, and upon commencement of each observation, I counted the number of ants within the flower. Floral visitation data was collected following Sidhu & Wilson Rankin (2016) with flowers considered "accepted" if the floral visitor landed for > 2 seconds, and considered "rejected" if the floral visitor approached < 1 cm from the front of the corolla opening, but did not land, or landed for < 2 seconds. I measured both the visitation rate (number of floral visitor acceptances per observation) and visitation duration (length of each visit in seconds). Visitors were identified to the lowest possible rank, or group (e.g. native bees).

5.2.5 Artificial inflorescence experiment

I conducted a manipulative experiment over three days at Karekare in March 2017, using artificial floral arrangements that could be easily manipulated into two treatments where ants were either able to access the flowers or were excluded (accessible flowers, inaccessible flowers). Each floral arrangement was an 'inflorescence' consisting of plastic stems and 40 individual small, white cloth flowers. They most closely mimicked 'open' type mānuka/kānuka

flowers (Newstrom-Lloyd, 2013). Inflorescences were arranged in pairs (n=20 for each treatment) by inserting the stem directly into the ground at a distance of 50 cm, with at least 8 m separating each pair from neighbouring pairs.

To exclude ants, I applied Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI, USA) to the base of the inflorescences in the inaccessible flowers treatment, rendering these inflorescences inaccessible to ants and other crawling invertebrates. At the beginning of each observation day, each of the 40 flowers within each inflorescence were provided with two drops of sucrose sugar water (2:1) using a Pasteur pipette. To allow ant recruitment, observations commenced 1 hour after sugar water had been applied. Recruitment of ants was observed for all inflorescences in the accessible flowers treatment.

Observations occurred between 1000h and 1800h during periods of fine weather. Each observation period consisted of a ten-minute focal observation, where floral visitor frequency and duration (seconds) was recorded for each inflorescence within a treatment. Each inflorescence within a treatment was observed separately, and the order of observation was randomised. A total of two observations were made for each inflorescence.

Floral visits were recorded as either "accepted" or "rejected". Accepted floral visits were defined as a floral visit of more than 2 seconds, whereas rejected floral visits were when the floral visitor came within less than 1 cm of the inflorescence, but did not land (Sidhu & Wilson Rankin, 2016). The duration of all accepted floral visits was recorded in seconds.

5.2.6 Statistical analyses

All analyses were conducted in R v. 3.4.2 (R Development Team, 2017), data represent mean ±SE, and significance was assessed at $P < 0.05$.

Ant community sampling

To analyse ant community differences, lure sampling data was first converted into a presence/absence matrix. To assess the differences in the ant community between Anawhata, Karekare and Whatipu for the 2015/2016 sampling period, as well as the monthly variation of the ant community for the Karekare 2016/2017 sampling period, I performed: i) classical multidimensional scaling ordination (CMDS, also known as principal co-ordinates analysis),

ii) permutational multivariate analysis of variance (PERMANOVA; Anderson 2001), iii) permutational analysis of multivariate dispersions (PERMDISP; Anderson 2006).

I visually assessed variation within ant community composition using CMDS with the Bray-Curtis dissimilarity. PERMANOVA was used to test whether ant community composition differed between the three sites in 2015/2016, and temporally for Karekare in 2016/2017 using the 'adonis' function of the *vegan* package based on 999 permutations (Oksanen et al., 2018). For the 2015/2016 sampling period, site and month and the site × month interaction were included as fixed effects. For the 2016/2017 sampling period, month was included as a fixed effect. To assess any differences between levels of the fixed effect variables, I conducted pairwise comparisons using the *pairwise adonis* package (Martinez Arbizu, 2017).

To assess the differences in the variability (i.e. dispersion) of ant communities between sites for the 2015/2016 sampling and months for the Karekare 2016/2017 sampling, I applied PERMDISP analyses using the 'betadisper' function of *vegan*, then used the 'permutest' function to compare the mean dispersion between groups based on 999 permutations.

Floral visitation on plant community

Preliminary data analyses of generalised mixed effect models indicated a poor fit, and therefore I employed a Bayesian approach for these analyses. To determine the effect of exotic ants on the visitation of other invertebrates to inflorescences, I used two different structures of Bayesian generalised (non)linear models, implemented with the *brms* package (Bürkner, 2017b). Models were run for 2000 iterations, with a burn-in of 1000, and fitted with weakly informative priors on both fixed and random effects. I assessed chain convergence using the $\hat{R}$ statistic (Gelman & Rubin, 1992), and visualised posterior predictive checks using the *Bayesplot* package (Gabry & Mahr, 2017). Sampling season was included as a separate random effect for all models, to ensure inflorescence number in the second year of sampling was accounted for.

To determine whether the number of exotic ants affected visitation occurrence, I constructed two logistic regression models (family= 'bernoulli' argument); one model with native bee visitation as the binary response variable, and the other with honey bees as the binary response variable. In these models, the number of exotic ants (continuous) was the fixed effect, while

the random effects were the individual sampling season, and the nested site/date/hour and plantspecies/plantID.

To determine whether the presence of ants affected the number of floral visits, I again constructed two models for each taxon using a generalised linear mixed effect model (GLMM) with a zero-inflated poisson distribution and a log link (Bürkner, 2017a). For these models, the number of floral visits was the response variable, the number of exotic ants was the fixed effect, and sampling season, site/date/hour (nested) and plantspecies/plantID (nested) were included as random effects.

Harakeke floral observations

To analyse the effect of ants on harakeke floral visitations, I used a GLMM with a binomial error structure to compare the acceptances relative to rejections at each flower across treatments. To determine the response for native bees and honey bees, data was analysed in two separate models for these groups. The response variable was the ratio of acceptances to rejections for each observation, the number of rejections was the weighted variable, treatment the fixed effect and ant traffic, date and time as random effects.

To assess how visit duration was affected by ant presence on harakeke flowers, I used a GLMM where visit duration (in seconds) was log transformed and used as the response variable, while ant traffic and group (ants absent, ants present) were the fixed effects; and the observation period, time and date were random effects.

Artificial inflorescence experiment

Although I recorded six species of floral visitors during this experiment, the majority of visits were by exotic honey bees (Table 1) and all statistical analyses are limited to honey bee data.

I used two GLMM models to analyse the effect of ant presence on the floral visitation. The first model compared the number of flower 'acceptances' relative to the number of 'rejections' to assess the effect of ant presence on whether a bee accepted or rejected inflorescences. In this model, the ratio of acceptances to rejections for each observation was the response variable, number of rejections was the weighted variable, treatment (i.e., accessible flowers vs. inaccessible flowers) was the dependent variable, and individual inflorescence was assigned as a random effect. The second model used the same structure, but with visit duration (seconds)

as the response variable, to assess how visit duration was affected by ant presence. In the "accessible flowers" treatment, I used a second GLMM to determine if different ant species affected visitation duration. I used visit duration (in seconds) as the response variable, ant species as the fixed effect, and the floral arrangement as a random effect, as repeated observations were made. I recorded five different ant species (four exotic and one native) on flowers. All specimens were kept in 75% ethanol and later identified to species level using a key to New Zealand ant species (Landcare Research, 2016).

Table 1 – Percentage of total visits by taxa for the artificial inflorescence flowers accessible to ants compared to flowers inaccessible to ants.

	% of total insect visits (ants present)	% of total insect visits (ants absent)
Apis mellifera	79	83
Vespula sp.	17	13
Diptera	2	2
Polistes chinensis	2	1
Hylaeus sp.	0	1
Coptomma variegatum	0	1

5.3 Results

5.3.1 Ant community from lure stations

Ant communities were different among the three sites (Fig. 1; $F = 3.42$, $df = 2$, $P = 0.002$); however, there was no difference between months ($F = 1.18$, $df = 4$, $P = 0.269$), nor the site*month interaction ($F = 0.75$, $df = 8$, $P = 0.859$). Dispersion of the ant community was different between sites ($F = 3.44$, $df = 2$, $P = 0.024$) but not months ($F = 3.44$, $df = 2$, $P = 0.050$). Sampling detected the highest number of native species at Anawhata and exotic species at Karekare (Table 2).

Table 2 – Detection of ant species through ground lure sampling at three sites and through collection on flowers. X indicates the species was detected at the site either through ground lure sampling or via collection from flowers. Native ant species are in bold.

	Anawhata		Karekare		Whatipu	
	Lure	Flower	Lure	Flower	Lure	Flower
Iridomyrmex suchieri				x		x
Mayriella abstinens			x			
Nylanderia sp.	x	x	x	x	x	x
Ochetellus glaber	x	x	x	x	x	x
Rhytidoponera chalybaea			x			
Technomyrmex jocosus	x					
Tetramorium grassii	x		x		x	
Monomorium antarcticum	x	x			x	x
Monomorium antipodum	x			x		
Monomorium smithiii			x	x		
Huberia striata	x					

Sampling at Karekare during 2016/2017 indicated that spring and early summer ant community composition differed to later summer months (Fig. 2; F= 5.16, df= 5, P = 0.001), with October and November being different to all subsequent months (Table 3). However, dispersion did not differ between months (F = 1.21, df = 5, P= 0.301). SIMPER analyses revealed the two dominant exotic ant species, *Ochetellus glaber* and *Nylanderia* sp. accounted for the largest variation between months.
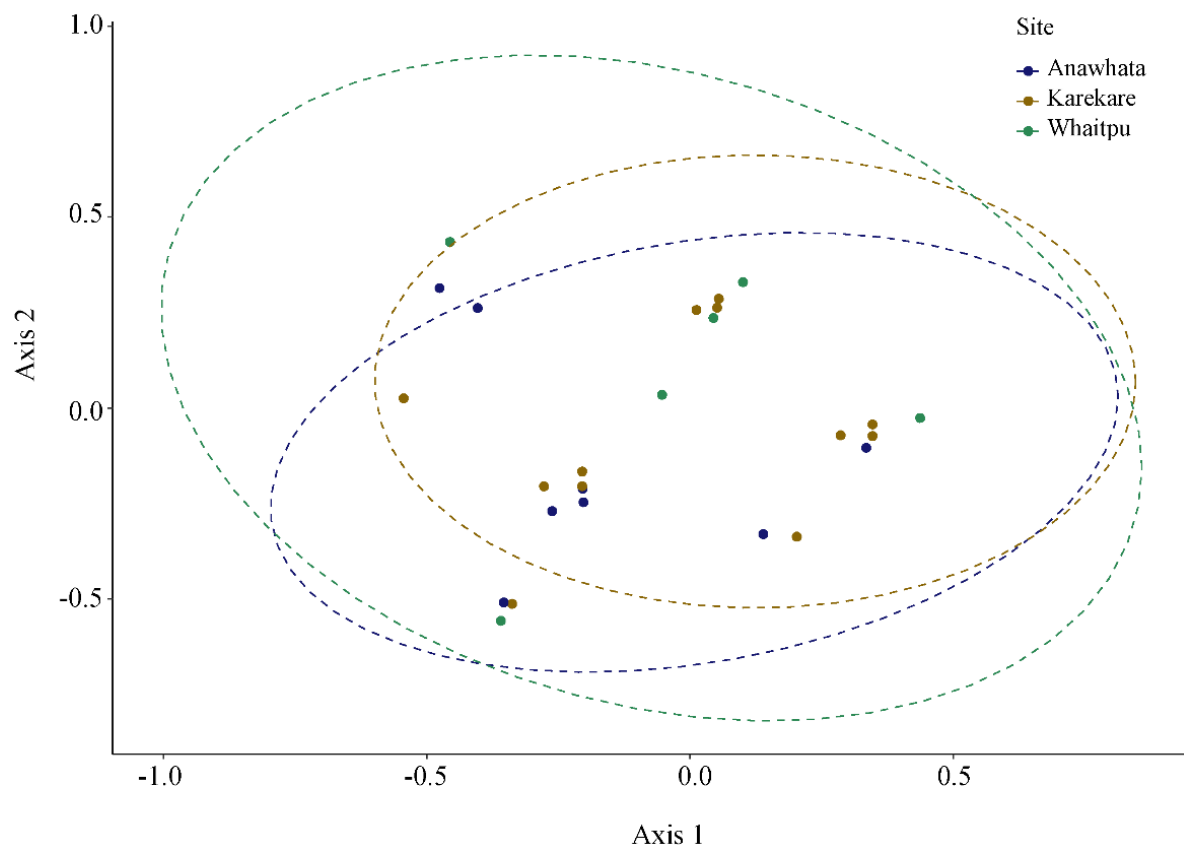
Figure 1 – Ordination of the ant community detected using lures for three sites, Anawhata, Karekare and Whatipu from 2015/16 Austral summer. The dotted ellipses denote 95% CIs for each site.
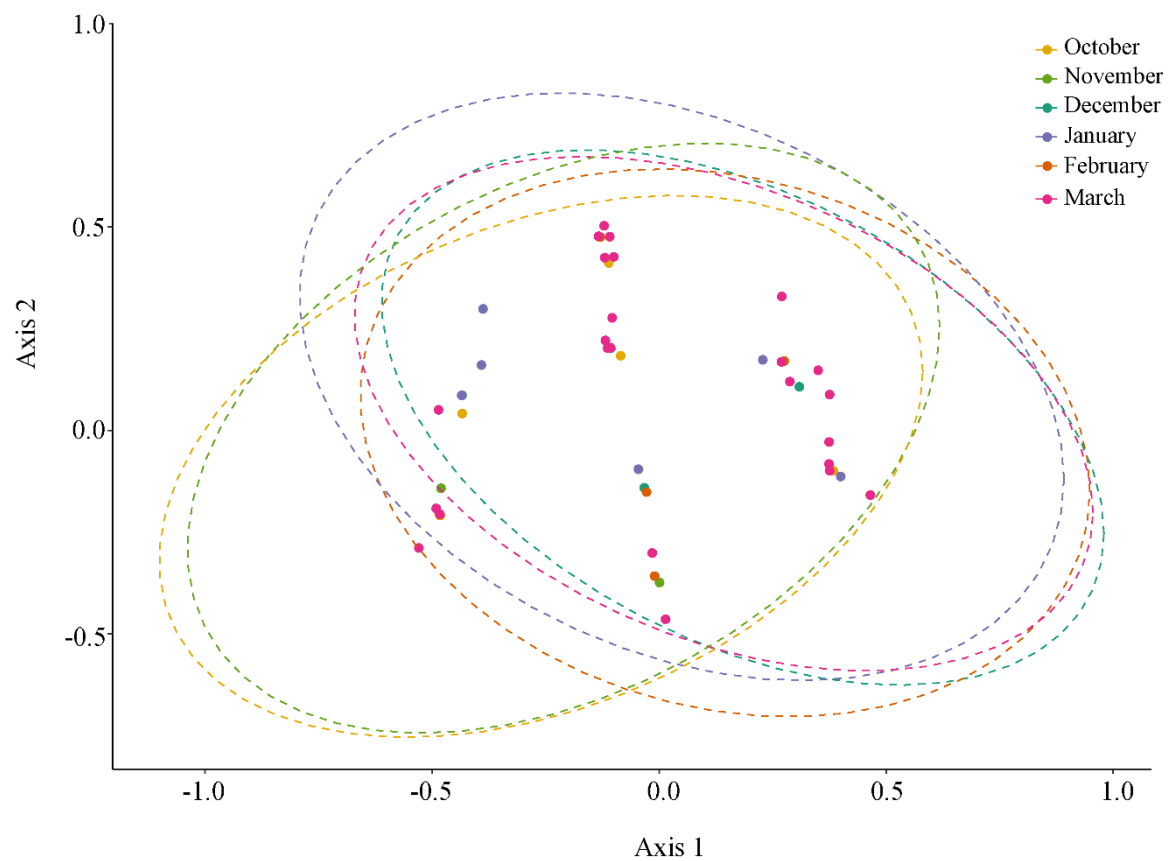
Figure 2 – Ordination plot detailing the variation of the ant community determined using lures at Karekare based on monthly sampling over the 2016/17 Austral spring and summer.

Table 3 – Results of pairwise comparisons between months at Karekare for ant community composition.

Comparison	F value	*P* value
October – November	3.16	0.420
October – December	8.84	0.015*
October – January	5.60	0.030*
October – February	8.98	0.015*
October – March	8.90	0.015*
November – December	9.39	0.015*
November – January	4.17	0.060
November – February	7.55	0.015*
November – March	6.95	0.015*
December – January	1.54	1.0
December – February	1.34	1.0
December – March	1.86	1.0
January – February	1.83	1.0
January – March	1.72	1.0
February – March	1.17	1.0

* Denotes significance after bonferroni adjustments

5.3.2 Floral visitation on plant community

Sampling across all three sites (2015/16) detected a total of 2390 flower visitor interactions, with 1012 of them being non-ant floral visitor interactions. However, ants were visitors in 11% of observations conducted at Anawhata, 12% at Whatipu, and 40% at Karekare. The ant species dominating flowering visitation across all three sites were *O. glaber* and *Nylanderia* sp. More intensive sampling at Karekare during 2016/2017 resulted in a total of 4500 flower visitor interactions; 33% were non-ant floral visitor interactions.

The probability of a visit occurring by either native bees or honey bees showed a negative relationship with increasing numbers of both *O. glaber* and *Nylanderia* sp. (Table 4; Fig. 3). The frequency of visits by native bees and honey bees was also found to be negatively affected by both ant species, with the exception of honey bees when *O. glaber* was present, where there was no effect (Fig. 4). In all cases data showed large variances, except when assessing the probability of a native bee visitation in response to *O. glaber* abundance.

Floral-visitor networks with or without exotic ants showed different interaction patterns (Fig. 5). For example, native bees dominated floral visitor observations when ants were not present on flowers, particularly at harakeke. *Ochetellus glaber* and *Nylanderia* sp. had the highest number of interactions compared to the other exotic ant species. Almost all observations conducted on *Vicia sativa*, a sprawling annual herb, included interactions with exotic ant species (*Nylanderia* sp. or *Tetramorium grassii*). Exotic ants were present for the majority of *Lilium formosanum* (exotic), and *Parsonsia heterophylla* (native) observations. All observations for *Agapanthus praecox* included exotic ant interactions, whereas no *Lycium ferocissimum* observations included any ant (exotic or native) interactions.

Table 4 – Average estimates and 95% posterior credible intervals (of Bayesian posterior distributions) for fitted parameters for the different models

Response variable	Ant species	B	Lower CI	Upper CI	R^2
Binary response models					
Native bee	*O. glaber*	-0.12	-0.16	-0.08	0.57
Native bee	*Nylanderia* sp.	-0.13	-0.26	-0.01	0.54
Honey bee	*O. glaber*	-0.02	-0.05	0.02	0.41
Honey bee	*Nylanderia* sp.	-0.16	-0.32	-0.02	0.41
Frequency response models					
Native bee	*O. glaber*	-0.01	-0.02	0.00	0.51
Native bee	*Nylanderia* sp.	-0.05	-0.12	0.01	0.51
Honey bee	*O. glaber*	0.00	-0.02	0.02	0.50
Honey bee	*Nylanderia* sp.	-0.05	-0.14	0.03	0.51
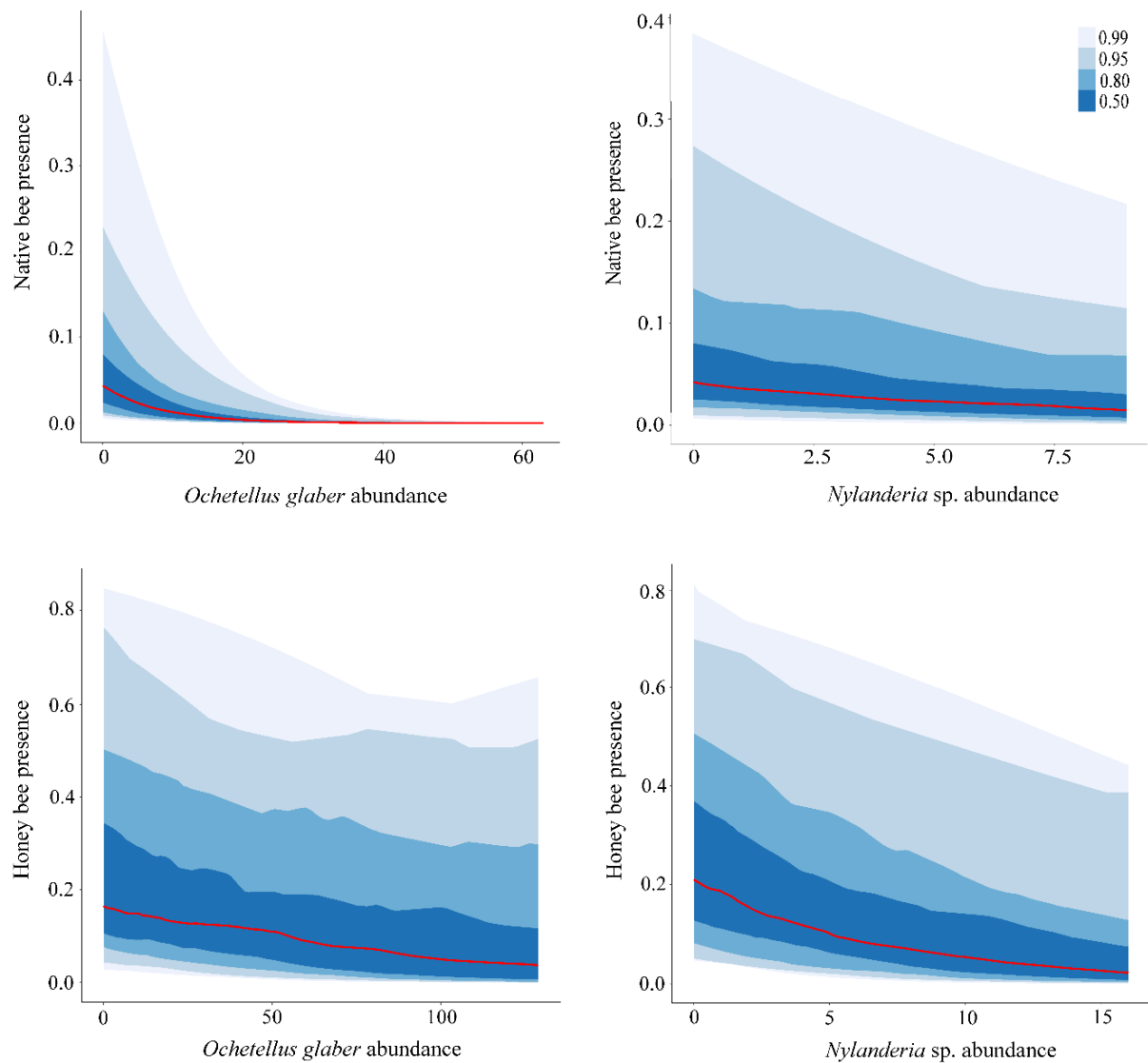
Figure 3 – Probability of a visit occurring from native bees and exotic honey bees to inflorescences across the plant community in response to two species of exotic ant, *Ochetellus glaber* and *Nylanderia* sp, based on posterior predictions (red line). Note different scales on the x and y axes. Different colours correspond to the 0.50, 0.80, 0.95 and 0.99 posterior credible intervals extracted from Hamiltonian Markov Chain Monte Carlo samples from Bayesian analysis.
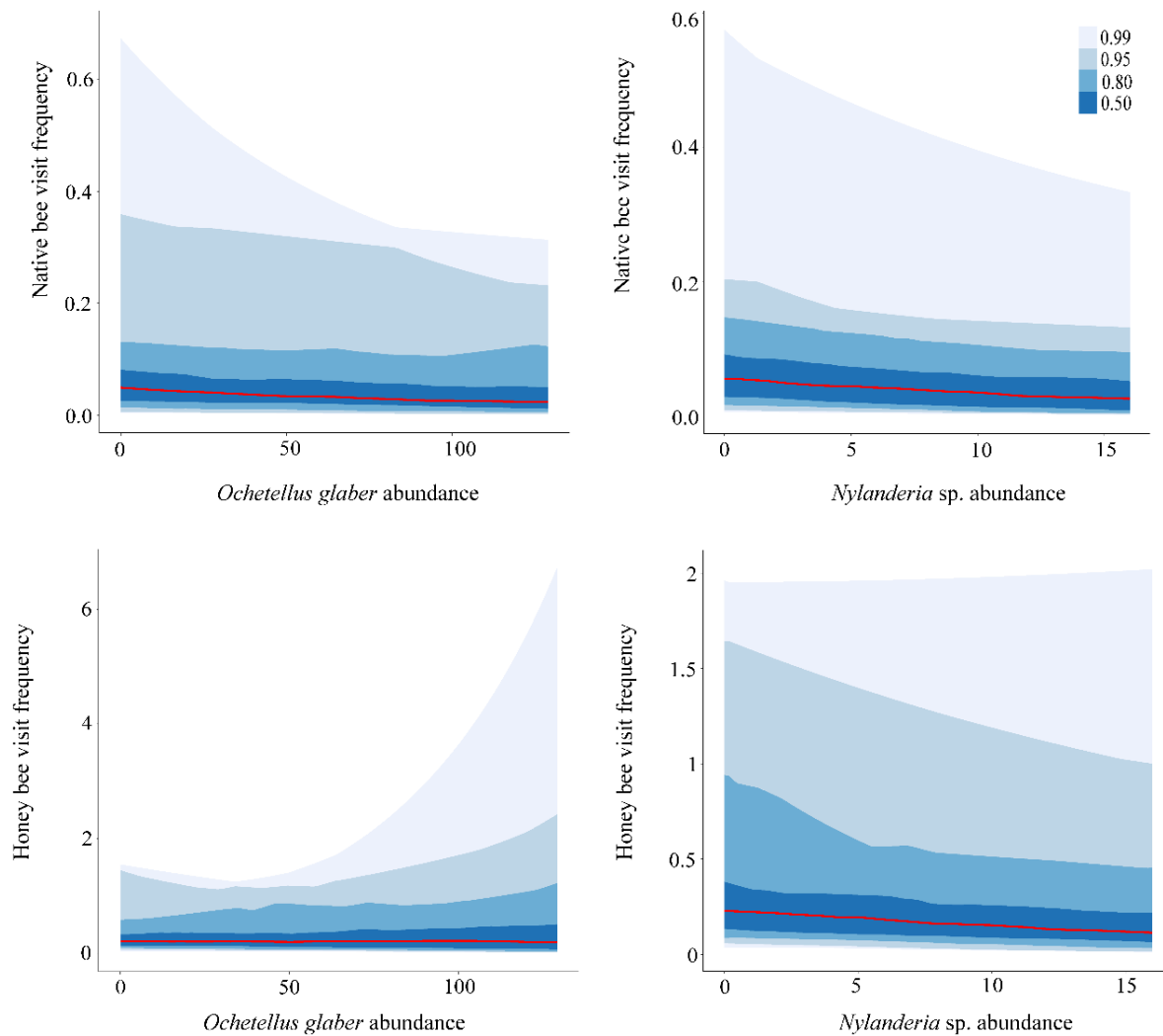
Figure 4 – Frequency of visits by native bees and exotic honey bees to inflorescences across the plant community in response to two species of exotic ant, *Ochetellus glaber* and *Nylanderia* sp, based on posterior predictions (red line). Note different scales on the x and y axes. Different colours correspond to the 0.50, 0.80, 0.95 and 0.99 posterior credible intervals extracted from Hamiltonian Markov Chain Monte Carlo samples from Bayesian analysis.
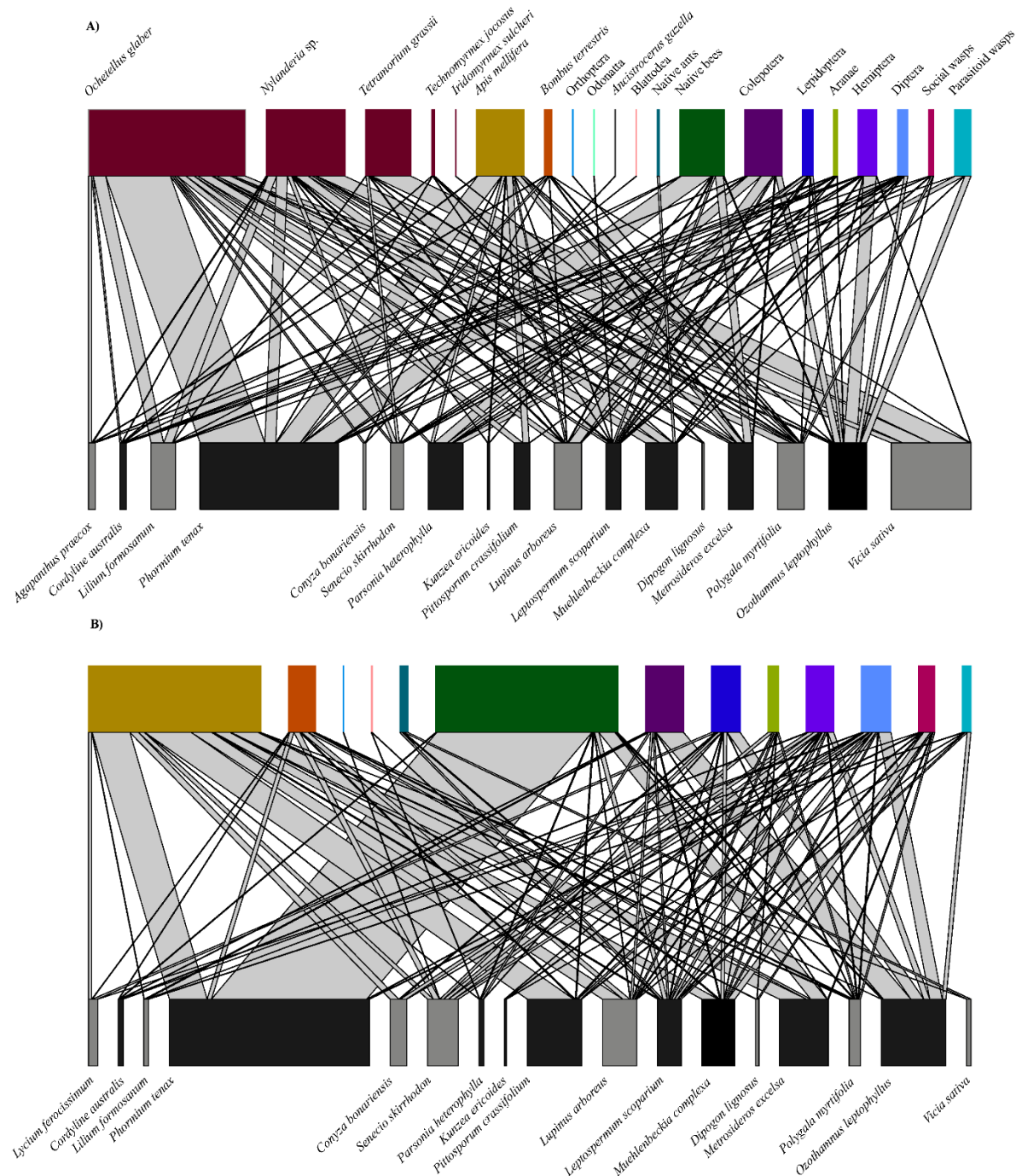
Figure 5 – Floral-visitor network from sampling at Karekare during the 2015/16 Austral summer for observations: A) exotic ants present or B) exotic ants absent. Top nodes represent floral-visitors: for exotic ant species node size is based on presence data, for all other floral visitors, node size is based on individual abundance. Colour for top nodes in A) represent the same taxa for top nodes in B). Bottom nodes represent plant species with native plant species.

5.3.3 Harakeke floral observations

There was no significant effect of ant presence ($z = 1.24$; $df = 1,41$; $P = 0.215$) or ant traffic ($z = -0.659$, $P = 0.510$) on the number of rejections for honey bees (47% ants present rejected vs 33% ants absent rejected). Nor was there was a significant effect of ant presence ($z = -0.686$, $P = 0.493$) or ant traffic ($z = -1.105$, $P = 0.269$) on the number of rejections for native bees (33% ants present rejected vs 47% ants absent rejected).

There was no difference in the duration of visit by honey bees when comparing between observations with ants present and ants absent (Fig. 6; ants absent 7.33 ± 1.82 s vs ants present 6.94 ± 1.10 s; $t = 0.48$, $P = 0.080$); however, duration was found to increase with increasing ant traffic ($t = 2.33$, $P = 0.030$). Conversely, the duration of visit by native bees differed between treatments (ants absent 9.58 ± 0.55 s vs 6.06 ± 0.30 s; $t = -2.184$, $P = 0.030$), with native bees visiting 1.4 times longer when ants were absent. There was no significant effect found of ant traffic on visit duration for native bees ($t = 0.028$, $P = 0.978$).
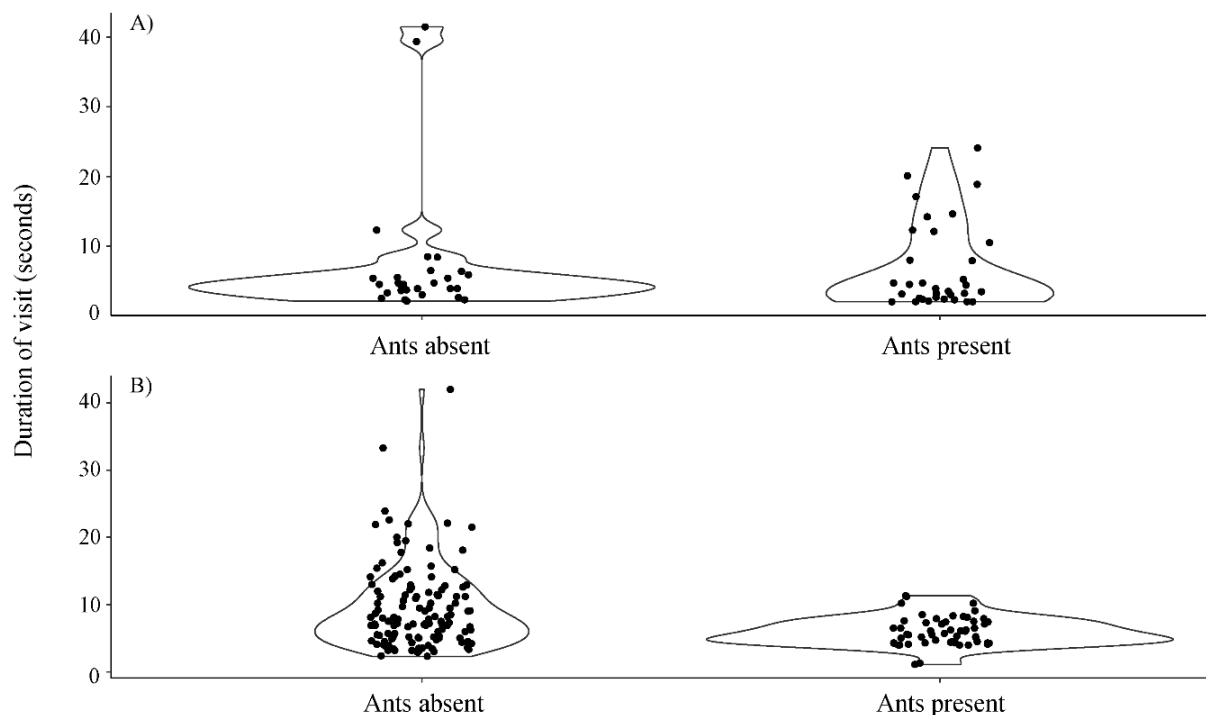
Figure 6 – Duration of visits by a) honey bees and b) native bees to harakeke when exotic ants are absent versus present.

5.3.4 Artificial inflorescence experiment

I recorded 279 total visits with 114 flower rejections by floral visitors. Six insect taxa from three orders were represented in my observations, with the majority of visits from honey bees (82%), and *Vepsula* wasps (14%) (Table 1).

Honey bees initially approached floral arrangements equally (accessible flowers 8.81 ± 2.33 [mean $\pm$ SE] approaches; inaccessible flowers 11.3 ± 2.79 [mean $\pm$ SE] approaches; $z = 0.850$; $P = 0.395$); however, honey bees were significantly more like to land on and accept inflorescences when ants were excluded, compared to when ants were present, with a 5-fold increase in acceptability ($z = 7.022$; $P = <0.001$). Whereas 19% of honey bees rejected ant inaccessible inflorescences, 42% of honey bees rejected ant accessible inflorescences. Furthermore, there was a significant effect of treatment on honey bee visit duration, with honeybees spending 1.7 times longer on inflorescences when ants were excluded (17.8 ± 2.08 s) compared to when ants were present (11.0 ± 1.8 s; Fig. 7; $t = 3.66$; $P = 0.001$).
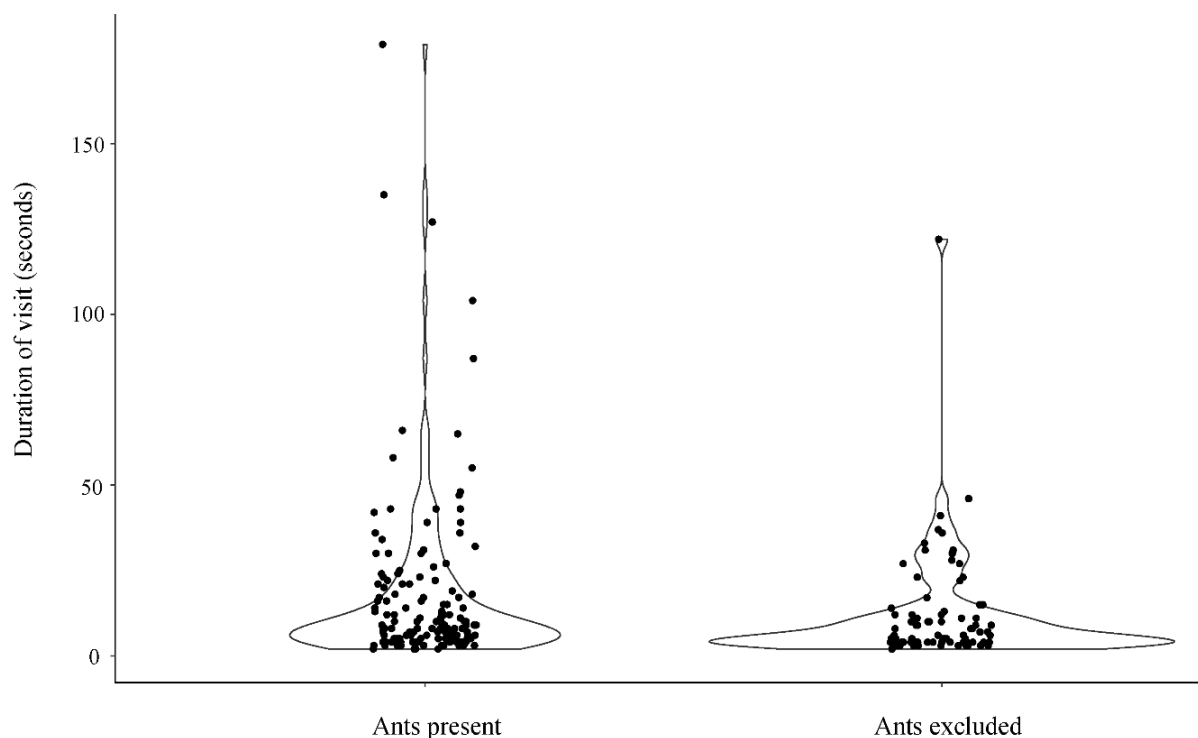
Figure 7 – Duration of visit honey bees spent at artificial inflorescences when they were accessible to exotic ants and when exotic ants were excluded.

5.4 Discussion

Across all three sites, the majority of ants present on flowers were represented by two species *Ochtellus glaber* and *Nylanderia* sp. At Karekare, these two species were also found to be dominant floral visitors when considering all taxa. These species have previously been found to be widespread and dominant in open-canopy, short stature natural ecosystems (Chapter 3), qualifying them as 'invasive' under the definition of Falk-Petersen, Bøhn, & Sandlund (2006). In general, native ants were not frequent floral visitors, and unsurprisingly, the majority of non-ant interactions with flowers were represented by honey bees and native bees. Flower visitor networks showed exotic ants exhibited interactions with all plant species within the community sampled. Further, networks revealed a strong difference for the interaction frequencies between native bees when comparing observations when ants were present versus absent. In focal observations on harakeke, native bees, but not honey bees, were found to negatively respond to exotic ants. On artificial flowers the visitation duration of honey bees was significantly lower when exotic ants were present.

The presence of an ant species at a site (as indicated by ground lure sampling) did not necessarily mean the species utilised floral resources. For instance, the native *Huberia striata*, was often detected though ground lure sampling at Anawhata, but never recorded as a floral visitor. Although this species is considered omnivorous (Don, 2007), it is likely that the relatively tall flowering plants at this site were outside of the foraging range of this species, which nests in soils and rotting wood. Similarly *Technomyrmex jocosus* was detected through ground lure sampling at Anawhata only in March, the end of the Austral summer, and never recorded on flowers. Previous sampling reveals a decline in *T. jocosus* occurrence at this site over a ten year period (Chapter 3), and it likely that in the area sampled, this species occurs at low levels. Whilst species in this genus are considered arboreal or sub-arboreal (Heterick, 2009) and can infest a high proportion of plants in their invaded ranges (Hansen & Müller, 2009; Lach, Tillberg, & Suarez, 2010), another study in Auckland looking at carbohydrate resource use reported *T. jocosus* (then considered *T. albipes*) absent from all observed flowers and honeydew, but present via ground lure sampling (Gardner-Gee & Beggs, 2009). Temporal and spatial differences in carbohydrate resource reliance (Mooney & Tillberg, 2005) and the seemingly low abundances of *T. jocosus* may explain why this species was not found to visit flowers surveyed at Anawhata.

The presence of exotic ants on flowers was spatially variable, as evidenced through the first year of sampling at the three sites. For the 2015/16 sampling period, only ~10% of observations at Whatipu and Anawhata recorded flower-ant interactions (native or exotic), compared to 40% of observations at Karekare. This variation may be due to site differences in the relative abundances of ant species. The two of the most common exotic species *O. glaber* and *Nylanderia* sp. were detected on flowers as well as by ground lure sampling across all three sites. These findings are congruent to the risk profiles allocated in previous risk assessments for these species (Ward, 2007; Ward, Stanley, Toft, Forgie, & Harris, 2008). These previous risk assessments have used a conservative measure when information is uncertain, for example using a 0.5 score within a 0-1 scoring system. However, as such, scores may under- or over-predict risk. For instance, *O. glaber* ranks as medium risk (0.5) for impact to native environments *because* of the uncertainty *rather* than the known risk. My findings show that both these species are well established in many open-canopy, short-stature ecosystems (Chapter 3), and can be dominant floral visitors, providing evidence for this potential risk to pollinator-plant interactions.

The levels of infestation of exotic ants on plants were lower than reported for invasive ants outside of New Zealand (Hanna et al., 2015; Hansen & Müller, 2009; Lach, 2005, 2008); however, the majority of these studies focus on only a few species, commonly recognised as the most invasive ant species, and usually examine infestation for only one focal plant species. Lach (2008) found high infestation levels for Argentine ants (96%) and *Pheidole megacephala* (67-96%) in Ōhi'a *(Metrosideros polymorpha)* trees. Similarly, Hanna et al., (2015) reported 100% of surveyed morning glory *(Calystegia macrostegia* spp.*)* had Argentine ants present in flowers. Both these species are known to form supercolonies, thus facilitating numerical dominance, a characteristic associated with the high potential risk in ants (Ward et al., 2008). The lower infestation rates reported in this study are thus likely reflective of comparably smaller colony sizes of species that occur at the sampled sites.

Focal observations on harakeke suggested ant presence has no effect on whether flowers were rejected by honey bees, or on the duration of visits by honeybees. In fact honeybee visit duration increased with increasing ant traffic. It is possible that honey bees, being larger than native bees, are more robust to antagonistic interactions from ants (Barônio & Del-Claro, 2018). The longer visit duration may have occurred due to disruption by ants during nectar

feeding, meaning honey bees had to spend a longer time within each flower. Exotic ant presence also had no effect on whether native bees rejected harakeke flowers, however in contrast to honey bees, native bees were found to visit 1.4 times longer when ants were absent. Bees are known to use visual cues to detect and avoid potential predators on flowers, particularly forelimbs of predators (spiders) (Gonçalves-Souza, Omena, Souza, & Romero, 2008), thus the depth of the corolla of flax flowers may have prevented visual detection of ants causing no effect on the rejections observed in my results. Although honey bees may detect ant pheromones to inform flower visitation decisions (Sidhu & Wilson Rankin, 2016), the use of ant pheromones as a cue for flower avoidance is learned, and may be ignored (Ballantyne & Willmer, 2012), illustrating the complexities of understanding pollinator behaviour. Interestingly, a strong negative response between ant presence and honey bee behaviour was found in the artificial flower experiment. In contrast to the tubular harakeke observations, ants were very conspicuous on the open artificial inflorescences, meaning honey bees may rely more heavily on visual cues when they are available.

An implication of ants visiting flowers is the potential disruption of floral-visitor networks, which may lead to decreased pollination and seed set in some species. The mechanism for this is thought to be through interference competition (Hansen & Müller, 2009; Lach, 2005). However, the majority of studies investigating floral visitation and/or pollination effects of ants are focused on Argentine ants or other highly invasive species (Blancafort & Gómez, 2005; Gonçalves-Souza et al., 2008; Hanna et al., 2015; Hansen & Müller, 2009; Lach, 2005, 2007, 2008; Sidhu & Wilson Rankin, 2016; Sinu et al., 2017; Stanley et al., 2013; Visser, Wright, & Giliomee, 1996). As the displacement of pollinations by ants does not necessarily result in reduced seed set (Lach, 2007; Stanley et al., 2013), a good understanding the pollination biology for individual species is critical for accurate interpretation of results. For instance, whether a species is pollen limited is necessary to predict the potential magnitude of any effect of ants on reproduction (Hanna et al., 2015; Lach, 2007). Although this study provides evidence that ant species with lower risk profiles are also capable of altering visitation, whether this leads to a reduction in pollination services presents itself as an important knowledge gap that requires further research on pollination systems for different plant species.

This study demonstrates that invasive ants can be frequent floral visitors of both native and exotic plant species in natural ecosystems. Infestation levels vary spatially, and further work is

required to determine drivers. I found that exotic ants can influence the visitation behaviour of important pollinating species, and future directions should determine if invasive ants impact plant fitness via quantifying pollination success at infested versus non-infested flowers. Surveillance and monitoring of ant communities at high conservation value sites is recommended, particularly where threatened flowering plants occur.

Chapter 6 — General discussion

6.1 Key findings

A critical challenge facing pest managers is the uncertainty relating to the type and severity of impacts associated with invasive species. It is difficult for managers to produce evidence-based justifications for pest management, or eradication, when both knowledge of impact and resources are limited. Therefore, a primary component of my thesis was to develop an objective risk assessment framework to aid decision-making (Fig. 1). The framework focussed on risk associated with ecological impacts and was applied to exotic ants in New Zealand, particularly species with no or minimal previous impact-related research (Chapter 2). After reviewing the risk assessment literature across various sectors, I found that a generalised disaster risk assessment framework presented itself as a pragmatic foundation for ecological risk assessment. The framework integrates key characteristics of both the invading species and potential recipient environments, facilitating risk identification to inform management and restoration decisions. I identified several key knowledge gaps: 1) the recognition of natural assets (ecosystems) that are most vulnerable to invasion, and the scale-specific vulnerability within such ecosystems, and 2) understanding the mechanisms and magnitude of impacts that invasive ants exert on the recipient communities and ecosystems.

In Chapter 3, I addressed the '*exposed assets*' module from the framework (Fig. 1). I found that exotic ants were generally ubiquitous throughout open-canopy ecosystems, yet concentrated at the edges of closed-canopy ecosystems. Furthermore, by replicating an earlier study, I demonstrated that this pattern was evident even after exotic ants were present for a period of at least 10 years. The prevalence of certain species indicated their status as invasive species, according to the definition outlined in Falk-Petersen, Bøhn, and Sandlund (2006).

I then addressed the '*damage state*' module, in Chapters 4 and 5, by investigating the different mechanisms and the magnitude of impact for different exotic ant species. In Chapter 4, I assessed dietary-related impacts, finding nitrogen signatures for two exotic species, *Linepithema humile* and *Technomyrmex jocosus*, which indicated a strong reliance on carbohydrate-rich resources, a trait implicated with highly invasive ant species (Holway et al., 2002a). Additionally, I found that the highest level of isotopic niche overlap between two

species corresponded to a negative co-occurrence patterns, suggesting a form of competitive exclusion between the invasive *Ochetellus glaber* and the native *Monomorium antarcticum.*

In Chapter 5, I found that floral resources were exploited by exotic ant species, although there were spatial (site, plant species) and temporal differences in infestation levels. The presence of exotic ants altered the visitation behaviour of invertebrate flower visitors, some of which may act as important pollinators; native bees appeared to respond most negatively to exotic ants. The invasion of natural habitats by ants likely has important implications for the fitness of both floral visitors and plants.

Despite invasive ants being recognised as a major threat to biodiversity and ecosystem functioning (Holway et al., 2002a; Rabitsch, 2011), there is a lack of context-specific impact knowledge for exotic ant species established in New Zealand. Like many of the exotic insect species established in New Zealand (Edney-Browne, Brockerhoff, & Ward, 2018), the exotic ant fauna is largely represented by species of Australian origin that have not been introduced elsewhere; thus, there is little baseline information on how these species may interact with native biodiversity and a poor understanding of associated ecological risk. Using the risk framework presented in Chapter 2, my study demonstrated that open-canopy, short-stature ecosystems are exposed assets (i.e. vulnerable to ant invasion), and that the mechanisms by which ants may impact on native biodiversity and ecosystems are varied (Fig. 1). Although the direct impacts, such as predation on the invertebrate community are apparent, understanding the indirect impacts (i.e. the associated changes to soil characteristics or other ecosystem attributes due to predation pressure altering the invertebrate community) are more difficult to predict and quantify.
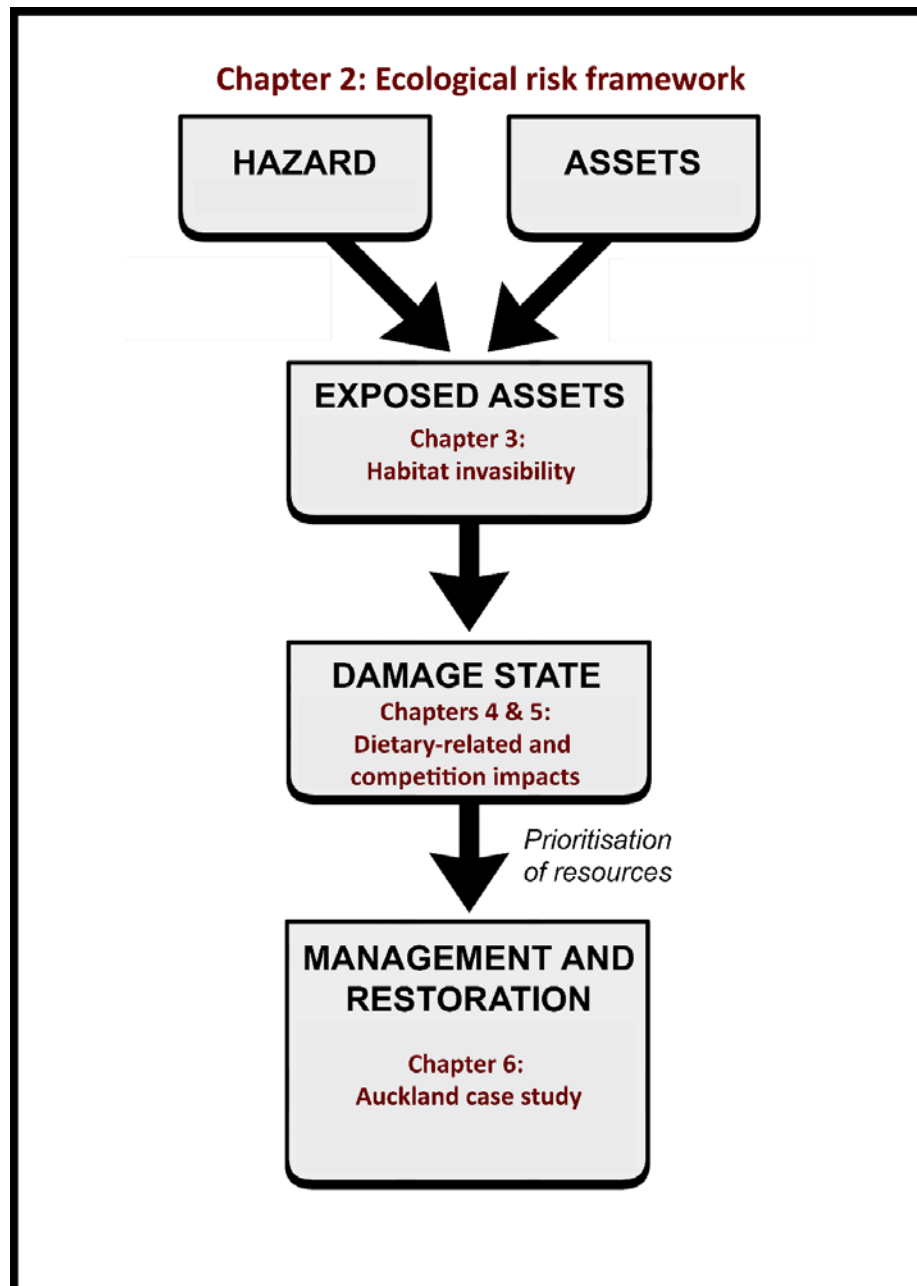
Figure 1 – The conceptual framework for ecological risk assessment presented in Chapter 2, embedded with the research from Chapters 3–5, and put in context of this thesis.

6.2 Application of framework to the Auckland region: a case study

Hazard: introduced species

The framework can be used in either a prospective or a retrospective approach, whereby it may be applied to exotic ant species that: 1) already occur within Auckland, or 2) have a high probability of arriving and establishing. Thus, the *hazard* module can easily be applied to the pest species or group of interest, with their traits and distribution identified and integrated with the *assets* module to inform vulnerability. In general, ants have a high capacity to be introduced outside of their native ranges as hitch-hiker pests (McGlynn, 1999). Although ~29 exotic ant species have established in New Zealand, there is limited information on the extent of their distribution across natural habitats with impact-focused research being almost non-existent for all species except the Argentine ant (Stanley et al. 2013; Stanley and Ward 2012; Cooling, Sim, and Lester 2015). Occurrence records for most exotic ant species are well represented within the Auckland region (Don, 2007); however, these are likely biased due to greater sampling efforts (Ward, 2012). Nevertheless, as a central port and New Zealand's most populous city, Auckland is a high risk area for new arrivals of exotic ants.

A priority at this step is to identify traits of the ant species that may facilitate establishment in Auckland. Climate suitability for the hazard species based on Auckland's temperature is thus central to this module and can be informative in determining risk (Barbet-Massin, Rome, Villemant, & Courchamp, 2018; Lester, 2005; Ward, 2007). Understanding the minimum ambient and soil temperature requirements for colony development can identify the probability of successful establishment for exotic ant species (Abril, Oliveras, & Gómez, 2010; Hartley & Lester, 2003). Given Auckland's subtropical climate, species from similar climate zones, or more cold-tolerant ant species, are thus associated with higher risk. In contrast, tropical species, such as the highly invasive yellow crazy ant (*Anoplolepis gracilipes*), which require specific minimum temperatures for colony development, are unlikely to establish due to temperature constraints across most of New Zealand (Abbott, Harris & Lester, 2005). Other general species traits that have generally found to predict invasiveness for ants, such as body size, colony size, reproductive and dietary characteristics (Holway et al., 2002a), should be identified for their suitability in risk prediction (Lester, 2005).

Assets: ecosystems

Terrestrial and wetland ecosystems within the Auckland region have been described by Singers et al. (2017). Within the classification, 36 ecosystems and their regional variants are outlined and geographically identified across the region. These represent the *assets* module in the framework from which ecosystem prioritisation may then be conducted. The Auckland Council has already prioritised ecosystems as Significant Ecological Areas (SEAs) based on criteria outlined in the Unitary Plan (Auckland Council, 2016). This provides a classification list identifying areas of high ecological value, which facilitates prioritisation of pest management.

Exposed assets: estimating vulnerability

Pest managers can use the traits of the ants (*hazards*), together with the classification of priority, high-value ecosystems, to predict the relative risk of ecosystem exposure to ant invasion, and in doing so, identify the *exposed assets*. Based on my findings in Chapter 3, open-canopy, short-stature ecosystems, which were coastal ecosystems, represent *exposed assets*. Unsurprisingly, coastal ecosystems are well represented as SEAs given the geography of the Auckland region (Auckland Council, 2016). However, ecosystems in coastal areas are also highly threatened by continued human development, and are generally under-represented as protected areas across New Zealand (Cieraad, Walker, Price, & Barringer, 2015).

Integrating the species' and ecosystem traits identified in the first two modules to evaluate the most *exposed assets* is central to this module of the framework. Open-canopy ecosystems differ in abiotic characteristics such as their light exposure, wind speed and temperature when compared to the closed-canopy indigenous forest (Davies-Colley et al., 2000). Soil temperature is often an important factor for epigeic ant assemblages, influencing distribution, species richness and colony sizes (Kaspari, Ward, & Yuan, 2004; Kwon, 2016; Sanders, Lessard, Fitzpatrick, & Dunn, 2007; Warren & Chick, 2013). Successful reproduction and colony development are associated with minimal soil temperature requirements (Abril et al., 2010). Therefore, open-canopy ecosystems provide favourable habitat for exotic ant species established in New Zealand (Chapter 3).

Damage state: impact

Chapters 3 and 5 revealed that exotic ant species usually dominated food resources (lures) in *exposed assets*, representing the numerically and behaviourally dominant ant species.

Specifically, *O. glaber* and *Nylanderia* sp., dominated lures within open-canopy, short-stature ecosystems; however, at some sites *Tetramorium grassii* and *Iridomyrmex suchieri* were also often detected at lures, demonstrating the site variability in ant assemblages. Invasive ant species may impact ant species richness at the local scale due to competitive exclusion (Parr, 2008; Parr, Sinclair, Andersen, Gaston, & Chown, 2005), with Chapter 4 suggesting a strong competitive effect between *O. glaber* and the native *Monomorium antarcticum*.

In Chapter 4, I used stable isotopes to investigate the diet of ants occurring in *exposed assets*. My findings revealed two species *L. humile* and *T. jocosus* feed at a lower trophic position than all other ant species. *Linepithema humile* has long been considered among the world's worst invasive species (Lowe, Browne, Boudjelas, & De Poorter, 2000; Luque et al., 2014), with the ability to exploit carbohydrate resources suggested to be a factor facilitating the invasiveness of ants (Holway et al., 2002a). Very little is known about *T. jocosus*, however, the finding that this species feeds at a similar trophic level to other highly invasive ant species warrants inclusion as a listed pest species in Auckland's Regional Pest Management Plan to allow for management at high-value sites (SEAs). Currently, only two ant species, *Linepithema humile* and *Doleromyrma darwiniana*, are listed on the Proposed Regional Pest Management Plan (Auckland Council 2017).

Chapter 4 revealed negative co-occurrence patterns between some species, suggesting some form of competitive exclusion may be structuring ant communities, as dominant ant species may directly affect ant occurrence patterns (Parr, 2008; Parr et al., 2005). I found negative co-occurrence patterns between *O. glaber* and *T. jocosus*, and *O. glaber* and *M. antarcticum*. *Monomorium antarcticum* was the most ubiquitous native ant species found across sites and it almost certainly represents a species complex (Dann, 2008). Nevertheless, my findings indicate that within the Auckland region, this species may be at risk.

Another impact associated with invasive ant species was highlighted in Chapter 5, where floral visitation differed for some taxa when ants were present on inflorescences. It is difficult to generalise the potential effects on plant fitness; in some cases invasive ant presence may increase plant growth and reproduction (Lach et al., 2010; Stanley et al., 2013), although any positive impacts are usually reaped by invasive, rather than native, plant species (Lach et al., 2010; Prior, Robinson, Meadley Dunphy, & Frederickson, 2014; Stanley et al., 2013). Although future research is required to determine the individual effects of invasive ants for

native plant species, SEAs containing threatened angiosperms should be prioritised for surveillance and monitoring of invasive ants.

Management and restoration

Based on existing knowledge, and the findings of Chapters 3, 4 and 5, invasive ant monitoring in areas designated as significant ecological areas is recommended within the Auckland region. Surveillance at high-risk sites (*exposed assets* = open-canopy, short-stature, coastal SEAs) for *L. humile, O. glaber, Nylanderia* sp. and *T. jocosus* is recommended, with the potential management of these species alongside current invasive mammal and weed control operations. It is important to consider decisions in conjunction with current management and restoration activities. For instance, previous restoration recommendations for the conservation of native honeydew-feeding geckos in Auckland have included the planting of carbohydrate-rich native plants such as ngaio (*Myoporum laetum*) and karo (*Pittosporum crassifolium*) (Gardner-Gee & Beggs, 2010). However, restoration planting of these trees may facilitate some invasive ant species, thus counteracting restoration objectives. This highlights the importance of considering restoration and management actions in context of the wider ecosystem scale.

Based on my findings, it is recommended that more research is conducted into the impacts of invasive ants in exposed assets, particularly those species highlighted as prevalent in Chapter 3 and 5. In particular, impacts on the local invertebrate community, and the potential impacts this may have on ecosystem functioning, warrant further investigation. Furthermore, understanding the impacts of ants on native plant and pollinator fitness highlights an important knowledge gap. The value of conservation practices that move beyond single species conservations to incorporate ecologically important species interactions (e.g. pollination, seed dispersal) is becoming increasingly recognised (Valiente-Banuet et al., 2015), signifying the importance and relevance of understanding floral visitor interactions within natural ecosystems.

Finally, it is recommended that future climate change projections are factored into long-term pest management practices in the Auckland region. This requires consideration early in the *hazard* and *asset* modules. Forecasting the likelihood of new ant species establishing under predicted increasing temperatures, but lower rainfall, will enable emerging risks to be identified and appropriate management plans to be implemented (Sheppard et al., 2014). Moreover,

identifying differences in asset traits (e.g. plant phenology, soil characteristics), likely to be altered under climate change, will enable further refinement of ecological risk predictions.

6.3 Wider applications of the conceptual risk framework

Although this thesis applies the conceptual framework presented in Chapter 2 to the ecological risk associated with exotic ants, it has broader applications and may be adapted and applied outside of the scope presented within this thesis. Specifically, the framework may be applied to any pest or pathogen species, identifying the traits of the *hazard* and the *assets*, then integrating this information to inform vulnerability. The characterisation of assets at a high level can provide an initial indication of the ecosystems most at risk. For instance, spatial mapping of the short-stature versus tall-stature ecosystems may be informative for a broad range of invertebrates native to warmer ranges, since native forest is unlikely to provide required temperatures for their development in New Zealand. Moreover, whilst I specifically developed this framework to assess ecological risk within the context of natural ecosystems, it could also be applied to productive systems, with *exposed assets* representing productive landscapes that could support pest populations. Incorporating components of both ecological and economic risk may be factored in when predicting both *exposed assets* and *damage state* to inform *management and restoration* decisions. Further, as the risk framework can be applied retrospectively, it can be used to forecast risk associated with predicted climate change scenarios for species already established.

6.4 Future research directions

Given the increasing establishment of species outside of their native ranges, assessing ecological risk is critical to mitigate potential impacts. It is often difficult to identify ecological risk, which facilitates the prioritisation of pest species to inform management decisions. There are still many research gaps that need to be addressed, including fundamental taxonomic and ecological information that enable improved risk prediction.

Basic biology

A major knowledge gap persisting in exotic ant risk assessment is the lack of basic taxonomic and biological information for many species established in New Zealand. This is exemplified in the misidentification of several species in New Zealand, leading to taxonomic

inconsistencies in the literature. For instance, *Technomyrmex jocosus* was originally thought to be *T. albipes,* a highly successful tramp species found in many parts of the tropics and subtropics (Bolton, 2007). Furthermore, the poor taxonomic resolution for native species (e.g. *Monomorium antarcticum* species complex) highlights how impact at the species level may be underestimated. Although closely related species are likely to be functionally similar, with comparable ecological impacts, ensuring accurate taxonomic information exists for exotic species is critical for robust biosecurity practices. Additional information relating to the biological characteristics for many species would be beneficial for more accurate risk assessment. Linking up species' traits to ecosystem traits is a fundamental component of the risk framework presented in Chapter 2, as using a predictive trait-based approach can be useful in broadly identifying risk associated with different ant species (Holway et al., 2002a; Rabitsch, 2011; Ward, Stanley, Toft, Forgie, & Harris, 2008). Although there are now excellent online resources that synthesise the global knowledge on ants (Parr et al., 2017), information on pest species is often poorly represented in the published scientific literature, disproportionately buried in grey literature (Lowry et al., 2013). Future work should focus on depositing information on exotic and invasive ant species into databases, enabling the dissemination of non-peer reviewed findings from researchers, regional councils and governments.

Ant distributions and habitat preferences

An understanding of the native and invaded ranges of invasive species is necessary for risk assessment. Current locality records provide baseline information allowing ecological niche modelling, which enables more accurate predictions of species' potential distributions, both now and under projected climate change. The ecosystem surveys presented in Chapter 3 represent an example of long-term datasets. Although long-term datasets for invasive ants do exist (e.g. Menke, Harte, and Dunn 2014; Menke, Ward, and Holway 2018), they are relatively rare, at least in the published literature, and have been highlighted as a central limitation in invasion biology (Blossey, 1999; Heger et al., 2013; Latombe et al., 2017). Given that a large shortcoming of invasive ant impact research relies on comparative analyses between invaded and uninvaded sites, and usually no monitoring occurs prior to invasion, it is difficult to disentangle the causative effects from baseline ecosystem differences prior to invasion. Funding limitations are usually the reason for lack of base-line monitoring as most grants only

extend to a few years of research. This highlights the critical need for open access data depositories to enable longitudinal monitoring via replication studies.

Diet analysis by metabarcoding

Chapter 4 highlighted the differences and difficulties in using stable isotope and DNA metabarcoding to analyse the diet of different ant species. Although stable isotopes have been widely used to investigate the diet of ants (Menke et al., 2010; O' Grady, Schmidt, & Breen, 2010; Roeder & Kaspari, 2017; Tillberg et al., 2007), to my knowledge, I am the first to use DNA metabarcoding. A study by Penn, Chapman, and Harwood (2016) suggested structures related to ant crops inhibit PCR, which led me to dissect out gut contents from individual ants. However, research focused on the microbial community of ant guts often homogenise ant gasters whole without related issues with PCR inhibition (Kautz, Rubin, Russell, & Moreau, 2013; Moreau & Rubin, 2017; Ramalho, Bueno, & Moreau, 2017). This may suggest a larger issue associated with the enzymatic breakdown of prey DNA resulting in negative PCR results. Ideally, future work will further investigate this in order to develop a protocol that can circumvent the arduous process of dissecting ant guts. Moreover, research is required to assess the efficacy of different PCR protocols for ants to optimise DNA amplification and sequence results (van der Reis, Laroche, Jeffs, & Lavery, 2018).

Alteration of floral visitation networks – implications for plants and their pollinators

Although Chapter 5 characterised the effect of ants on floral visitors with inferred implications for plant-pollinator networks, I did not quantify any consequences on pollinator or plant fitness. Although pollination studies often use visitation frequency as a measure of pollinator efficacy, visitation does not necessarily translate to successful pollen transfer (Ne'eman, Jürgens, Newstrom-Lloyd, Potts, & Dafni, 2010). Specific traits such as hairiness, body, and mouthpart length have been found to be better predictors of pollen transfer (Barrios, Pena, Salas, & Koptur, 2016; Garibaldi et al., 2015; Stavert et al., 2016). As my findings in Chapter 5 suggest that different floral visitors respond differently to exotic ants, understanding the effects on pollination requires research addressing the efficacy of different pollinators for different plant species. Priority for this research should focus on threatened, dominant or keystone species within high-value ecosystems, which heavily rely on native invertebrate pollinators.

6.5 Concluding remarks

Pest managers and conservation practitioners face time pressures and limited resources to make cost-effective decisions when managing natural ecosystems. Biosecurity practices employed within New Zealand's biosecurity system appear to be preventing the establishment of exotic species (Edney-Browne et al., 2018), despite increasing rates of establishment being observed at a global scale (Seebens et al., 2017). This highlights how biosecurity measures are functioning at the (pre-)border. However, once a species has established, risk assessment can be difficult due to uncertainties associated with evaluating ecological risk. In order to make justifiable management recommendations, pest managers must make decisions following processes that systematically evaluate the available information. Even when quantitative data is lacking, qualitative judgements drawn from previous knowledge relating to the species should follow a standardised decision-making process.

This study greatly contributes to the ecological risk assessment posed by exotic species in native ecosystems. Although a lack of information is clearly the largest impediment to risk assessment, I provide a step-wise process to enable risk prediction even when information is limited, to better inform management decisions. Despite the difficulties in assessing ecological risk, predicting ecological impact is a critical component of robust risk assessment and thus must be effectively evaluated to inform policies and pest management decisions.

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