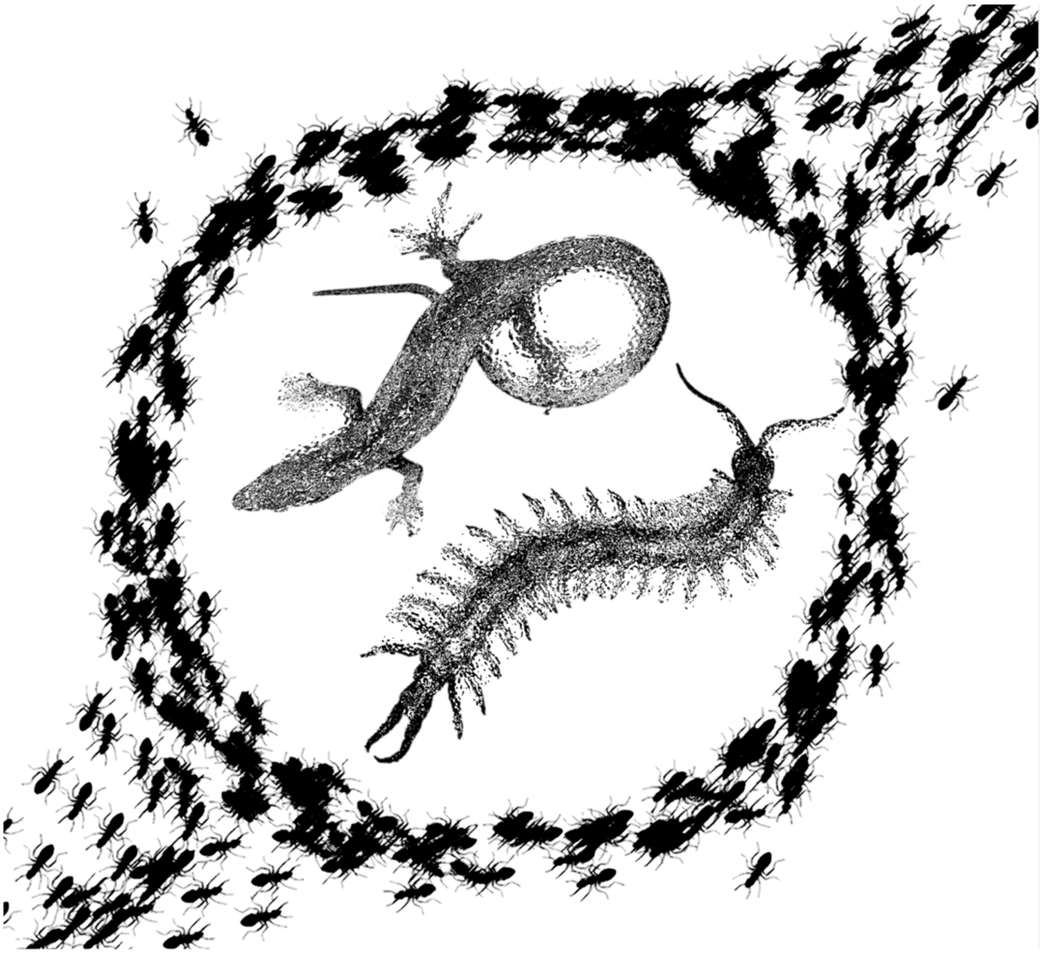
The Trophic Ecology of Non-Native Ants on Round Island, Mauritius



A thesis submitted to Cardiff University for the degree of
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by

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Summary

Non-native ants are implicated in the demise of native species around the world, though their trophic ecology remains poorly understood. Non-native ants have invaded Round Island, a globally significant site of biodiversity conservation located 21 km north-east of Mauritius in the Indian Ocean, but it is unclear how they are affecting the unique ecological community found there. To reveal their potential impact, I conducted a meta-analysis into the effects of non-native ants on animal community diversity in relatively undisturbed areas around the world, showing that non-native ants drive diversity declines in local animal communities by approximately 50 % on average (Chapter 2). I then examined the ecological role of non-native ants on Round Island specifically and first determined, using dietary DNA metabarcoding, whether an abundant native omnivore, Telfair's skink, consumed non-native ants. Skinks do consume ants, though it was unclear to what degree these detections were deliberate or accidental (Chapter 3). I then identified the diet of the 12 most numerous non-native ant species on Round Island using dietary metabarcoding, revealing that all ant species showed unique generalist diet profiles and together consume over 150 species of animals and plants. The diet of the ant community was also driven by seasonal changes in food availability. This presents the first study to date detailing the diet of individual ants at the community level and that a community of generalist non-native species exhibit dietary niche separation (Chapter 4). I compared the diet of native skinks and centipedes with that of non-native ants, finding that skinks are not competing with ants for food, whilst centipedes are (Chapter 5). Overall, our results suggest, through five separate lines of evidence arising from the study, that non-native ants are having a significant impact on the Round Island ecosystem.

Lay Summary

Ants are very important animals. They keep much of the natural world on land running normally by hunting prey, eating plants, farming insects and fungi, and moving more soil than earthworms. If all the ants on Earth were dried and weighed together, they would weigh about 12 megatons. That's the same as 33 Empire State Buildings or 876,000 Big Bens. They outweigh all wild mammals and birds combined and about the same as 1.4 billion people. Because there are so many ants, they can radically change the environment around them. International trade has led people to accidentally transport some ant species all over the world. Unfortunately, many of these ant species can cause severe harm to animals and plants in these new areas. These are called "invasive ants". This document contains information from a 4-year study that reveals how invasive ants affect a unique community of extremely rare species on an island located just north of Mauritius in the Indian Ocean, called Round Island.

I first looked at many studies from across the globe that showed how invasive ants influence other animals. Then I combined the results from all of these studies together. In doing so, I calculated that invasive ants halve the number of animal individuals and species. This is extremely important because it shows that invasive ants can have a huge impact on other creatures when they move into a new area.

Our next step was to examine how invasive ants might be affecting the Round Island ecosystem specifically. Invasive ants are very numerous on Round Island, and I therefore wanted to see if native animals might be eating them. I discovered that an important species on the island, Telfair's skink (a type of lizard), do consume them. I did this by sequencing the DNA of what they ate, i.e., their faeces. However, skinks ate ants accidentally in most cases because ants swarmed over whatever they consumed! I also used the DNA method to look at the diet of the ants themselves and found that they consume over 150 species of animals and plants, some of which are only found on Round Island. Most of these species were other insects. Given that invasive ants are extremely numerous on Round Island and consume so many species, I wanted to test whether they are competing with native animals for food. I thus described the diet of another important predator, the Serpent Island centipede, using our DNA method and were then able to compare the diets of the native lizards and centipedes with that of the invasive ants. From our results, Telfair's skinks are not competing with ants, though unfortunately it appears very likely that the centipedes are. Moreover, the centipedes are almost always found in areas of the island with low numbers of ants, and I discovered that the ants also hunt the centipedes when possible. There are tens of millions of ants on Round Island, and this is bad news for the extremely rare centipedes.

Overall, our results suggest that invasive ants dominate the Round Island ecosystem and may influence the food available to native animals. Ants may also be directly hunting many extremely rare species on Round Island that may not be found anywhere else. In light of these findings, the native invertebrate community of Round Island might be considered one of the most threatened on Earth.

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It's been a long and winding road, this PhD. Studying insects has been a life-long dream and, over the last four-and-a-bit years of study, I've been able to live that dream. I've been to six countries across three continents to give talks, attend courses, or conduct fieldwork. I've seen things and been to places few people have ever had the privilege. I've been able to observe organisms I couldn't have imagined I would ever actually see with my own two eyes as I read about them in my childhood encyclopaedias. Ants have been my world. I've made friendships that will last for the rest of my life. I was literally put on a helicopter to go to an uninhabited island paradise to study the insect community. *It's been absolutely amazing.* If someone told six-year-old me that all this would happen, I think I would be extremely joyous… and somewhat disbelieving… and then probably unbearably irritating! It hasn't all been helicopter rides to paradise, however. At times, it's been hard on my personal relationships, physical and mental health, self-confidence, and motivation for pretty much everything. Through both the highs and lows, a battalion of amazing people and organisations have helped me get through life and stuck with me.

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Some people are born into huge amounts of money or power. Others win the genetic lottery and might go on to become Olympians or models. I have none of those things, but I’d argue I’m just as lucky. Instead, I won the lottery by being the son to my impossibly amazing parents, Patrisha H. Townsend Green and Paul G. Tercel. It’s difficult to fully convey the scale of gratitude I have for them, who have been the most tear-jerkingly supportive human beings I could ever imagine. Since childhood I’ve been on this strange obscure quest to examine ‘the little things that run the world’, and every single step of the way my parents have been supportive but not pushy, critical but not unfair, realistic but not defeatist. I remember having a conversation with my dad and him telling me that people can actually get paid to do this and they’re called “field biologists”. It’s hard to overstate the significance this conversation has had on my life so far. My parents have provided the perfect environment to learn. They are the parents they want to be, not the parents they themselves had growing up. This is no mere ‘ordinary’ lottery win, however. This is some Euromillions rollover stuff right here. Because I also get to call Oscar T. T. G. Tercel my brother. Oscar is my greatest teacher of all. Through our discussions, debates, and verbal sparring over the course of life thus far, Oscar has fundamentally helped me become the scientist I am today. Together, my parents and Oscar have taught me so much about how my own mind works. I owe much of this scientific work to their lessons, and thus dedicate this thesis to them.

Thesis outline

Study system

Round Island is a globally significant site of biodiversity conservation located 21 km north-east of Mauritius in the Indian Ocean. It harbours a very high level of endemism and is the last expanse of native lowland palm forest habitat in Mauritius. Round Island forms one of the largest population reservoirs for several species that are listed as ‘threatened’ on the IUCN Red List; some of these species are found only on Round Island. The reptile and bird communities are particularly well-studied, and these native taxa appear integral to the functioning of the wider ecosystem. Far less is known about the native invertebrates. Goats and rabbits were introduced to the island by European mariners at some point between the 17th and 19th centuries and caused severe habitat degradation. Goats and rabbits were eradicated in 1979 and 1986, respectively, but the loss of habitat led to significant soil erosion and created large expanses of barren rock slab over much of the island. Since 2002, there have been extensive efforts to restore the lost hardwood forests and to enhance the natural regeneration of the native palm habitat.

Non-native ants are implicated in the demise of native species around the world and have colonised Round Island. These ants are hyper-abundant and represent approximately 85 % of all invertebrates captured in pitfall traps across the island’s various habitats. Determining the trophic ecology of the non-native ants on Round Island may identify how they are influencing the wider ecosystem.

Thesis objective

To identify the trophic ecology of non-native ants on Round Island, whether they are being eaten by or competing with native consumers, and how they may be influencing the wider ecosystem.

Chapter summaries

Chapter 1 provides a general introduction to non-native ant ecology and includes a section about identifying trophic interactions using DNA metabarcoding.

Chapter 2 details a meta-analysis that quantifies the effect of non-native ants on animal community diversity in many locations around the world. Our results show that non-native ants approximately halve animal community abundance and species richness in areas relatively unimpacted by other environmental stressors, isolating non-native ants as the causal agents of these declines.

Chapter 3 is the first dietary analysis presented in the thesis and focusses on Telfair's skink, *Leiolopisma telfairii,* a keystone native omnivore. I see that Telfair's skink are highly generalist, consuming many animal and plant taxa of native and non-native origin. However, the majority of dietary detections originate from non-native taxa, and many of these detections are of ants. It is unclear exactly how Telfair's skinks are consuming ants so frequently, but it is likely the majority of these detections arise due to accidental consumption of ants swarming over other food items.

Chapter 4 focusses on the ecology of the non-native ant community itself and provides the first study to identify the diet of individual ants at the community level (across 12 species), the first study to show a community of non-native generalist species showing dietary niche separation, and the first to show that non-native ants consume one another very frequently. I also discuss the role of 'dominance' and how this might partly structure the ant community and affect diet.

Chapter 5 presents a comparative dietary analysis between two native consumers, Serpent Island centipedes (*Scolopendra abnormis*) and Telfair's skinks, and the non-native ant community to determine to what degree the consumers prey upon or compete with one another. I found that Telfair's skink are probably not competing with ants, whilst it appears highly likely that Serpent Island centipedes are. Ants consume all species consumed by centipedes, ants themselves consume centipedes, and centipedes are almost always found in areas of low ant activity. Ants appear to affect centipedes through influencing the availability of food resources as hyper-abundant generalist consumers, as well as through competition, predation, and possibly sub-lethal effects.

Chapter 6 draws from the previous Chapters to provide a conclusion relevant to the conservation context of Round Island. Though our conclusions are primarily made through inductive rather than deductive inference, five separate lines of evidence suggest non-native ants are having a significant impact on the Round Island ecosystem. In light of these findings, the high number of endemic species, and very small area of occupancy, the native invertebrate community of Round Island might be considered one of the most threatened on Earth.

All photos and artwork are my own, unless otherwise stated.

Scientific articles published during the PhD studentship

2023

***Tercel, M. P. T. G.,** Cuff, J. P., Symondson, W. O. C. and Vaughan, I. P. (in press *Insect Conservation and Diversity)*. Non-Native Ants Drive Dramatic Declines in Animal Community Diversity: a Meta-Analysis. https://resjournals.onlinelibrary.wiley.com/doi/full/10.1111/icad.12672

Cuff, J. P., Windsor, F. M., **Tercel, M. P. T. G.,** Bell, J. R., Symondson, W. O. C., and Vaughan, I. P. (2023). Temporal variation in spider trophic interactions is explained by the influence of weather on prey communities, web building and prey choice. *Ecography*, 7, e06737. https://onlinelibrary.wiley.com/doi/full/10.1111/ecog.06737

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Cuff, J. P., Kitson, J. J. N, Hemprich-Bennett, D., **Tercel, M. P. T. G.,** Browett, S. S. and Evans, D. M. (2023). The predator problem and PCR primers in molecular dietary analysis: swamped or silenced; depth or breadth? *Molecular Ecology Resources,* 23(1), 41-51. https://onlinelibrary.wiley.com/doi/abs/10.1111/1755-0998.13705

2022

***Tercel, M. P. T. G.** and *Cuff, J. P (2022). The complex epistemological challenge of data curation in dietary metabarcoding: Comment on 'The precautionary principle and dietary DNA metabarcoding: commonly used abundance thresholds change ecological interpretation' by Littleford-Colquhoun et al., (2022). *Molecular Ecology*, 31(22). https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.16576. (Joint 1st/corresponding author)

Cuff, J. P., Windsor, F. M., **Tercel, M. P. T. G.**, Kitson, J. J. N. and Evans, D. M. (2022) Overcoming the pitfalls of merging dietary metabarcoding into ecological networks. *Methods in Ecology and Evolution,* 13(3), 545-559. https://besjournals.onlinelibrary.wiley.com/doi/abs/10.1111/2041-210X.13796

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Cuff, J. P., **Tercel, M. P. T. G.,** Drake, L. E, Vaughan, I. P., Bell, J. R., Orozco-terWengel, P., Müller, C. T. and Symondson, W. O. C. (2021). Density-independent prey choice, taxonomy, life history, and web characteristics determine the diet and biocontrol potential of spiders (Linyphiidae and Lycosidae) in cereal crops. *Environmental DNA*, 1-16. https://onlinelibrary.wiley.com/doi/full/10.1002/edn3.272

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Cuff, J. P., Drake, L. E., **Tercel, M. P. T. G.**, Stockdale, J. E., Orozco-terWengel, P., Bell, J. R., ... & Symondson, W. O. C. (2020). Money spider dietary choice in pre-and post-harvest cereal crops using metabarcoding. *Ecological Entomology*, 46, 249-261. https://onlinelibrary.wiley.com/doi/full/10.1111/een.12957

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Glossary:

Haplometrosis: where a single queen forms a colony.

Independent colony formation: the more common form of colony formation in ants. This involves an alate virgin queen flying from the nest on her "nuptial flight" where she mates with a male and subsequently forms a colony independently after finding a suitable substrate.

Invasive species: a non-native species that establishes a stable presence and population in a non-native location that detrimentally affects native organisms and/or people.

Monodomy: where an entire colony is found/based in a single nest site.

Non-native species: a species which has been transported to an area outside of their native range, where they typically reproduce and grow in population.

Polydomy: the use of more than one nest by a single colony. Each nest is spatially separate and may or may not have differing functions to the original nest.

Polygyny: where multiple queens produce eggs in the same colony. This can arise in one of two main ways: through pleometrosis, whereby two queens cooperatively form a colony (primary polygyny), or through accommodation of additional queens after colony formation by a single queen (secondary polygyny).

Propagule: a (usually very small) fraction of a colony that splits off with a mated female to form a new colony in ant species that colonise via budding.

Supercolony: a collection of colonies sharing a common source colony that have spread over a larger range. Ants from different colonies within the same supercolony behave to one another as if they are from the same colony.

Tramp species: a non-native species of ant that typically remains in or near human habitation, e.g., within cities or buildings. Not necessarily considered 'invasive'.

Unicoloniality: unicolonial species are those that show little to no aggression between closely-related colonies. These often reproduce to form dense collections of nests, between which workers are freely exchanged.

Glossary words are marked bold in the text upon first use.

Chapter 1: General Introduction

Anoplolepis gracilipes, Chiang Mai, Thailand.

1.1 Introduction

Through their enormous abundance and biomass, ants influence terrestrial biota between the poles on a scale unmatched by almost all other animal groups except humans (Parker and Kronauer 2021; Schultheiss et al. 2022). They are keystone members of terrestrial ecosystems, fulfilling a wide range of ecological functions, e.g., as parasites, predators, herbivores, decomposers, soil turners, and mutualists (Hölldobler and Wilson 1990). Ants exceed the combined biomass of all wild birds and mammals and equate to approximately 20% of the total biomass of humans (Schultheiss et al. 2022). Over 14,000 species of ants have been formally described (*AntCat* 2022) and many play important roles in nutrient cycling and soil turning, as well as forming mutualistic or symbiotic relationships with other organisms dating back 45-60 Mya (Stadler and Dixon 2005). Ants are involved in seed dispersal, farming exudate-producing insects and fungi, protection of myrmecophytes, being hosts and mutualists of myrmecophiles, and acting as specialist predators, for example. They form dominant ecological roles in most terrestrial systems and are major conduits of organic material and energy; ants are the leading predators of invertebrates in boreal, temperate, and tropical forests (Hölldobler and Wilson 1990; Philpott and Armbrecht 2006) and leaf-cutter ants consume 13-17% of foliar biomass in Neotropical cerrado savannah (Costa et al. 2008). Ants impose survivorship bias on species inhabiting terrestrial ecosystems outside of Antarctica and the Arctic, and much of extant terrestrial life has passed through an "ant-shaped selective filter" (Parker and Kronauer 2021).

Increasingly over the last century, organisms have been accidentally or deliberately introduced outside of their native ranges through the proliferation of global trade and travel. Many species then colonised these new areas because of a combination of poor identification resources and decontamination programmes at international borders. These species are variously called "**non-native**", "introduced", "**tramp**", or "alien" species; throughout this thesis I will use the term "non-native species" because it does not come with unintended linguistic loading. Moreover, the term "**invasive species**" is reserved for non-native species that have been shown to detrimentally affect native species, human well-being, or industries such as agriculture or construction. Approximately 200 ant species have been transported around the world into new areas (McGlynn 1999; Suarez et al. 2009; *AntCat* 2022). Of these, five are listed on the IUCN's "100 of the World's Worst Invasive Alien Species" list (Luque et al. 2014), primarily because of their threat to biodiversity; these are *Anoplolepis gracilipes* (yellow crazy ant), *Linepithema humile* (Argentine ant), *Pheidole megacephala* (big-headed ant), *Solenopsis invicta* (red imported fire ant), and *Wasmannia auropunctata* (little fire ant). Invasive ants have been observed to dramatically change ecosystems into which they have been introduced (O'Dowd et al. 2003), and many studies show that invasive ants pose a substantial threat to native biodiversity (see Chapter 2). This

Chapter will examine the taxonomy and biogeography of non-native ants, the invasion process, mechanisms by which invasive ants may detrimentally affect native species, and examples of ecosystems and species under threat by invasive ants, as well as the application of molecular methods to the study of ant ecology. Much of the literature on invasive ants focusses on the above five species (Holway et al. 2002; Krushelnycky et al. 2009) and, whilst some inferences can likely be made about broader non-native ant biology, it may be incorrect to extrapolate findings onto other less studied species.

1.2 Evolutionary and biogeographical perspectives of invasion success

1.2.1 Evolutionary patterns of invasive ants

Non-native ant species generally follow the same taxonomic distribution across subfamilies as total ant species (Figure 1.1). Though 9 of 16 subfamilies are represented by non-native ants, the absent 7 subfamilies contain only 242 described species in total, just 1.72 % of total ant diversity. Moreover, the bulk of non-native ants (72.3 %) are myrmicines, formicines, dolichoderines and ponerines, the four most speciose subfamilies of ants, and thus approximately proportional to general ant diversity (the same four families comprise 87.5% of total ant species). Consequently, the chances of successful establishment of a non-native ant species appears independent of phylogeny at the subfamily level. Indeed, the five most widely studied invasive ant species listed above are also members of the most speciose subfamilies (Dolichoderinae, Formicinae, Myrmicinae). Though ants seem to become established at approximately the same proportion as the number of species there are at the subfamily level, it is not known whether this rule is consistent across genera (Suarez et al. 2009).

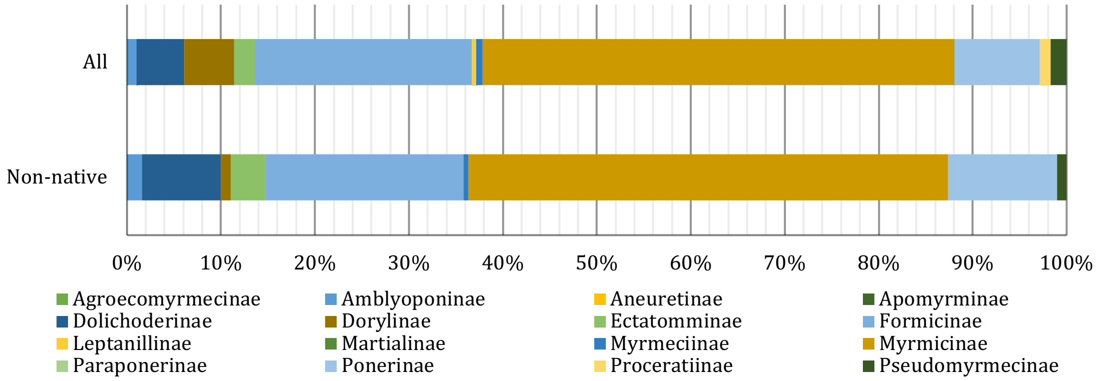


Figure 1.1. Distribution of ant species across subfamilies. The upper bar shows all named ant species (14,084); the lower bar shows all recorded non-native ant species (186). Data retrieved from AntCat and AntWeb (17/10/22).

Rather than a phylogenetic bias in invasion success, certain biological characteristics or life-history traits are more reliable predictors of invasion success. For example, dispersal methods differ in invasive ants relative to ant species generally (Hölldobler and Wilson 1977). In ants, two main dispersal strategies exist. The basal trait in ants, and most common strategy, is independent monogynous **haplometrosis** (Hölldobler and Wilson 1977), where an alate unmated queen conducts a nuptial flight, during which a male finds and inseminates her. She then breaks off her wings, finds a suitable substrate to begin excavating a nest, and lays her eggs within, feeding the brood with either foraged food or nutritional eggs. Colonies formed in this way are usually mutually antagonistic and **monodomous**. The second (derived) strategy is dependent colony foundation and is less common across ant species but found in the majority of invasive ants (Hölldobler and Wilson 1977; Holway et al. 2002). This involves a mated queen travelling from her natal source nest with a number of workers (a '**propagule**') on foot and establishing a new nest nearby. These nests are usually mutually tolerant of each other and are often called '**supercolonies**' due to the lack of aggression between workers; indeed, workers are often interchangeable between nests. Moreover, dependent colony foundation leads to **polygyny** in almost all cases, with each newly formed nest in the supercolony containing one or more queens from a nearby source nest and are also therefore **polydomous** (Hölldobler and Wilson 1977; Hölldobler and Wilson 1990). Whilst dispersal distance is greater in species using **independent colony formation**, because the queen takes flight, the primary modes of dispersal of non-native species to new areas are overwhelmingly anthropogenic (McGlynn 1999; Suarez et al. 2001; Rizali et al. 2010), and thus, on a global scale, chances of introduction should not be dependent on dispersal method (Hölldobler and Wilson 1990; McGlynn 1999). Invasion success after introduction, however, is likely to be dependent on dispersal method and life-history traits.

1.2.2 Patterns of life-history traits in invasive ants

Dependent colony formation and **unicoloniality** (formation of supercolonies) is disproportionately common in invasive ant species (Krushelnycky et al. 2009), including all five of the 'worst' invasive ant species listed above, obligately or facultatively. The ecological consequences of unicoloniality are similar across ant species: colonies eliminate some intraspecific competition and territorial conflicts as well as increasing the success rate of colony establishment by newly mated queens, but trade-off lower dispersal distance and the number of dispersing reproductive individuals compared to multicolonial species (Keller et al. 1989; Keller 1991). Moreover, unicolonial species, such as *L. humile*, where seasonal colony fusion can occur in its native range, are able to better command space than species with a fixed, non-plastic colony structure. Due to seasonal flooding in its native south America, unicolonial *L. humile* colonies in danger of being waterlogged will retreat to and fuse with colonies in drier areas. This increased worker density allows for better microclimatic regulation within the nest (Krushelnycky et al. 2009) and the ability to expand and recede when dry areas become available. Compared to typically multicolonial species, this translates into a more facultative and robust use of space by workers where resource availability can be tracked and reacted to with an increase in worker density to those colonies near a given resource. Ultimately, these benefits afford unicolonial species a greater chance of survival after establishment and thus persisting in the environment.

Most non-native ants are omnivorous (Holway et al. 2002). Omnivory is defined as having a diet that includes both animal and plant tissues. Ants that forage epigeally commonly feed on dead animals, capture small invertebrates, and consume sugary plant and insect exudates (Hölldobler and Wilson 1990). Omnivores are the most "generalist" of consumers, interacting with a wide range of different dietary taxa. Generalism more broadly can be difficult to define across a very wide range for taxa. Within a taxon or feeding guild, however, generalism/specialism can be usefully defined in the context of a specific aspect of the niche. A generalist species, for example, might be a species that can tolerate a broad range of abiotic conditions, nest in a variety of substrates, or consume many different species relative to other members of the same wider feeding guild. Specialist species therefore have narrower tolerances or interact with only one or a few species for food. Non-native ants are mostly generalist ground-nesting and ground-foraging species that exhibit generalist omnivorous diets, though there are differences between some species. For example, *P. megacephala* appears to more readily consume seeds than other invasive ants (Hoffmann 1998) and *S. invicta* and *W. auropunctata* have powerful stings that can paralyse prey and thus may rely more on live animal food sources. A broad diet increases the likelihood a non-native species will be able to find and persist on food sources present in their non-native range, and thus increases the chance of successful colonisation (Kolar and Lodge 2001). Furthermore, unicoloniality necessarily requires a species to have a broad diet to support such large colony populations relative to most multicolonial species. This increases the chances they might compete with a wide range of other species for food (Hölldobler and Wilson 1977).

1.2.3 Abiotic patterns of invasive ant distribution

The abiotic characteristics of environments that non-native ants are introduced to may modify the chances of successful colonisation. Non-native ants are often found in highly anthropogenically disturbed habitats (King and Porter 2007) for several reasons, so much so that invasive ants are often thought of as "disturbance specialists" (Vonshak and Gordon 2015; Andersen 2018). Disturbed areas are typically characterised as more open environments with less shade, higher temperature, lower humidity, and less structural heterogeneity than many natural systems (e.g. forests). Non-native ants are predisposed to these disturbed open areas, though their macro-climatic preferences generally track those of their native regions (Suarez et al. 2001; Holway et al. 2002; Suarez et al. 2009), e.g., non-native ants from temperate zones will not begin colonising the tropics and *vice versa*. Moreover, the primary mode of dispersal in non-native species is anthropogenic transportation from one highly disturbed area to another (McGlynn 1999), increasing the likelihood non-native species will be found in such locations. A high level of disturbance typically also lowers native ant abundance and species richness, and may suppress competitive interactions, releasing non-native ants from high levels of 'biotic resistance' (Elton 1958; Diamond and Case 1986; Parker et al. 2013). Despite the correlation between disturbance and invasion, however, non-native species often spread to natural systems after successful establishment in a disturbed area, e.g., in *A. gracilipes* (O'Dowd et al. 2003), *L. humile* (Slingsby and Bond 1984; Krushelnycky et al. 2005; Oliveras et al. 2005), *P. megacephala* (Heterick 1997b; Hoffmann and Parr 2008), and *W. auropunctata* (Dunham and Mikheyev 2010).

Certain abiotic conditions, often at small spatial scales, such as maximum and minimum day temperature, humidity level, soil temperature etc., determine whether an area is physiologically suitable for invasion. For example, in southern California *L. humile* invaded only irrigated plots and failed to spread to dry plots (Menke et al. 2007), whereas in New Zealand high vegetation cover and low soil temperatures prevent *L. humile* invasion into some areas (Hartley and Lester 2003; Ward and Harris 2005). Because of the different tolerances to abiotic conditions across species, some areas may be physiologically viable to certain invasive species but not others. The ability of invasive ants to extirpate native species can also depend on abiotic conditions at small temporal scales, e.g., changes in temperature throughout the day can prevent *L. humile* from extirpating native dominant *Iridomyrmex* species in Australia and halt their spread (Thomas and Holway 2005). *Iridomyrmex* species are better able to persist in hot, dry conditions, whereas *L. humile* prevails in warm and wet conditions. Furthermore, rainfall was the best predictor of invasional spread of *L. humile* in California, where hot and dry areas are a barrier to invasion (Heller et al. 2008).

Ergo, the chance of successful introduction for a non-native ant species depends on abiotic conditions and interacting ecological factors (such as competitiveness of native species). This is combined with the specific life-history traits of non-native ants that may afford them an increased ability to persist in foreign environments.

1.3 Invasional process

1.3.1 Competition with native ants

Hölldobler and Wilson (1990) state that interspecific competition is the "hallmark of ant ecology", claiming it is the primary driver of ant community composition. A large body of research supports this to some extent, suggesting that ant-ant interactions help to shape the spatial distributions of ant species at both large-scales, e.g., between 1-100 m distances (Greenslade 1971; Vepsalainen and Pisarki 1982; Savolainen and Vepsäläinen 1988; Andersen 1992; Andersen and Patel 1994), and small-scales, e.g., 1-100 cm (Savolainen and Vepsäläinen 1988; Bestelmeyer 2000; LeBrun 2005). Moreover, dominance hierarchies are thought to exist in ant communities, whereby ecologically dominant ants exert strong top-down effects on the rest of the ant community. Almost all highly invasive ant species behave as "dominants" (see definitions below in section 1.3.2.) and are therefore assumed to affect native communities through top-down processes such as competition and predation (Holway et al. 2002), though few studies measuring the impacts of ant invasion explicitly identify these processes.

If ant communities are structured to some degree by competition, might native ants be able to halt or slow the spread of invasive species through competition for resources or predation, i.e., the 'biotic resistance hypothesis' (Elton 1958)? The evidence supporting this is mixed. For example, *L. humile* were able to spread along riparian corridors in northern California over a 4-year period independent of the number and abundance of native species (Holway 1998), suggesting competition from native species has little to no effect on the rate of spread of *L. humile*. However, further research in California in the same system found that the spread of *L. humile* is accelerated when native ants are removed (Menke et al. 2007), suggesting the opposite: native species may limit resources available to *L. humile* or show direct aggression. Instead of a clear relationship on either end of the 'biotic resistance hypothesis', the ability of invasive ants to spread may be dependent on biotic processes, such as competition, that are mediated by abiotic variables, such as temperature. As described above in section 1.2.3, native dominant *Iridomyrmex* species are able to outcompete invasive *L. humile* in Australia because of different thermal niches and tolerances between them (Thomas and Holway 2005). *Iridomyrmex* can forage throughout the thermal peak of the day, whereas *L. humile* forage most effectively during milder more humid conditions (Menke et al. 2007). Therefore, temperature and humidity mediate the competitive outcomes and dominance of these species, as has been seen in other locations with different native and non-native ant species (Hartley and Lester 2003; Ward and Harris 2005; Heller et al. 2008).

1.3.2 'Dominance' of invasive ants

Dominance between ant species is hypothesised to be an important factor that helps determine the composition of a given community. Parr and Gibb (2009) define dominance broadly as: *when a single species makes up a large proportion of community biomass or numbers*. However, there are several aspects to dominance in ant ecology. *Behavioural* dominance is determined by observing interactions between individuals, typically at food baits; species showing aggression that force other individuals from food baits are considered behaviourally dominant (Parr and Gibb 2009; Cerdá et al. 2013). This usually involves direct physical or chemical attacks and vigilance in the presence of other ant species – behaviourally dominant species will attack in the absence of food resources to defend a territory e.g., in *Atta*, *Eciton*, *Formica*, *Iridomyrmex*, and *Oecophylla* (Cerdá et al. 2013). *Numerical* dominance simply refers to the abundance or biomass of a species at resources and can be measured by examining abundance and/or frequency of occurrence at traps or baits, or the proportion of baits occupied by a species in a given area (Parr and Gibb 2009; Cerdá et al. 2013). *Ecological* dominance, sometimes also called 'functional dominance', is a combination of behavioural and numerical dominance and can be quantified by measuring the abundance of species at baits versus in pitfall traps; ecologically dominant species are found in higher proportions at baits relative to pitfall traps and thus represents a measure of foraging success relative to abundance in the surrounding environment (Parr and Gibb 2009). Dominance in ant ecology therefore usually refers to the amount of resources controlled by a given species in the context of the rest of its community.

In native ant communities, it is hypothesised that a "dominance-discovery" trade-off exists (Fellers 1987). This entails some species being good at finding food (discovery), whilst others are good at defending or seizing food resources from other ants (dominance). The evidence for whether the dominance-discovery trade-off exists is somewhat mixed, however. Parr and Gibb (2012) conducted a systematic literature review and field study, revealing a paucity of evidence supporting a trade-off between the ability to discover and dominate resources. Instead, they found a positive correlation between species that first arrived at baits and their ability to defend them – the opposite of results expected if a dominance-discovery trade-off existed. Their search did, however, reveal discovery-dominance trade-offs amongst some ant communities in the presence of natural parasitoids (LeBrun and Feener 2002; LeBrun 2005; Adler et al. 2007). Presence of predators could have a similar effect, though has not been studied. One criticism of the methods employed by studies investigating the dominance-discovery trade-off is the use of large stationary baits to test which species find and defend food resources. These may not accurately represent the natural spatial spread of food resources and may hinder "discoverer" species that rely on finding and transporting small food items quickly back to the nest. It is possible a discovery-dominance trade-off may only be seen when natural distributions of food resources are present, as opposed to large baits placed on homogenous surfaces. Many other factors also influence ant community dynamics, such as tolerances to abiotic conditions and what time of day or night species are active (Cerda et al. 1997; Bestelmeyer 2000; Thomas and Holway 2005; Heller et al. 2008).

Almost all invasive ants exhibit dominant characteristics, both behaviourally and numerically, and are usually able to extirpate native dominant species (including non-ant invertebrates) from resources (O'Dowd et al. 2003; Touyama et al. 2003; Carpintero and Reyes-López 2008; McNatty et al. 2009), though there are exceptions to this rule (Majer et al. 1994; Way et al. 1997; Walters and Mackay 2005). Mechanistically, this may not be simple aggression (Bertelsmeier et al. 2015a) but rather the overall ability of these species to control a resource using various strategies. For example, *L. humile* employs a 'bourgeois strategy' (Carpintero and Reyes-López 2008), where individual workers are submissive to other species that are numerous, aggressive, or defending a bait, but behaviourally dominant when in a large group. This allows them to avoid conflicts they are likely to lose whilst increasing the probability of successful conflicts once *L. humile* individuals have gathered in large numbers. In addition, invasive ants are thought to be able to "break" the discovery-dominance trade-off, finding and recruiting to food resources faster than native species, whether such a trade-off exists or not (Holway 1999). Invasive ants are also known to extirpate native species through nest raiding even when diets do not overlap. This is probably the cause of many extirpation events in certain groups of ants with specialised diets e.g., in harvester ants that share very little of their diet with invasive ants (Hook and Porter 1990; Human et al. 1998; Zee and Holway 2006).

1.3.3 Consequences of ant invasion

Given the ability of invasive ants to dominate ant communities, how is it that these species cause ecological damage at great spatial scales? Through competition for food resources, predation, and non-lethal effects, invasive ants directly and indirectly detrimentally affect a range of native species that can have cascading effects on entire native ecosystems. For example, on Christmas Island, *A. gracilipes* extirpated the abundant native keystone detritivore, the red land crab (*Gecarcoidea natalis*), and in doing so slowed leaf litter decomposition and increased seedling recruitment (O'Dowd et al. 2003). This led to a change in the physical structure and composition of the plant community on the forest floor in invaded areas. Invasive scale insects were also supported in much higher abundance on trees where *A. gracilipes* was present, which further led to growth of sooty moulds sustained by the high quantities of honeydew on leaf surfaces. This in turn caused canopy die-back and tree death in invaded areas through a combination of increased competition from plants on the forest floor, widespread sooty mould infection, and grazing by scale insects. These effects radically altered the basal level of the food web on Christmas Island and substantially altered the composition of the associated ecological community. Further examples of tree death indirectly caused by *A. gracilipes* have been seen on Bird Island, Seychelles, due to increases in scale insect abundance and concomitant grazing damage (Feare 1999; Hill et al. 2003).

Detrimental effects of invasive ants can also stem from the extirpation of native ant species. For example, *L. humile* invasion led to lower abundance of horned lizards in California, USA, by outcompeting and extirpating their main food source - native ants (Fisher et al. 2002). Horned lizards are unable to exist on a diet of *L. humile*, and therefore cannot replace the absent native ants with the invasive species (Suarez and Case 2002). In Gabon, *W. auropunctata* invasion leads to an increase in phloem-feeding insect abundance (scale insects), and a decrease in leaf-chewing insects (e.g. Lepidoptera larvae), with an overall large decrease in leaf herbivory damage (Dunham and Mikheyev 2010) that may have indirect effects on invertebrate and plant community composition in invaded areas.

The consequences of ant invasion are highly diverse, and this is probably a function of the diversity of interactions invasive ants are involved in. Through their generalist diets and high abundance, invasive ants come into direct contact with many species. Despite this, the indirect effects of ants may be more damaging to the functioning of native ecosystems. For example, whilst almost all invasive ant species probably prey on native invertebrates, the functional roles of these invertebrates may be invaluable to the functioning of a given native ecosystem. Consumption of, or aggression towards, native pollinators and seed dispersers, for instance, may present substantial negative implications for how an ecosystem persists. The diversity and scale of such impacts are discussed in Chapter 2.

1.3.4 Abundance of invasive ants in their non-native range

Invasive ants can reach densities far exceeding those of native ants and it is the very high number of interactions between invasive ants and native species that can lead to ecological damage. Despite this, it is unclear exactly how invasive ant colonies reach such huge densities. The research that does exist suggests biological release from natural enemies could partly explain such large invasive populations, as in *S. invicta*, whereby invasive populations in north America are 4-7 times larger than in their native south American range (Porter et al. 1997). By accounting for seasonal and temporal conditions, climatic variables, land management regimes, habitat type, and level of polygyny, Porter *et al.* (1997) suggest low natural enemy species richness is a good explanation for the abundance of *S. invicta* in its non-native range. It has not been studied whether this holds for other invasive ant species (Krushelnycky et al. 2009). Invasive ant abundance tends to increase further once native ants have been extirpated, presumably because all nesting and food resources are made available to the invasive ants in the absence of competition (Holway et al. 2002).

Another explanation for high abundance is the reduction in intra-specific territorial conflict of many invasive ant species because of unicoloniality (Macom and Porter 1996). The workers that would be reserved for defence can be reallocated to other tasks, such as nest maintenance, foraging, and brood care (Hölldobler and Wilson 1977; Hölldobler and Wilson 1990). Though the broad ecological effects of unicoloniality have been discussed, the genetic bottleneck that results from introduction can increase the prevalence of unicoloniality by reducing the ability to discriminate nestmates and non-nestmates (Tsutsui et al. 2000). Ultimately, these factors do not work in isolation; as mentioned previously, the ability of invasive ants to dominate a range of resources behaviourally and ecologically also helps them attain very high populations.

Mutualisms between non-native ants and exudate-producing insects and plants bearing extra-floral nectaries have also probably facilitated invasion and high abundances of both ants and their mutualists through a positive-feedback loop, though the evidence for this is clearer for some species than others. For example, *A. gracilipes* has been shown to tend hemipterans in its non-native range (O'Dowd et al. 2003; L. Abbott and T. Green 2007; O'Loughlin and Green 2015), and experimentally-increased sugar concentrations (small containers of sugar water attached to tree trunks) led to 400% higher worker recruitment to carbohydrate resources versus normal sugar concentrations available from extra-floral nectaries and hemipterans (Savage et al. 2011), suggesting sugar concentrations greatly benefit *A. gracilipes* colonies. Similarly, experiments providing varying amounts of sugar water available to colonies showed that *L. humile* brood mass, foraging activity, and aggression increased proportionally with sugar quantity (Grover et al. 2007; Kay et al. 2010), in addition to total colony size being much greater with high sucrose volumes (Schindelin et al. 2012). In *S. invicta*, colonies in their non-native range rely more on honeydew than in those found in the native range (Wilder et al. 2011), essentially occupying a lower trophic level. Moreover, the invasive mealybug, *Antonina graminis*, accounts for 70% of Hemiptera tended by *S. invicta* and the size of colonies in field sites in Texas, USA, were significantly correlated with the abundance of the mealybugs and indirectly by their host plants (Helms and Vinson 2002; Helms et al. 2011). *Pheidole megacephala* tended the invasive scale insect, *Pulvinaria urbicola*, on Cousine Island, Seychelles, protecting it from natural enemies, but after ant eradication, *P. urbicola* was eventually exterminated solely by natural enemies (Gaigher et al. 2013), suggesting *P. megacephala* presence facilitated invasion by *P. urbicola* and both species' abundances were then increased through a positive feedback loop. More generally, *P. megacephala* has been shown to tend and promote exudate-producing hemipterans throughout its non-native range (Wetterer 2007; Tanaka et al. 2011). The importance of sucrose has not been experimentally quantified in *P. megacephala* as has been done with *A. gracilipes*, *L. humile*, and *S. invicta* (Helms 2013). Likewise, in *W. auropunctata*, though exudate-producing hemipterans are readily tended (Dunham and Mikheyev 2010), the importance of this source of sucrose has not been quantified for colonies (Helms 2013). Invasive ant species for which the importance of sucrose has been quantified show numerous positive effects on total colony size and growth rate, brood mass, individual worker aggression, and recruitment rate at food resources proportional to the availability of sucrose resources. All five of the most studied and ecologically damaging invasive ant species readily tend exudate-producing hemipterans, some of which are themselves invasive. Thus, both native and invasive honeydew-producing hemipterans may increase the total number of interactions invasive ants can undertake, essentially increasing the destructive potential of these species. Furthermore, invasive ants are better at protecting and extracting honeydew from these hemipterans than native ants (Holway et al. 2002), allowing them to exceed natural populations, further adding to the positive feedback loop. It is possible interactions with honeydew-producing insects are important during invasion events, though have not been explicitly studied (Holway et al. 2002).

Invasive ants are able to monopolise a greater proportion of resources than native ants and this creates a positive feedback loop further increasing population size and monopolisation of more food resources. Moreover, invasive ants are released from natural enemies in their introduced range, which may further increase abundance and worker efficiency. In addition, unicoloniality mitigates some factors limiting population size, such as intraspecific competition and territorial conflict, promoting higher abundances again. The number of ecological interactions permitted by these relatively high abundances amplifies any ecological ramifications presented by non-native ant species, and these appear to be negative in the majority of empirical examples. See Chapter 2 for a quantitative analysis of these effects.

1.4 Molecular determination of trophic interactions

1.4.1 Overview

A key objective of ecology is to determine the structure of complex species-interaction networks and analyse the processes driving their dynamics. Trophic interactions help shape the diversity of life, including species' distributions, behaviour, anatomy, and abundance. Analysing the diets of consumers is therefore fundamental to discovering the processes underpinning the ecology of life on Earth. However, identifying exact species that make up the diet of a consumer has historically been fraught with methodological challenges.

Before the advent of molecular methods, diet analyses were conducted using morphological methods whereby gut contents, faecal samples, or regurgitates were physically dissected and food taxa were identified visually using classical morphological identification. Behavioural observations of direct feeding events have also been used. These methods suffer from several issues. For example, researchers need high levels of taxonomic training to physically extract and identify prey. For generalist consumers eating many species, it may not be feasible for a single researcher (or several researchers) to identify all prey items based on small body fragments found in diet samples. Moreover, morphological methods cannot reliably identify soft-bodied prey or the diet of fluid feeders, i.e., most life on Earth. These methods are also time-consuming, and the taxonomic resolution attained is rarely at the species-level for dietary items. Furthermore, some consumers are too small to be reliably observed or dissected i.e., many arthropod predators (Symondson 2002; Harper et al. 2005). These methods are still often used today, despite the shortfalls.

Early molecular methods overcame some of these issues. Protein electrophoresis, for example, can be used to identify the diet of fluid-feeders and small invertebrates by homogenizing their guts (or entire bodies) and analysing them on polyacrylamide gels by staining for enzymatic activity (Symondson 2002). The band patterns are then compared to those of potential prey species. However, identification is still a challenge using these methods and banding patterns of generalist predator diets may be too complicated to identify prey with reliability (Walrant and Loreau 1995; Symondson 2002). Better still is the use of monoclonal antibodies that allow for more accurate analysis of predator diets. By fusing myeloma cells and antibody-producing B-lymphocytes, hybridomas are made *in vitro* that can be used to produce specific antibodies that are selected based on potential prey species proteins to which they bind (Symondson 2002). This method reduces the chances of cross-reactions relative to polyclonal studies because of the greatly reduced number of different antibodies that can bind to the target protein (Symondson 2002). Use of monoclonal antibodies can therefore identify and quantify the predators feeding on a prey species, such as important invertebrate crop pests. However, these methods both fall short when trying to reveal the diet of generalist predators (Symondson 2002).

DNA-based methods to determine predator diet quickly became widely accepted after their first use (reviewed in Symondson (2002) and Pompanon *et al.*, (2012)). Initially, this used Sanger sequencing, which produces a single forward and reverse read with a high degree of accuracy for a given gene sequence (Symondson 2002; Deagle et al. 2005; Harper et al. 2005). The technique is typically limited to a few samples because only one sequence can be read at a time. Recently, massively parallel high-throughput sequencing (HTS), also known as next-generation sequencing (NGS), has been used to improve upon all previous methods of diet analysis, visual and molecular, in terms of accuracy, speed, and price per sample (Pompanon et al. 2012; Symondson and Harwood 2014). It involves the massively parallel sequencing of millions of fragments of target DNA simultaneously. DNA-based approaches involve the use of a broadly accepted barcoding region for a given taxon, which is a standardised section of DNA, either genomic or from organelles within the cell, containing high levels of species-level variability. Kress and Erickson (2012) suggest a barcode requires three main attributes:

> *"(1) contain significant species-level genetic variability and divergence, (2) possess conserved flanking sites for developing universal PCR primers for the widest taxonomic application, and (3) be of appropriate sequence length so as to facilitate current capabilities of DNA extraction and sequencing.",*

For animals, the mitochondrial cytochrome *c* oxidase subunit I (COI) gene is commonly used. After extracting DNA from a sample and sequencing a given barcode marker, a barcode library can be generated that is used to identify other sample sequences. These barcode libraries can be made open-source and freely available to interested researchers. GenBank and Barcode of Life Database (BOLD) databases are examples of barcode libraries, allowing organisms to be identified easily from faeces, gut contents, or environmental samples (Ficetola et al. 2008). This greatly increases the potential scope of DNA-based analyses of diet. These libraries also present one of the potential shortfalls of the methodology. Barcode libraries can have an uneven taxonomic coverage and require skilled taxonomists to identify sources of new sequences. Indeed, some sequences added to barcode libraries are unreliable because of inaccurate identification of specimens from which the sequence came. Certain groups that may not be comprehensively described at species-level (e.g., insects) suffer from a lack of recorded sequences across barcode libraries. Ergo, when a library is searched using a tool such as the Basic Local Alignment Search Tool (BLAST), a given species may not appear because it has not yet been submitted to the library. Therefore, it is good practice to identify, sequence, and barcode a range of specimens that could be potential prey items of the focal predator(s), though this mitigates some of the benefits of HTS for gut analysis in terms of price and time, and may introduce sampling biases (Clare et al. 2018).

An option for network analyses using genetic data is to use molecular operational taxonomic units, MOTUs (Clare et al. 2018), also called Amplicon Sequence Variants (ASVs), to overcome taxonomic challenges in barcode libraries. This technique essentially uses MOTUs as a measure of prey genetic diversity, and although each MOTU may not represent a species, it can be used to generate ecological networks and study prey consumed (Clare et al. 2016; Clare et al. 2018). Each MOTU is created using a MOTU threshold, which is the genetic similarity required for unidentifiable species to be grouped together, and typical values range from 92-99% depending on the study (Clare et al. 2018; Cuff et al. 2021a; Tercel et al. 2022). The MOTU threshold should be ideally determined by the genetic diversity of the group of prey being studied (Clare et al. 2016), but this is not always possible. For example, when the prey taxa are varied in a generalist predator. Both MOTUs and taxonomic data can be used in tandem to examine prey choice in a more robust way than using only one method (Clare et al. 2018), though a comprehensive taxonomy-based sequence library is often unachievable.

Another difficulty of DNA-based methods stems from the inconsistencies of PCR primer pairs. These often have patchy and sometimes confusing taxonomic biases in the organisms whose DNA they amplify. For example, primers can be designed to primarily amplify a section of the mitochondrial COI gene in insects but may also amplify the same region in jellyfish and Carnivora; these are highly divergent evolutionary lineages that have happened to convergently possess similar mutations to the same region, allowing the PCR primers to bind to flanking sites. Primers targeting a different section of the same gene, a longer amplicon length of the same section, or a different gene entirely may have different biases. Furthermore, within-group predation can be difficult to detect because of the number of predator DNA reads generated during sequencing. For instance, in studies investigating insect predators of other insects, it is likely the primers will amplify both the predator and the prey. One way to mitigate this issue is to dissect and extract DNA solely from the gut or crop (depending on the species) to reduce the total quantity of predator DNA extracted. However, this can be impossible in very small predators. Moreover, even if guts can be successfully dissected, much host DNA may still remain. The problems associated with the amplification of host reads in diet analyses (the "predator problem") are reviewed comprehensively in Cuff *et al*. (2022).

Finally, one problem associated with using metabarcoding to determine diets emerges when the focal consumer is omnivorous. The problem, in essence, relates to how certain taxa present in the sequencing results may not be ecologically meaningful, and that it may be difficult to determine which taxa these are. For example, a hypothetical generalist vertebrate scavenger is known to feed on vertebrates, invertebrates, and plants. Researchers are collecting fresh faecal samples to elucidate trophic interactions using DNA metabarcoding (Figure 1.2). The omnivore commonly feeds on fallen fruit, but flies and ants colonise the fruits as soon as they reach the ground. The omnivore indiscriminately consumes the fallen fruit, accidentally ingesting many ants and flies at the same time. When primer pairs amplifying plants and insects are used, the flies, ants, and fallen fruit are all amplified and detected in the sequencing results. Researchers are then tasked with determining if these detections are meaningful. Given that the omnivore is known to feed on invertebrates, this may be a difficult question to answer. The ant species itself is an abundant generalist omnivore and colonises most food items before the hypothetical vertebrate, and thus appears in many faecal samples. The issue is exacerbated if little is already known about specific trophic interactions between the omnivore and its prey, making disentangling what has happened more challenging. The flies and ants in the above scenario may be assumed to be highly important to the focal omnivore because they frequently occur in faecal samples, but this may not be true biologically. The omnivore may not benefit from ingesting these species at all. In fact, the vertebrate may actually be harmed by feeding on the ants, which could possess distasteful compounds and/or venom (Blanchard & Moreau, 2017; Hölldobler & Wilson, 1990; Schmidt, 2009).

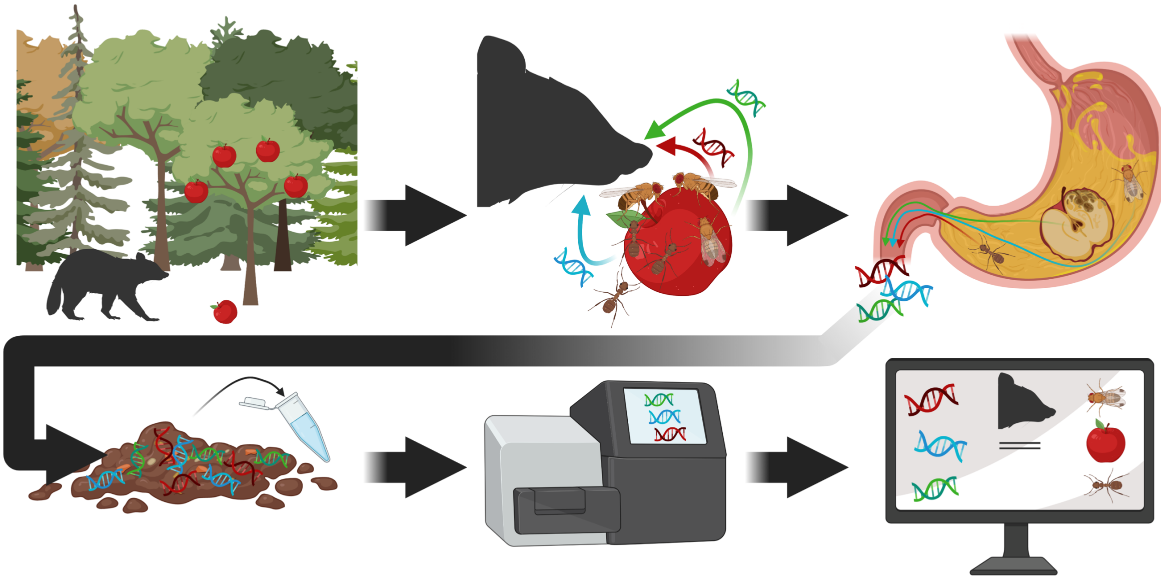


Figure 1.2. A hypothetical generalist vertebrate omnivore feeding on a fallen fruit colonised by insects. The apple is intentionally consumed and the insects accidentally, resulting in detection of both plant and insects in the DNA metabarcoding output, and likely equivalent representation in subsequent analyses. Figure created in Biorender.com. Figure taken from Tercel *et al*., 2021.

The difficulties associated with studying the diet of omnivores using dietary metabarcoding are reviewed in further detail in Tercel *et al.* (2021).

1.4.2 Molecular analyses of ant diet

Most previous analyses of ant diet have been through visual observation of feeding (reviewed in Hölldobler and Wilson (1990) and Lach *et al.* (2010)) or pre-HTS molecular methods (e.g. protein electrophoresis (Morris et al. 1999)). Though much useful information has been gleaned using behavioural observations, in many cases they investigate the broad macronutrient preferences of species, life-stages, or track seasonal changes in diet (Hölldobler and Wilson 1990; Lach et al. 2010) and have not resolved dietary preferences to particular prey species in generalist ant predators.

To the author's knowledge, only five published studies have been conducted that attempt to elucidate ant diet using DNA-based methods. Muilenberg *et al.* (2008) showed an economically important crop pest, *Enaphalodes rufulus* (Coleoptera: Cerambycidae), were predated upon by ants (*Camponotus pennsylvanicus* and *Aphaenogaster tennesseensis*) but detection rates were very poor even when *E. rufulus* eggs were fed to laboratory-reared colonies of the ant species, suggesting a problem with PCR inhibitors. Fournier *et al*. (2008) investigated ant predation of *Homalodisca vitripennis* (Hemiptera: Cicadellidae), an important pest of several crop species, and failed to detect any DNA in the guts of ants using DNA-based methods, despite 12% of ants showing positive results with enzyme-linked immunosorbent assays for *H. vitripennis*. In light of the above studies having trouble picking up prey DNA, Penn *et al*. (2016) examined the effect of PCR amplification inhibitors across several ant species, finding that certain structures in the gaster significantly inhibited amplification of prey DNA. The main source of inhibitors originated in the crop, and thus the authors suggest isolation of the gut and sequencing DNA solely from there, instead of homogenizing the whole ant body or gaster, might yield more reliable results. Jiménez-Carmona *et al*. (2019) attempted to detect the presence of *Hypothenemus hampei* (Coleoptera: Scolytidae), the coffee bean borer, using three separate primer pairs, but detected DNA in 82.73% of negative control treatments during diagnostic testing, suggesting the primers are not specific to the prey and thus unreliable. These studies focussed on the role of ants as biocontrol agents of crop pests but suffered from methodological difficulties that led to unreliable results (Penn et al. 2016; Penn and Harwood 2016). Finally, Hoenle *et al*. (2019) go a step further than all previous studies by resolving many dietary items of army ants to species-level using a scaled-up barcoding approach. Whilst they do not have individual-level dietary data, because the authors intercepted ants carrying prey items and sequenced the prey items directly, rather than what was actually consumed by individual ants, their study is the most comprehensive dietary analysis of ants published thus far. This work allowed them to construct a large trophic network from over a thousand individual prey items from 11 specialist ant species. Despite the lack of individual diet data, this study goes some way to confirm that army ants exhibit a high level of dietary specialisation on other ants.

1.5 The study system

1.5.1 Round Island description

Round Island (Figure 1.3) is a basaltic cone that reaches 280 m above sea level and retains the last remnant of native lowland palm habitat within the Mascarenes (Cheke and Hume 2008), which has been recovering since non-native vertebrate herbivores were eradicated (Merton 1987; Cheke and Hume 2008). This habitat is primarily dominated by the blue latan palm, *Latania loddigesii*, and, to a lesser extent, *Pandanus vandermeschii.* Non-native herbaceous plants, such as *Achyranthes aspera* and *Tridax procumbens*, form swathes of invaded habitat in large open clearings between thickets of native trees. Before the 1980s, much of the island suffered deforestation caused by non-native rabbits, *Oryctolagus cuniculus*, and goats, *Capra aegagrus hircus,* resulting in the loss of all but two hardwood tree species represented by a single individual bois buis tree, *Fernelia buxifolia*, and a few individuals of acacia indigéne, *Gagnebina pterocarpa* (Strahm 1993). Goats and rabbits were eradicated in 1979 and 1986, respectively, but the loss of habitat led to extensive soil erosion and created large expanses of barren rock slab over much of the island (Figure 1.4; see "rocky slab" habitat description below). Since 2002, there have been extensive efforts to restore the lost hardwood forests and to enhance the natural regeneration of the palm habitat (Jones 2008).

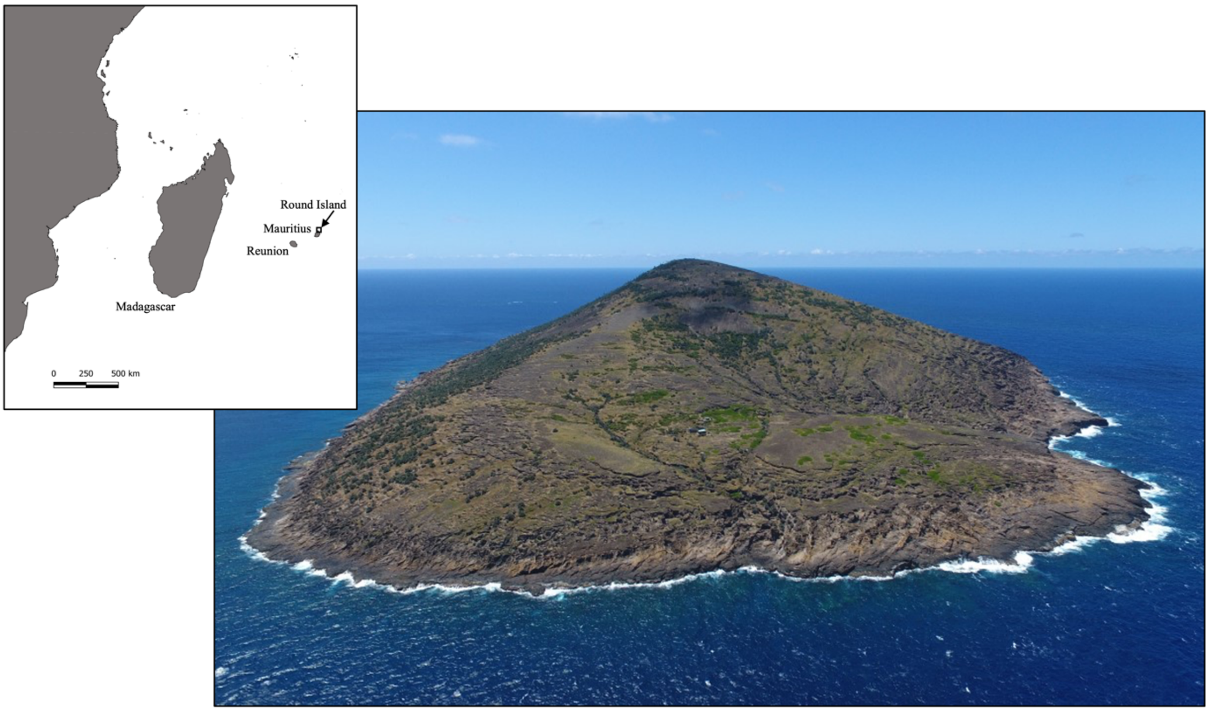


Figure 1.3. Round Island and its position in the Indian Ocean.

Lambdon (2019) groups the habitats of Round Island into 14 broad categories (Figure 1.4):

i. Coastal rocks. These are typically barren areas of shoreline flushed by large waves and generally inhospitable for vascular plants. These expanses can reach 60 m altitude and provide minimal opportunities for plant growth.
ii. Inland cliffs and rocks. Steep rocky faces which lack the maritime element of coastal rocky habitats. *Ipomoea pes-caprae* can hang from above or colonise areas of the cliff where soil has accumulated. Dominant plants typically include *Dactyloctenium ctenoides, Chloris barbata, Boerhavia coccinea* and *Achyranthes aspera*.
iii. *Ipomoea* sward. The creeping vine *Ipomoea pes-caprae* dominates this habitat type, which is characterised by high proportions of bare rock substrate and minimal soil accumulation. Though it lacks biodiversity, this habitat type is nevertheless important, as it represents the first stage of colonisation of the heavily eroded substrate. This eventually aids in soil accumulation and plant community succession.

iv. Open gulley. These habitats are a combination of permanently bare rock, dense herbaceous cover and established palm thickets. Gullies do not contribute a large proportion of total land area on Round Island, but are important landscape features for accumulating soil, providing refuges and nesting sites for geckos *Phelsuma guentheri, Phelsuma ornata,* and *Nactus durrellorum* and providing ideal conditions for rapid palm establishment. Dominant plants include *Latania loddigessi, D. ctenoides, Cenchrus echinatus, Digitaria horizontalis, Tridax procumbens,* and *Solanum americanum.*

v. Open weed. This habitat provides a matrix of herbaceous cover between the denser thickets of palm woodland and can be considered one of Round Island's most important communities. It is reasonably diverse, with 9.4 plant species per 25 m^2. The grasses *C. echinatus, Cymbopogon caesius* and *D. ctenoides* dominate, with at least 10 % cover provided by *A. aspera, D. horizontalis* and *I. pes-caprae*. Isolated *L. loddigessi* trees may be present. This habitat provides many resources for Round Island's fauna: hunting grounds for the boas and lizards, forage for the tortoises, and is likely to contribute the largest proportion of nectar and seeds of any habitat on Round Island, which are both important sources of food for invertebrates.

vi. Palm glade. Primarily dominated by *L. loddigessi*, providing substantial shade and a unique understorey community. Only shade-tolerant plants are likely to persist in these areas, though there is potential for rapid growth because summer die-back is somewhat mitigated by the canopy cover. The canopy consists of 50-90 % *L. loddigesii*, and the understorey is dominated by the creeping herb *Commelina benghalensis* in the darkest areas, rarely found elsewhere on Round Island. Taller herbs *Abutilon indicum* and *Solanum americanum* predominate away from the darkest areas. This habitat is likely to become more abundant as latan palms continue their regenerative expansion and it currently represents one of the most biodiverse habitats on Round Island.

vii. Palmoid thicket. These areas represent a mid-late successional stage in the Round Island canopy regeneration project, and thickets are typically of a similar age to those of the palm glade. Sites are dominated by either *L. loddigesii* or *Pandanus vandermeschii*, or both. In contrast to palm glade, palmoid thicket tends to be species-poor, perhaps owing to the heavy blanketing of the substrate by fallen leaves and almost complete canopy cover.

viii. Restoration areas. Though the entirety of Round Island could be considered a "restoration area", these areas are the most artificially selected of Round Island's plant communities. There is considerable variation in which species are planted in which areas, and management practices vary between them. Broadly, these areas consist of planted native trees in a matrix of non-native ruderal weeds such as *Ageratum conyzoides*, *Conyza bonariensis, Oxalis corniculate* and *Sonchus oleraceus*.

ix. Rocky slab. Large swathes of Round Island are covered by bare rock though it is not certain whether these are naturally occurring or were caused by deforestation and subsequent soil erosion from the early 1800s onwards. Old 18th and 19th century accounts do not mention striking expanses of rock slab, which are a characteristic of Round Island today (Cheke and Hume 2008). Lichen colonies on bare rock are also universally small, and these mature over decades and centuries, suggesting soil may have covered the entire island ~350 years ago. Moreover, several standing dead endemic ebony trees show a distinct "soil line", where soil presumably reached when it may have covered Round Island. These habitats are largely barren of plant life, though small patches of weed can be found, which adhere to the characteristics of *Ipomoea* sward and open weed habitats.

x. *Scaevola* scrub. These areas are found primarily in southern coastal areas and are dominated almost exclusively by *Scaevola taccada*. This species was introduced in 2002 to provide habitat structure in previously bare coastal ridges, and it was either this or *Tournefourtia argentea* that was present along the coasts prior to habitat degradation. Telfair's skinks feed on the fruits of *S. taccada* and forage for invertebrates, and boas are often seen hunting in the dense stands. However, *S. taccada* outcompetes native habitats, and it may need to be controlled in the near future to allow xeric weed communities to expand.

xi. Summit habitat. Though unique, the summit consists of a mosaic of existing communities, all influenced by the exposed location. Strong winds make this area unfavourable for tree growth, though the western side is notably dotted with patches of the Critically Endangered bottle palm, *Hyophorbe lagenicaulis*. However, bare rock predominates and the plant communities resemble those of rocky slab habitats. Importantly, the flatter areas of the summit are populated by relatively rare plants, *Cyperus exilis*, *Sida pusilla* and *Phyllanthus mauritianus.*

xii. Tall weed. Averaging 60-200 cm in height, these often vast swathes of herbaceous cover dominate sheltered areas at higher altitude. Plant communities primarily consist of *A. aspera* and *Bidens pilosa*, though these are often mixed with *Desmanthus virgatus*, *C. echinatus* and *A. indicum*. These areas are broadly considered undesirable because they consist almost entirely of non-native species, occupy areas which would be ideal for hardwood tree growth, and they provide few resources for native animals. Indeed, several areas of wedge-tailed shearwater, *Ardenna pacifica*, nests have been completely overgrown, leading to nest-site abandonment.

xiii. Tortoise-grazed sward. These habitats began proliferating in 2007 once tortoises were introduced and are similar in composition to open weed or tall weed habitats. However, the structural heterogeneity is much different, with tortoises heavily grazing a broad range of plants. The movement of tortoises also creates areas of bare earth. Promisingly, the Mauritian native grass *C. compressus* is spreading rapidly in these areas because it seems to be able to respond to grazing pressure. Non-native ruderal weeds also proliferate in the tortoise-disturbed patches, notably *A. conyzoides, Amaranthus viridis, C. bonariensis* and *Oxalis corniculata*.

xiv. Xeric weed. This represents the only predominantly native habitat remaining on Round Island, as measured by species richness. It may be the last remnant of a former tortoise-grazed community, which would have been curated by the now-extinct native *Cylindraspis* spp. tortoises. Indicator species include *C. exilis, Euphorbia thymifolia, Fimbristylis cymose, Ipomoea pes-caprae,* and *Lepturus repens*.

Figure 1.4. Habitats of Round Island. From left to right, top row: coastal rocks, inland cliffs and rocks, *Ipomoea* sward, open gulley; second row: open weed, palm glade, palmoid thicket, restoration areas; third row: rocky slab, *Scaevola* scrub, summit habitat, tall weed; fourth row: tortoise-grazed sward and xeric weed. Categories and images from Lambdon, 2019. All images taken by Phil Lambdon.

See Chapter 3 for a description of the vertebrate community on Round Island and Chapter 4 for a more in-depth description of the invertebrate fauna.

Round Island may be significantly affected by invasive ants, which have now established a strong presence on the island since the early 2000's (Motala et al. 2007a; Smith and Fisher 2009; Dunlop et al. 2016). Other island systems have been badly affected by invasive ants (see Chapter 2), and Mauritius may have historically suffered a similar scale of biodiversity loss. However, it is not possible to describe and quantify this here because there are no usable records of invertebrate biodiversity before invasive ants colonised Mauritius. Nevertheless, no studies to date have described the species-level trophic interactions of invasive ants anywhere in the world at the community scale. By using DNA metabarcoding I will be able to identify the trophic interactions of invasive ants on Round Island. Do invasive ants truly have broad diets? Are ant-ant interactions important factors governing invasive ant ecology? How do invasive ants potentially affect other consumers when there are shared food resources? These are the conservation-relevant questions I will attempt to answer by using DNA-based dietary analyses of invasive ants.

1.6 Conclusions

The destructive potential of invasive ant species and their ability to invade a new environment depends on high abundances and certain life-history traits, as opposed to a taxonomic bias at the sub-family level. For the five most studied invasive ants, traits such as behavioural dominance and dietary generalism help them control resources in their non-native range and thus build colonies to ecologically important abundances. Much of the published research focusses on how invasive ants affect native ants, and although some studies have shown responses of non-ant invertebrate communities to invasive ants, relatively little is still known at the community level. Quantifying the responses of native ants and wider animal communities to non-native ants might help inform management decisions by stakeholders. I present a meta-analysis of this in Chapter 2.

The use of dietary DNA metabarcoding has not yet been used to study ant ecology. However, as evidenced in other systems and consumers, it appears to have the potential to reveal the processes underpinning the trophic ecology of ant communities. The method therefore is likely to have broad relevance to the field of ant ecology. This includes studying the diets of non-native ants in their non-native ranges. In doing so, it may be possible to identify the ecological roles they play in the wider food webs of which they are a part and analyse the processes driving ant trophic dynamics.

Diacamma sp., Chiang Mai, Thailand.

Chapter 2: A Meta-analysis reveals that Non-Native Ants Drive Dramatic Declines in Animal Community Diversity

Anoplolepis gracilipes and *Monomorium* sp., Khao Yai National Reserve, Thailand.

The results of Chapter 2 have been published in *Insect Conservation and Diversity*

Citation: Tercel, M. P. T. G., Cuff, J. P., Symondson, W. O. C., and Vaughan, I. P. (2023). Non-native ants drive dramatic declines in animal community diversity: a meta-analysis. *Insect Conservation and Diversity*. Early View: https://resjournals.onlinelibrary.wiley.com/doi/10.1111/icad.12672

2.1 Abstract

Non-native ants can cause ecosystem-wide ecological change and these changes are generally assumed to be negative. Despite this, the evidence-base has never been holistically synthesised to quantify whether and to what degree non-native ants impact native species diversity positively or negatively across different taxa and environments globally. In this study, I performed a meta-analysis of the effects of ant invasion on animal community diversity. I extracted data from 48 published articles investigating abundance (159 effect sizes) and richness (58 effect sizes) responses of taxa to ant invasion in locations relatively unimpacted by other stressors (e.g., human disturbance, other non-native species) to help isolate the effects of invasion. Overall, local animal diversity declined severely, with species abundance and richness lower by 36.23 % and 53.35 %, respectively, in areas with non-native ants compared to intact uninvaded sites. Moreover, I combined extracted data across all taxa in a given article into a single response to represent the "community" abundance (41 effect sizes) or richness (29 effect sizes) response to non-native ants. Local communities decreased starkly in total abundance (50.25 %) and species richness (52.06 %) in invaded sites. These results unequivocally highlight the destructive potential of non-native ants and their threat to biodiversity in relatively undisturbed systems, approximately halving local species abundance and richness in invaded areas for which data were available. Improved international prevention processes and local control measures deployable by conservation practitioners are urgently needed if these effects are to be mitigated, prevented, or reversed.

2.2 Introduction

The diversity of life on Earth is integral to a healthy and stable environment, ensuring environmental resilience (Folke et al. 2004) and providing all organisms, including humans, with the life systems required to survive. Invasive species (organisms introduced outside of their natural range that negatively affect native species) are a threat to global biodiversity (Simberloff et al. 2013; Luque et al. 2014), often leading to the homogenisation of ecosystems (McKinney and Lockwood 1999). In "100 of the World's Worst Invasive Alien Species", the International Union for the Conservation of Nature (IUCN) lists five invasive ant species (Lowe et al. 2000; Luque et al. 2014). Ants are ecologically important social insects, participating in a wide range of species interactions, e.g., as predators, parasites, herbivores, granivores, prey, mutualists, and hosts, across almost all terrestrial environments and all continents except Antarctica (Hölldobler and Wilson 1990; Stadler and Dixon 2005; Lach et al. 2010). Invasive ants possess adaptations such as supercoloniality and dietary generalism to establish themselves outside of their natural ranges and subsequently ecologically dominate native communities (Holway et al. 2002). Studies investigating native species responses to ant invasion tend to show negative consequences, but many studies cannot isolate non-native ants as the causal factor of these changes due to environmental differences between uninvaded and invaded sites or other confounding variables (Hill et al. 2003; King and Tschinkel 2008; Vonshak et al. 2010; Narendra et al. 2011; King and Tschinkel 2013; Stuble et al. 2013a; Sakamoto et al. 2019). Typically, non-native ants are found in heavily disturbed habitats because of their transportation by humans around the world (McGlynn 1999; Suarez et al. 2009) and because they are thought to be disturbance specialists (Holway et al. 2002; Berman et al. 2013), thriving in structurally open and homogenous environments. Measuring local community responses to non-native ants in these areas might therefore confuse results because of an already diminished native community and the presence of other non-native species (Berman et al. 2013; Stuble et al. 2013a).

Invasive ants are generally expected to lower native species diversity through direct predation and competition, as well as indirect effects arising from the extirpation of certain species. Such effects have been observed from studies examining native ant responses to invasive ants (Hoffmann et al. 1999; Hoffmann and Parr 2008; Dunham and Mikheyev 2010; Cooling and Hoffmann 2015), but there are mixed responses from other taxa (Porter and Savignano 1990; Dunham and Mikheyev 2010; Estany-Tigerström et al. 2010; McPhee et al. 2012; Alvarez-Blanco et al. 2017). The fate of a given species is probably determined primarily by the way in which it might interact with any incoming non-native ants, if they interact at all. For example, native scale insects may benefit from highly aggressive non-native ants that can protect them more effectively from natural enemies than a native ant. However, necessarily the natural enemies of the scale insect and the native ants might be adversely affected in this scenario. Furthermore, local species might be indirectly affected by incoming non-native ants at the community level. Studies describing “invasional meltdown” support this idea, whereby invasive ants cause ecosystem-wide devastation as a result of cascading direct and indirect species responses to invasion (O’Dowd et al. 2003; Handler et al. 2007; O’Loughlin and Green 2015).

The current evidence-base suggests the impact of non-native ants can range from ecologically damaging at the ecosystem scale to beneficial for some native taxa. This body of research has not yet been synthesised holistically in relatively intact natural systems. Robustly measuring local animal community responses to non-native ants in primarily native undisturbed habitats will help isolate the role non-native ants play in affecting those communities. A better quantitative understanding of the consequences of invasion by ants might be a timely and useful addition to the ecological knowledgebase and could yield insights that can be used to inform biodiversity conservation.

Here, I conduct a quantitative assessment of local species responses to non-native ants across many taxa and environments around the world using a meta-analytical approach. I use the term ‘local’ instead of ‘native’ because, although our studies were stringently selected solely in undisturbed natural systems, I cannot rule out that a small percentage of species in these areas may be non-native. I quantify the effects of non-native ants on local animal abundance and richness at both the level of an individual taxon (e.g. Coleoptera, Lepidoptera, birds, reptiles) and averaged across all taxa in a local community (the mean response of all taxa in a given article). I compute local responses by comparing abundance and species richness values in native habitats invaded by non-native ants (but otherwise undisturbed) to paired uninvaded control sites with nearly identical environmental conditions. In doing so, I answer four key questions: (1) What is the overall impact of non-native ants to local species abundance and richness around the world? (2) Are responses taxon-specific? (3) Are responses dependent on the unique local community being invaded? And (4) to what extent are responses determined by non-native ant species, habitat type, or location of the study?

2.3 Methods

2.3.1 Data collection

I aimed to collect a comprehensive database of articles reporting the effect of non-native ants on local species richness and/or abundance that adhered to our criteria. These articles were identified using Web of Science as our search engine, using the Web of Science Core Collection, BIOSIS Citation Index, KCI-Korean Journal Database, MEDLINE, Russian Science Citation Index and SciELO Citation Index databases for articles published between 1900-2021 using a Boolean search string (Chapter 2 Appendix S2.1). This returned 740 articles on 9th August, 2021. A PRISMA flow diagram (Chapter 2 Appendix Figure S2.1) shows the stages at which articles were disqualified or eventually used in the current study.

To be suitable for our database, articles needed to adhere to the following criteria: (1) report the abundance and/or species (or morphospecies) richness of local species in paired uninvaded and invaded sites, before and after invasion by invasive ants or before and after eradication of invasive ants (for the latter two, multiple years of sampling and environmental variables were required to account for interannual differences in local community); (2) investigate community-wide effects, not the response of a single species, unless recording the response of native vertebrates to invasion, which are typically single-species studies; (3) undertake observations in semi-natural or natural environments primarily made up of native vegetation; (4) sites were not "cherry-picked" within each treatment, i.e., sites could not be selected solely due to high or low densities of non-native ants within a treatment type and sampling locations were random within habitat types; (5) any changes to local species diversity were directly attributable to, or very likely to be caused by, non-native ants (i.e., no other non-native species were highly abundant); (6) report data with mean, sample size, and variance (standard deviation, standard error, or confidence intervals), or in another format that allowed these statistics to be inferred from the reported results, such as plots; (7) published in English. Sample sizes for uninvaded and invaded groups were the number of distinct sites reported by the authors.

I extracted data for each local taxon response to non-native ants from each article using a data extraction spreadsheet (Chapter 2 Appendix Table S2.1), and hereafter refer to these as separate "studies". These are observations of the species richness or abundance of a given taxon in geographically discrete paired sites, one with introduced ants present (invaded), the other with introduced ants absent (uninvaded). Articles may report more than one study, e.g., an article may report the species richness of beetles (one study) and the abundance of native lizards (a second study). From studies reporting local species diversity changes before and after non-native ant eradication/control, I used mean values for before and after years but, where possible, excluded diversity values from the first year after eradication to allow local communities to respond to the removal of introduced ants. To be considered "uninvaded", non-native ants had to be completely removed or in very low numbers (0-1 per uninvaded site, which were typically ≥ 20 m^2).

In total, I extracted data from 213 studies published in 48 peer-reviewed journal articles (Figure 2.1), of which 54 and 159 were richness and abundance responses, respectively. I separated local taxon responses by order or class for invertebrates (e.g. Coleoptera, Chilopoda, Araneae), and class for vertebrates (e.g. Reptilia, Amphibia). I did this because of 1) the ecological and biological similarity within these groups and 2) because studies typically report local responses using these taxonomic groups. The exception to this is for native ants, which I separated from other Hymenoptera in all analyses because they are likely to present unique responses. If order- or class-level changes to invertebrates are not reported, these are simply reported as “invertebrates”. In addition to invasive ant species and local taxon responses in the paired sites, I extracted the following data for each study: coordinates of study sites, location, whether the site is an island, habitat type, duration of study, sampling method, use of formicides (and active ingredient if so), and number of samples per site. All codes for these variables and the data extraction spreadsheet used to aid the data extraction process can be found in the Chapter 2 Appendix. I also provide the full meta-analysis protocol designed to ensure robust and repeatable results (Chapter 2 Supplementary File). Data in tables or text were directly extracted and used. When data were expressed only graphically, I used WebPlotDigitizer to extract data values. This program can be found and downloaded here: https://automeris.io/WebPlotDigitizer/.

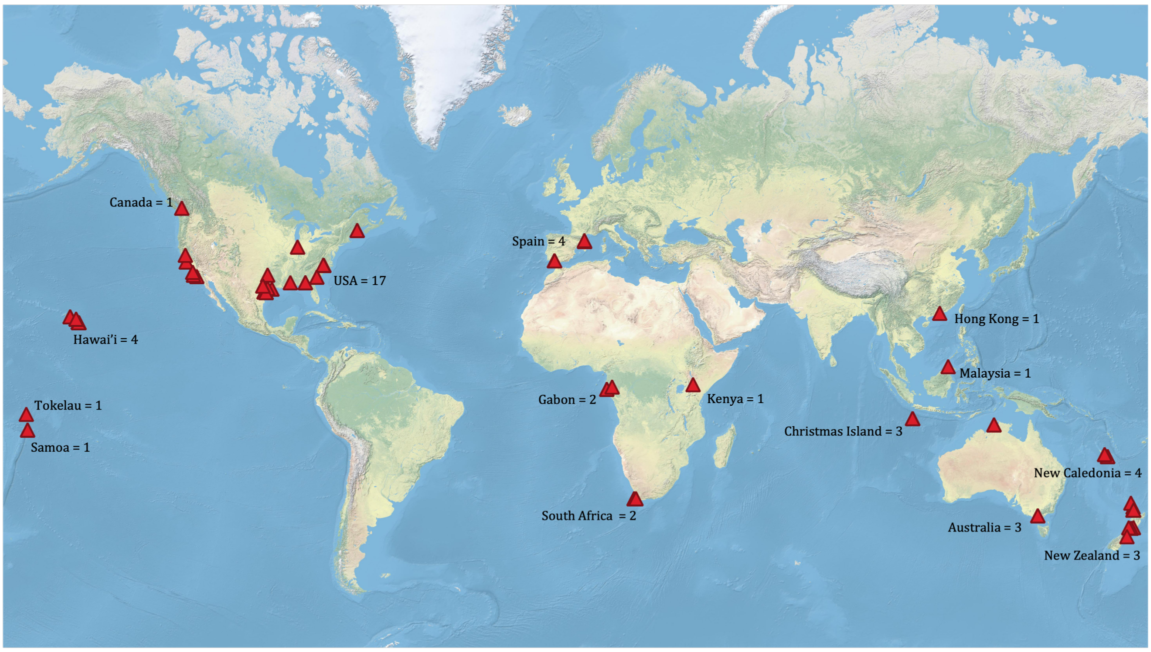


Figure 2.1. Locations of studies included in meta-analysis. Red triangles indicate individual study sites; numbers indicate the number of articles included from each country from which data were extracted. There are more triangles than articles because articles typically used several study sites. Note: points may overlap if study sites are near one another.

A second meta-analyst conducted article screening, data-extraction and analysis on a subset of articles post-abstract filtering (26 of 115) to ensure reliable and repeatable results. Article choice and data extracted were not significantly different between the two meta-analysts (Chapter 2 Appendix S2.2, Table S2.2), and raw extracted data were on average 87.5 % similar (range = 72-100 %) for richness data and 83.5 % (range = 62.1-96.1 %) for abundance data. This ratified our robust data extraction protocol. Data extracted by the first analyst were therefore used in all meta-analyses after these checks.

2.3.2 Meta-analysis

I measured the magnitude of local responses to invasion by nine non-native ant species, covering all classes of terrestrial vertebrate and a wide array of invertebrate taxa on five continents and in a range of habitats, including tropical, temperate and boreal forests, and grasslands (Chapter 2 Appendix Table S2.3). I did this using a standardised mean difference (SMD) approach and a random-effects model (Koricheva et al. 2013) using R package "metafor" (Viechtbauer 2010) in R version 4.1.0 (R Core Team 2014). I chose Hedges' g as our effect size measurement because it is not affected by unequal sampling variances in the paired groups and includes a correction factor for small sample size (Koricheva et al. 2013). To do this, I extracted the mean species richness or abundance value in paired uninvaded and invaded sites in each study and the associated standard deviations (SD) for each mean. All studies that reported means reported either standard deviation or standard error (SE). If SE was reported rather than SD, I calculated SD using Equation 2.1:

$$SD = SE\sqrt{n} \tag{2.1}$$

The Hedges' g value of a study was calculated using Equations 2.2 and 2.3:

$$g = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}}}J, \tag{2.2}$$

where

$$J = 1 - \frac{3}{4(n_1+n_2-2)-1} \tag{2.3}$$

is a correction for small sample size, and where subscripts $_1$ and $_2$ denote the uninvaded and invaded groups, respectively; thus, $\bar{x}_1$ and $\bar{x}_2$ are the mean local species response values across uninvaded and invaded sites, n_1 and n_2 denote sample size of uninvaded and invaded sites, and s_1 and s_2 are the standard deviations of uninvaded and invaded groups.

To compute the percentage change in local species diversity and abundance between uninvaded and invaded sites, I calculated the response ratio R and subsequently its natural logarithm using Equation 2.4

$$\ln R = \ln\left(\frac{\bar{x}_1}{\bar{x}_2}\right) \tag{2.4}$$

and the weighted mean percentage change using Equation 2.5.

$$(e^R - 1) \times 100 \tag{2.5}$$

I separated abundance and richness responses into two analyses and used random-effects models for both to allow for variance of effects between studies due to factors such as different invasive ant species, local communities, and habitat types, as well as within studies due to sampling variance and sampling error. The abundance model consisted of 159 studies from 42 articles, whilst the richness model consisted of 58 studies from 29 articles. Each "study" accounted for a separate local taxon responding to non-native ants in each article, or a conglomerate of taxa, such as "invertebrates". All models weighted each study by the inverse of its variance as well as between-study variance.

I verified the robustness of our meta-analysis using the checklist of Koricheva and Gurevitch (2013; 2014). Though this checklist was designed for meta-analyses in plant ecology, it is applicable here. All quality criteria were fulfilled (Chapter 2 Appendix Table S5). For all four models, I conducted sensitivity analyses to explore whether the results are sensitive to certain selection mechanisms. Firstly, I created funnel plots to detect whether publication bias was skewing results (Chapter 2 Appendix Figures S2.2-2.5) using the "funnel" function in metafor. I then statistically tested for publication bias with the trim-and-fill method using the "trimfill" function and adjusted the overall effect size accordingly if publication bias was revealed. None of the models showed evidence of publication bias from these analyses. I also ran leave-one-out analyses, where each study is sequentially omitted from the results to explore to what degree the overall results depend on each individual study. No outlier studies were found. Finally, I calculated Rosenthal's Fail-safe N for all models. Rosenthal's Fail-safe N denotes the number of studies/articles with an effect size of zero that would need to be added to the analysis to overturn the results into non-significance: abundance by taxon $N = 3{,}918$ (24.6 times the original sample size), richness by taxon $N = 4{,}237$ (77 times the original sample size), abundance by article $N = 816$ (19.4 times the original sample size), richness by article $N = 1{,}196$ (39.9 times the original sample size). All functions were from the "metafor" package (Viechtbauer 2010) in R version 4.1.0 (R Core Team 2014).

2.4 Results

Both models revealed highly heterogenous responses by local taxa (abundance: Cochran's Q_M(df = 157) = 340.77, p = <0.0001, I^2 = 71.49 %, H^2 = 3.51, τ^2 = 1.064; richness: Cochran's Q_M(df = 53) = 320.02, p = <0.0001, I^2 = 95.08 %, H^2 = 20.31, τ^2 = 6.89). I accounted for potential non-independence of studies from each article by including article identity as a moderator variable in a mixed-effects model (Koricheva et al. 2013), which was the best explanation for heterogeneity in each, accounting for 54.66 % and 37.6 % of heterogeneity in the abundance and richness models, respectively (abundance: Q_M(df = 40) = 75.46, p = <0.0006, R^2 = 54.66 %; richness: Q_M(df

= 26) = 30.95, p = 0.23, R^2 = 37.6 %). I verified the robustness of all moderator analyses using permutation tests (Chapter 2 Appendix Table S2.4), which did not significantly change results. I tested additional moderator variables using mixed-effects models to explain heterogeneity across models, such as invasive ant species, location, and habitat (Table 2.1), but found that article identity explained the greatest amount of variability.

Table 2.1. Moderator analyses were conducted by running separate meta-regression mixed-effects models ("Model") to explicitly characterise if a given variable ("Moderator variable") explained a significant or large proportion of the variety of native responses to ant invasion ("R^2").

Model	Moderator variable	Q_M(df)	p	Sig.	R^2
Abundance by native taxon	Native taxon	43.42 (30)	0.054		25.45%
Abundance by native taxon	Invasive ant species	5.95 (8)	0.65		0.67%
Abundance by native taxon	Habitat	5.8 (9)	0.76		1.79%
Abundance by native taxon	Article	75.46 (40)	0.0006	*	54.66%
Abundance by native taxon	Island or continental	0.1119 (1)	0.74		0.00%
Abundance by native taxon	Use of formicides	3.96 (4)	0.41		0.00%
Richness by native taxon	Native taxon	3.26(9)	0.95		0.00%
Richness by native taxon	Invasive ant species	13.37 (7)	0.064		25.36%
Richness by native taxon	Habitat	5.64 (7)	0.58		9.51%
Richness by native taxon	Article	30.95 (26)	0.23		37.6%
Richness by native taxon	Island or continental	0.071 (1)	0.79		0.00%
Richness by native taxon	Use of formicides	4.1 (3)	0.25		0.00%
Abundance by article	Invasive ant species	5.69 (8)	0.68		0.00%
Abundance by article	Habitat	4.87 (8)	0.77		0.00%
Abundance by article	Island or continental	0.33	0.57		0.00%
Richness by article	Invasive ant species	9.3 (7)	0.23		27.33%
Richness by article	Habitat	5.78 (7)	0.57		10.4%
Richness by article	Island or continental	0.03 (1)	0.85		0.00%

Article identity moderated the effect size more than all other tested variables and I attributed this to the fact that each article examined a unique ecological community of interacting and dependent species that responded to non-native ants. I attempted to account for this by running two additional random-effects models to measure article-level abundance and richness responses. I did this by combining raw response results for each article (the mean of individual taxon responses). On average, article-level abundance and richness responses combined 3.88 (SD ± 4.77) and 2 (SD ± 1.92) taxon-specific results, respectively. Both article-level models were highly heterogenous (abundance: Cochran's Q_M(df = 40) = 152.99, p = <0.0001, I^2 = 84.68 %, H^2 = 6.53, τ^2 = 1.99; richness: Cochran's Q_M(df = 28) = 169.49, p = <0.0001, I^2 = 92.54 %, H^2 = 13.4, τ^2 = 3.54). No moderator variables significantly explained this heterogeneity (Table 2.1).

Invasion by non-native ants was associated with large significant decreases to local species abundance and richness across all four analyses. For analyses where articles were split into separate studies for each responding local taxon (Figure 2.2), both local species abundance and richness were significantly lower in areas invaded by ants (abundance response by local taxon: mean Hedges' *g* [± 95% CI] = 0.46 [0.26 to 0.66], *p* = <0.0001; richness response by local taxon: mean Hedges' *g* [± 95% CI] = 1.81 [1.064 to 2.55], *p* = <0.0001). Because article identity explained the greatest amount of heterogeneity in both abundance and richness models, I ran two additional meta-analyses looking at the combined responses reported in each article (Figure 2.3) to account for the potential non-independence of taxon responses in each article. These showed a similar trend, but with community abundance responses stronger and community richness responses slightly weaker (abundance by article: mean Hedges' *g* [± 95% CI] = 0.822 [0.33 to 1.31], *p* = <0.0001; richness by article: mean Hedges' *g* [± 95% CI] = 1.48 [0.73 to 2.22], *p* = <0.0001). Other moderator variables did not sufficiently explain the heterogeneity between studies, and I therefore attributed these differences to the highly variable and unique ecological communities examined in each article that responded to introduced ants.

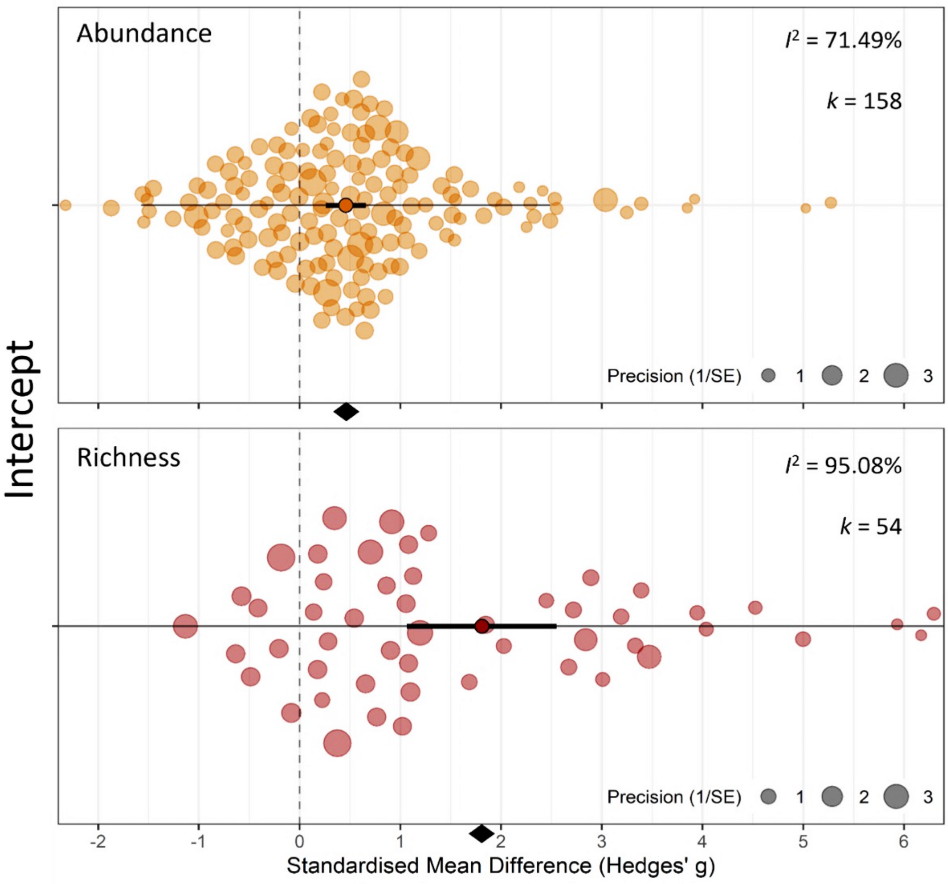


Figure 2.2. Local responses to invasive ants by taxon. The upper plot shows abundance responses, the lower plot shows richness responses. Solid dots with black bars represent the overall standardised mean difference (Hedges' *g*) and 95% confidence intervals, respectively. Translucent circles represent individual taxon responses extracted from each article. The size of each circle is proportional to its relative weighting in the overall model and the inverse of its variance. A positive effect size means that invasive ants are reducing local diversity. The diamond at the bottom of each plot shows the overall effect size (abundance: mean Hedges' *g* [± 95% CI] = 0.46 [0.26 to 0.66], p = <0.0001; richness: mean Hedges' *g* [± 95% CI] = 1.81 [1.064 to 2.55], p = <0.0001). The k value denotes the number of data points ("studies") in the model, whilst I^2 denotes the level of heterogeneity between effect sizes in the model. The position on the y-axis ("intercept") ensures that points are visible and do not overlap.

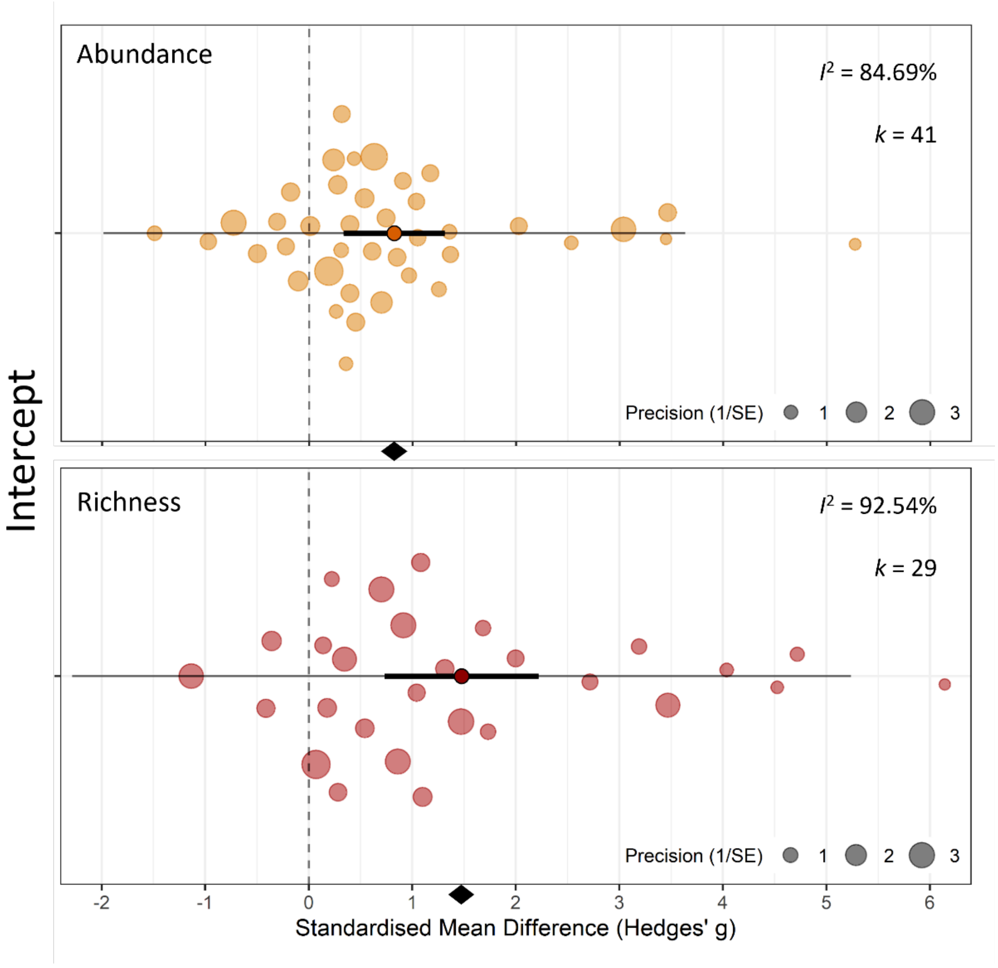


Figure 2.3. Local community responses to non-native ants. The upper plot shows abundance responses, the lower plot shows richness responses. Solid dots with black bars represent the overall standardised mean difference (Hedges' *g*) and 95 % confidence intervals, respectively. Translucent circles represent local community responses to invasive ants by combining taxon-specific responses within each article. The size of each circle is proportional to its relative weighting in the overall model and the inverse of its variance. A positive effect size means that invasive ants are reducing community diversity. The diamond at the bottom of each plot shows the overall effect size (abundance: mean Hedges' *g* [± 95 % CI] = 0.822 [0.33 to 1.31], *p* = <0.0001; richness: mean Hedges' *g* [± 95 % CI] = 1.47 [0.73 to 2.22], *p* = <0.0001). The k value denotes the number of data points ("studies") in the model, whilst I^2 denotes the level of heterogeneity between effect sizes in the model. The position on the y-axis ("intercept") ensures that points are visible and do not overlap.

A positive effect size denotes that local taxa are lower in abundance or richness in areas invaded by ants. Cohen (1988) suggests Hedges' *g* can be interpreted as follows: 0.2 = a small effect that cannot be discerned by the naked eye; 0.5 = a medium effect; 0.8 = a large effect immediately noticeable. This rule of thumb is designed for meta-analyses in the social sciences and therefore may be less relevant to ecological meta-analyses.

Native ants showed some of the strongest negative responses of all local taxa to invasive ants (Figures 2.4 and 2.5). Where species level response data were combined by authors (termed "invertebrates" in our analyses), I similarly saw strong abundance and richness responses.

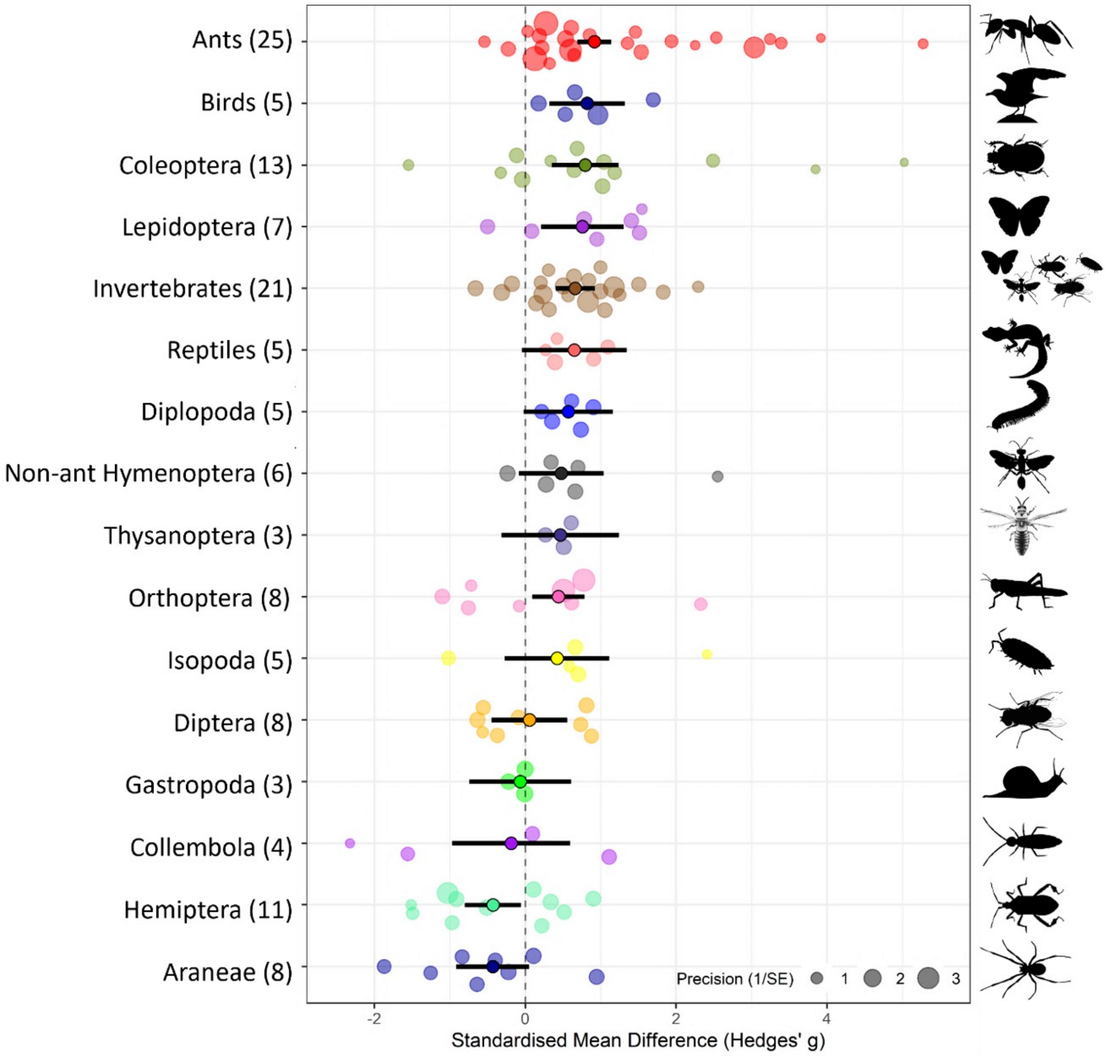


Figure 2.4. Local abundance responses to invasive ants separated by taxon. The number of studies *k* for each taxon is in parentheses by taxon labels. Solid dots with black bars represent the overall standardised mean difference (Hedges' *g*) and 95 % confidence intervals, respectively. Translucent circles represent individual taxon responses. The size of each circle is proportional to its relative weighting in the overall model and the inverse of its variance. A positive effect size means that invasive ants are reducing taxon abundance. Taxa with fewer than three studies were omitted from the plot to aid visualisation. The position on the *y*-axis ("intercept") ensures that points are visible and do not overlap and is also determined by the taxonomic grouping variable.

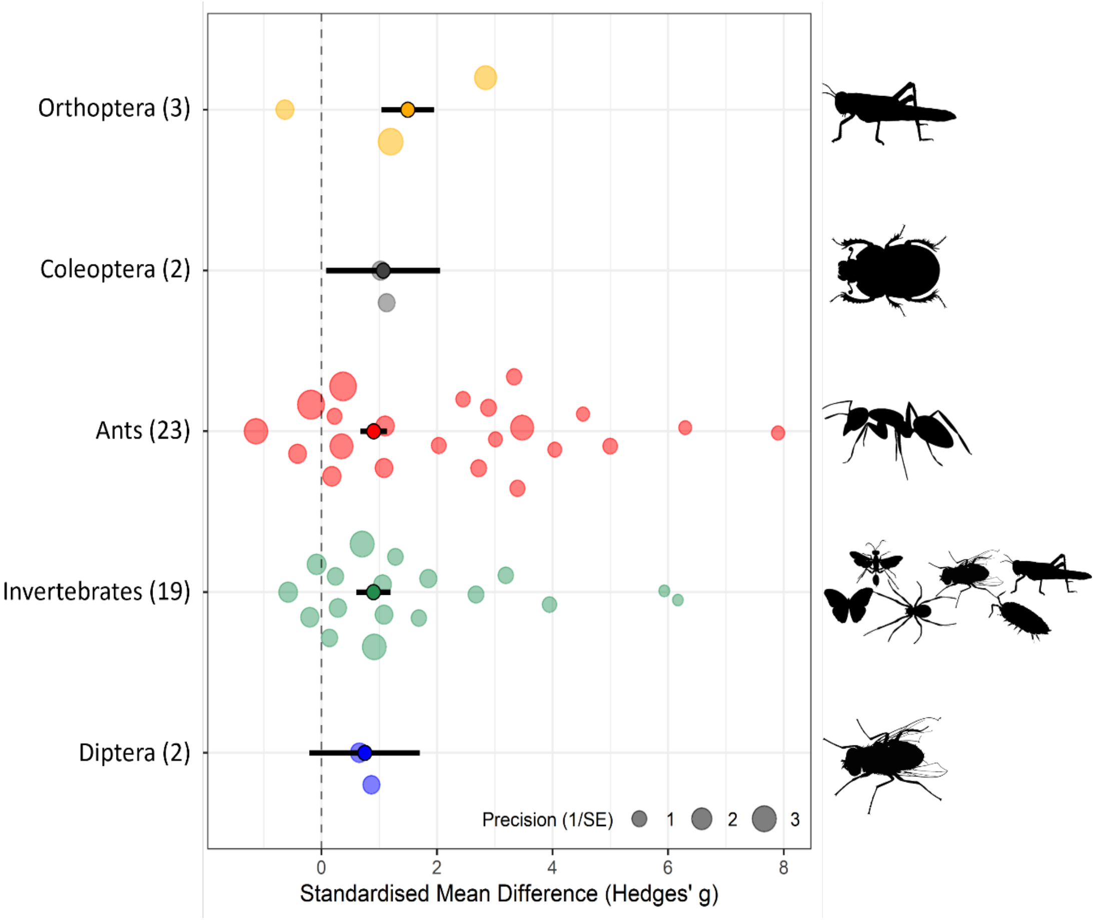


Figure 2.5. Local richness responses to invasive ants separated by taxon. The number of studies for each taxon is in parentheses by taxon labels. Solid dots with black bars represent the overall standardised mean difference (Hedges' *g*) and 95 % confidence intervals, respectively. Translucent circles represent individual taxon responses. The size of each circle is proportional to its relative weighting in the overall model and the inverse of its variance. A positive effect size means that invasive ants are reducing taxon richness. Taxa with fewer than two studies were omitted from the plot to aid visualisation. The position on the y-axis ("intercept") ensures that points are visible and do not overlap and is also determined by the taxonomic grouping variable.

See Chapter 2 Appendix S2.3 for a discussion of the limitations of the study.

2.5 Discussion

Our results show that non-native ants severely reduce animal community diversity in relatively undisturbed natural systems across continents and habitat types. This study thus supports the idea that non-native ants present a significant threat to animal biodiversity across much of the globe. Moreover, our meta-analytical design restricted studies to intact natural areas free from other stressors, identifying non-native ants as the primary drivers of biodiversity change rather than passengers of other anthropogenic impacts (Stuble et al. 2013a). Our results broadly conform to a previous meta-analysis by Cameron *et al.* (2016) investigating the impacts of non-native terrestrial invertebrates more generally, of which non-native ants were a large proportion, but I found stronger impacts on community abundance (29% vs. 50% reduction) and richness (33% vs. 52% reduction). I were unfortunately unable to extract the effect of invasive ants solely from that of other non-native taxa in their study, and thus cannot make any strong inferences about the discrepancy in the strength of the results. The key difference between our study and that of Cameron *et al.*'s is that our meta-analysis used studies conducted in intact natural areas. Of the 48 articles I selected, only 15 were shared with Cameron *et al.*'s study, presumably because 1) our search was conducted seven years later, resulting in more studies being available, 2) I disqualified many of the studies included in the Cameron *et al.* meta-analysis due to our focus on undisturbed habitats, and 3) our search may have more comprehensively identified studies that conformed to our specific inclusion criteria, which was heavily focussed on capturing all studies relating to non-native ant species rather than non-native terrestrial invertebrates more generally.

The severity of a given response to non-native ants appears to be primarily determined at the community-level. Given that almost all non-native ants are highly abundant generalist species (Holway et al. 2002; Tillberg et al. 2007), they are probably capable of directly and indirectly influencing a very large proportion of animal species in areas they colonise (Hölldobler and Wilson 1990). These indirect effects may somewhat confound taxon-specific results. For example, our analyses show that native ants decrease dramatically in diversity in areas colonised by non-native ants. Even in this group, however, some native ants appear to benefit from non-native ants, as shown by the small number of studies showing positive responses. One possible explanation for this is that non-native ants indirectly benefit some native species by removing their predators or competitors, for instance, and similar results may be true of other taxa. Such indirect effects likely have multiple levels, cascading though an ecological community in unpredictable ways and partly confounding taxon-specific responses. Moreover, it may be the unique community composition that can determine whether the fundamental function of an ecosystem alters after non-native ant invasion, ultimately leading to 'invasional meltdown' (O'Dowd et al. 2003; Handler et al. 2007; Rowles and O'Dowd 2009; Stuble et al. 2013a; O'Loughlin and Green 2015). Such case-studies unanimously detail or suggest very large direct and indirect impacts by non-native ants.

The mechanistic underpinnings as to why native diversity falls substantially once non-native ants have invaded are likely to be multifaceted, incorporating direct, indirect, lethal, and sub-lethal interactions. Both predatory and competitive processes appear to govern the responses of native ants to non-native ant invasion, for example (Human and Gordon 1996; Holway 1999; Holway and Case 2001; Zee and Holway 2006; Rowles and O'Dowd 2007). Invasive ants can "break" the discovery-dominance trade-off thought to structure many native ant assemblages (Bertelsmeier et al. 2015; Arnan et al. 2018; though see Parr and Gibb 2012), ultimately allowing them to dominate food resources to such an extent that native ants are unable to coexist. Invasive ants are typically also hyper-abundant because of their ability to control resources and their release from natural enemies and strong competitive forces (Porter et al. 1997). Most research identifying the mechanisms behind diversity declines relate to native ants responding to invasive ants. Our results suggest that entire communities of disparate animal taxa respond negatively to non-native ants and the mechanistic cause of these declines may vary between communities and taxa. Generating accurate species-level interaction data of invasive ants using high-throughput DNA-based methods (e.g., dietary metabarcoding), for example, could help pinpoint the mechanisms behind certain taxon or community responses. These methods could reveal competition for food resources between invasive ants and native species or if predation of particular groups during the initial stages of invasion might be the cause of diversity declines. Research generating species-level interaction data of invasive ants paired with surveys of native diversity at different stages of invasion therefore merits further exploration.

Our results also suggest that there are some taxon-specific responses to non-native ants that are predictable enough to identify certain trends. Native ants, birds, reptiles, beetles, and Lepidoptera all show very strong negative abundance responses to non-native ants overall. Whilst the specific mechanisms underpinning these responses are outside the remit of the current review, it is clear that these results might be useful for conservation managers aiming to protect certain threatened species or communities. Furthermore, the conglomerate group “invertebrates” responded strongly to non-native ants, providing definitive evidence that non-native ants can deconstruct and diminish invertebrate communities in undisturbed systems (Berman et al. 2013). Given that invertebrates fulfil almost every ecosystem process (Prather et al. 2013), significant declines in invertebrate diversity will probably affect the wider functioning of the ecosystem of which they are a part.

These trends raise serious concerns about the future and long-term existence of endemic species in natural systems where ants are invading. Invasive species are currently the second largest threat to biodiversity after land-use change (Clavero and Garcia-Berthou 2005; Simberloff et al. 2013; Luque et al. 2014), and it is therefore critical to identify the specific impacts of invasive taxa in natural areas. This evidence must then be used to prioritise control measures and prevent further spread of particularly damaging groups. I observe that invasive ants are a high-risk group, posing a serious threat to native species in intact native habitats. Natural systems typically hold higher overall species richness than degraded habitats and associated native communities react more strongly and more predictably to ant invasion than non-native species in the same system (Krushelnycky and Gillespie 2008). Increasing the available resources to conservation managers could facilitate regional reversal or prevention of such severe biodiversity declines, though implementing control of invasive ants is not without issues (Hoffmann 2010; Hoffmann et al.

2014; Hoffmann and Broadhurst 2016). This synthesis suggests there are crucial considerations for conservation policy. Though there have been many ant eradication attempts, less than half are successful and most are extraordinarily costly to employ financially and logistically for conservation managers considering the large land areas many invasive ants have colonised. For example, eradication regimes cost on average $2,885 and $822 per ha for aerial and hand toxin broadcast methods, respectively (Hoffmann et al. 2016). Well defined and resourced measures to prevent the further spread of invasive ants are urgently required in addition to more effective control strategies for non-native ants that have already colonised native areas. For example, improving the efficacy and rigour of inspection of living plants in international shipments (McGlynn 1999).

This study presents data definitively showing that non-native ants are the drivers of strong negative biodiversity declines at the taxon- and community-level across multiple habitats and geographical locations around the world. I see these impacts affect both vertebrate and invertebrate taxa. The responses are observed in relatively undisturbed environments where habitats consist entirely or almost entirely of native plant species, showing that the impact of non-native ants is not limited to disturbed habitats. Reductions to animal community biodiversity may have severe consequences for ecosystem functioning and the long-term future of endemic species. Improved control measures are therefore urgently needed for conservation managers and policymakers if these dramatic responses are to be avoided or reversed.

Dolichoderus sp., Chiang Rai, Thailand.

Chapter 3: The Omnivorous Diet of Telfair's Skink (Leiolopisma telfairii) on Round Island, Mauritius

Telfair's skink (*Leiolopisma telfairii*), Round Island, Mauritius.

The results of Chapter 3 have been published in *Ecology and Evolution*.

Citation: **Tercel, M. P. T. G.,** Moorhouse- Gann, R. J., Cuff, J. P., Drake, L. E., Cole, N. C., Goder, M. G., Mootoocurpen, R. and Symondson, W. O. C. (2022). DNA metabarcoding reveals introduced species predominate in the diet of a threatened endemic omnivore, Telfair's skink (*Leiolopisma telfairii*). *Ecology and Evolution*, 12(1), pp. 1-14. https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.8484

3.1 Abstract

Telfair's skinks are locally abundant endemic omnivores living on Round Island, Mauritius, a globally significant site of biodiversity conservation. It was not previously known to what extent these globally threatened species rely on non-native animals and plants as sources of food in the Round Island ecosystem. I aimed to determine the dietary diversity and key trophic interactions of Telfair's skinks, whether non-native species are frequently consumed, and if diet composition changes seasonally between male and female skinks. I used DNA metabarcoding of skink faecal samples to identify animals (COI) and plants (ITS2) consumed by skinks. There were 389 dietary detections belonging to 77 dietary taxa found across the 73 Telfair's skink faecal samples. Non-native taxa were cumulatively consumed more frequently than other categories, accounting for 49.4 % of all detections, compared to cryptogenic (20.6 %), native (20.6 %), and endemic taxa (9.5 %). The most frequently consumed non-native species was the ant, *Pheidole megacephala*, present in 40 % of samples. Blue latan palm, *Latania loddigesii*, was the most frequently consumed endemic species, present in 33 % of samples but was only detected in the dry season, when fruits are produced. I found a strong seasonal difference in diet composition explained by the presence of certain plant species solely or primarily in one season and a marked increase in the consumption of animal prey in the dry season. Male and female skinks consumed several taxa at different frequencies. These results present a valuable perspective on the role of non-native species in the food web of their invaded ecosystem. Both native and non-native species provide nutritional resources for skinks and this may have management implications in the context of species conservation and island restoration.

3.2 Introduction

Non-native species are typically associated with net-negative effects on the ecosystems they invade, supported by a large body of empirical evidence showing native biodiversity declines (Luque et al. 2014), extirpation and extinction of native species (Clavero and Garcia-Berthou 2005), and, in some cases, ecological collapse (O'Dowd et al. 2003). Despite this, the many ways a non-native species might interact with a native community may also be neutral or net-positive, e.g. as a mutualist (Kaiser-Bunbury et al. 2011), competitor (Cole and Harris 2011), prey (Li et al. 2011), or parasite (Arbetman et al. 2013) and through network and community restructuring (Memmott et al. 2000; Russo et al. 2014). For example, non-native species can directly benefit native species through beneficial interactions, such as non-native trees providing nesting sites to threatened birds (Schlaepfer et al. 2011) and non-native plants providing floral resources to a range of threatened native pollinators (Baldock et al. 2015). Non-native species have been studied extensively as invasive predators and herbivores, but their role in the diet of native species has been given less attention. A few studies examine this subject explicitly. For example, Ando *et al.* (2013) showed that the critically endangered red-headed wood pigeon, *Columba janthina nitens*, consumed non-native plants more frequently than native species on the Ogasawara Islands, Japan. Similarly, non-native species were consumed frequently by the Ogasawara buzzard, *Buteo buteo oyoshim*, with 90% of its diet consisting of non-native animals (Kato and Suzuki 2005), despite these islands harbouring high levels of endemism. Non-native species are typically associated with disproportionately negative effects on island biodiversity relative to mainland invasions (Sax and Gaines 2009), but are shown to provide nutritional resources to these endemic species. This may be more common than currently acknowledged, with non-native species representing a significant dietary element for some native consumers.

Round Island, situated 22.5 km north-east of Mauritius (Figure 3.1), is a globally significant site of biodiversity conservation and now represents the last remnant of native lowland palm habitat in the Mascarenes (Cheke and Hume 2008). The palm habitat has been recovering since the eradication of goats, *Capra aegagrus hircus*, in 1979, and rabbits, *Oryctolagus cuniculus*, in 1986 (Merton 1987; Cheke and Hume 2008). At just 2.19 km^2, it is home to several reptile species extirpated from mainland Mauritius by non-native species and habitat destruction. Telfair's skinks, *Leiolopisma telfairii*, (Figure 3.2) are vulnerable omnivorous reptiles, typically growing to approximately 30 cm in total length and are endemic to Mauritius. They became restricted to Round Island by the mid-1800s because of the introduction of non-native predators, such as rats (Cole et al. 2018a). The species has now been reintroduced to the island Nature Reserves, Ile aux Aigrettes, (0.26 km^2, located 600 m from south-east Mauritius) and Gunner's

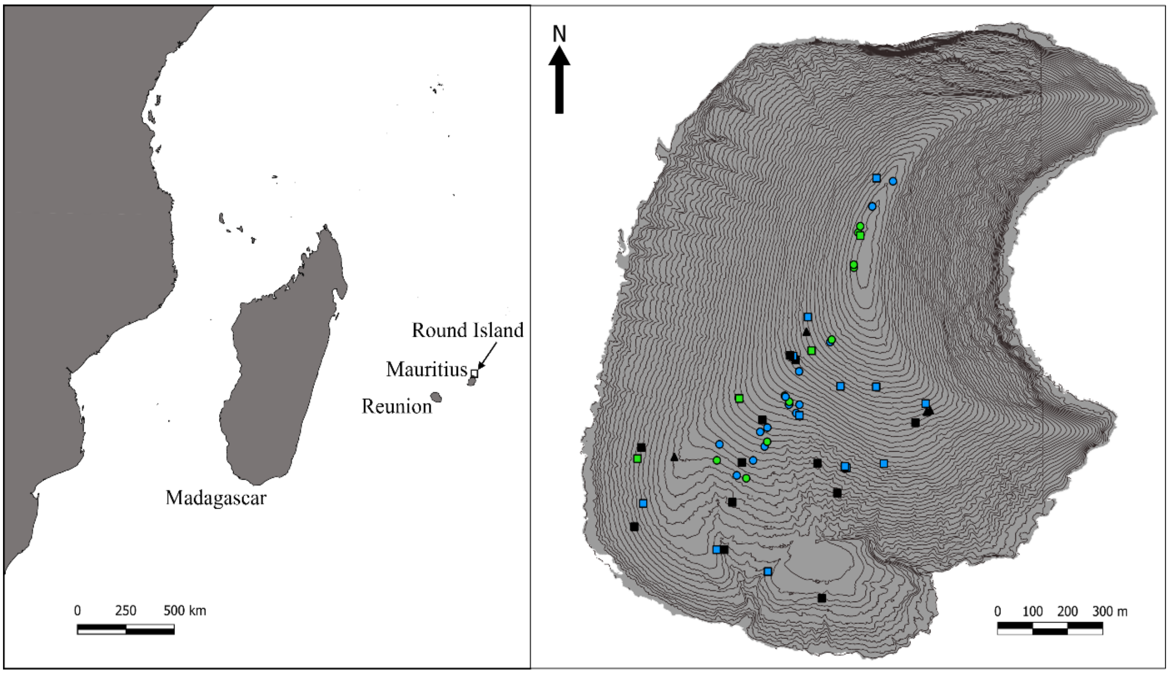


Figure 3.1. Location of the study. The left map shows the location of Round Island in the Indian Ocean. The right map shows the topography of Round Island (5 m contour lines) and the sampling locations for each skink. Symbol shape denotes the season that samples were collected: wet = square, dry = circle, unknown = triangle. Symbol colour denotes the sex of the skink a sample was collected from: females = green, males = blue, unknown = black.

Quoin (0.7 km2, 5 km to the north of Mauritius; Cole et al. 2018a). Round Island has been a designated nature reserve since 1957 and has never suffered from non-native terrestrial vertebrate predators, which have caused the extirpation and extinction of multiple Mauritian species elsewhere (Cheke and Hume 2008). Habitat restoration efforts on Round Island since the 1980s have led to the recovery of its reptile populations, which includes seven species, four of which became restricted to the island by the mid-19th century (North et al. 1994; Cole et al. 2018a). Previous dietary analyses of Telfair's skinks include morphological identification of food items and molecular analyses (Pernetta et al. 2005; Zuël 2009; Brown et al. 2014; Moorhouse-Gann et al. 2022). Morphological examination of faeces show that Telfair's skinks consume a variety of non-native and native species of fruit, seeds, arthropods, and vertebrates (Pernetta et al. 2005; Zuël 2009). However, morphological methods of diet analysis can be unreliable and taxonomically imprecise, even when researchers are skilled. These methods also fail to adequately detect small or soft-bodied prey (Symondson 2002; Pompanon et al. 2012). Molecular approaches, especially those using high throughput sequencing (HTS), can provide much greater precision, frequently identifying taxa in faecal samples to species-level (Symondson 2002; Pompanon et al. 2012; Taberlet et al. 2018). Previous HTS-based faecal analysis of Telfair's skinks targeting plant (Moorhouse-Gann et al. 2022) and animal (Brown et al. 2014) food resources on Ile aux Aigrettes and Round Island confirmed skinks eat a diverse range of taxa.

Figure 3.2. Telfair's skink, *Leiolopisma telfairii*.

Identifying the diet of omnivores is challenging (Tercel et al. 2022), but a few studies have facilitated the most comprehensive complex dietary assessments to date using DNA metabarcoding (de Barba et al. 2014; Robeson et al. 2018; Bonin et al. 2020; da Silva et al. 2020). Trophic generalists may be central to ecological networks and can elicit top-down effects across their entire breadth and depth. Deciphering the structure and dynamics of these interactions is therefore valuable, especially within a conservation context. Telfair's skinks are large, locally highly abundant trophic generalists endemic to Mauritius (Vinson and Vinson 1969; Jones 1993; Cole et al. 2018a), and their trophic interactions may influence the population dynamics of some species in the wider biological community of Round Island.

Here, I aimed to study the complete diet of Telfair's skinks on Round Island by using broad-coverage plant and animal DNA metabarcoding primers. In doing so, I aimed to show: 1) the dietary diversity and key trophic interactions of Telfair's skinks; 2) whether non-native species, such as ants and weeds, feature prominently in the diet; 3) whether diet composition changes between seasons; and 4) whether diet composition is different between male and female skinks, which may have implications for conservation management and reintroduction initiatives.

3.3 Methods

3.3.1 Study site

See Chapter 1.5.1 for a description of the geography and habitats of Round Island.

The vertebrate community of Round Island consists of globally important populations of birds and reptiles. Seabird colonies of wedge-tailed shearwater, *A. pacifica*, the Round Island petrel species complex, *Pterodroma* spp., red-tailed tropicbird, *Phaethon rubricauda*, and white-tailed tropicbird, *Phaethon lepturus*, can be found in high-density populations across Round Island. At least three non-native land bird species are commonly encountered on Round Island: Zebra dove, *Geopelia striata,* house sparrow, *Passer domesticus*, and red-whiskered bulbuls, *Pycnonotus jocosus*, (Cheke and Hume 2008; Cole et al. 2018a).

Seven native reptile species survive on Round Island. Five of these are listed as Threatened on the IUCN Red List: Bojer's skink, *Gongylomorphus bojerii*, Durrell's Night gecko, *Nactus durrellorum*, keel-scaled boa, *Casarea dussumieri*, Round Island day gecko, *Phelsuma guentheri*, and Telfair's skink, *Leiolopisma telfairii*. The remaining two non-threatened reptiles are the ornate day gecko *Phelsuma ornata*, and Bouton's skink, *Cryptoblepharus boutonii*. Additionally, two tortoise species, Aldabra giant tortoise, *Aldabrachelys gigantea*, and radiated tortoise, *Astrochelys radiata*, have been introduced to Round Island as "ecological replacements" for extinct Mauritian tortoises, *Cylindraspis* spp. (Griffiths et al. 2010). Round Island was also the last known location for the now-extinct burrowing boa, *Bolyeria multocarinata*, a monotypic genus of bolyeriid snake. Bolyeriidae are endemic to Mauritius and includes only *Bolyeria* and *Casarea* genera. As the largest and one of the most abundant of the island's lizards, Telfair's skinks are likely to constitute a major component of animal biomass on the island (Cole et al. 2018c) and are thought to have a significant role within the island's food web dynamics.

Broad dry and wet seasons exist in Mauritius (Senapathi et al. 2009). The dry season typically begins in May and is characterised primarily by low rainfall, mean air temperature of ~20.5 °C and stronger winds, with the driest months being September and October. The wet season typically begins in December and is characterised by much more frequent rainfall, mean air temperature of ~24.5 °C and minimal winds, with the wettest months being January and February (Senapathi et al. 2009).

See Chapter 4 for a description of the invertebrate community of Round Island.

3.3.2 Skink sampling on Round Island

Faecal samples were collected in March, June, July and December 2015 (Figure 3.1). Skinks were caught opportunistically by noose or hand after which defecation was induced using a gentle abdominal massage. The faecal samples were placed in polythene bags and dried over silica gel. Telfair's skinks are present over the entire island but, unfortunately, some areas of the island are too dangerous to capture these fast-moving reptiles. Skinks were released unharmed within ten minutes of capture at the locations where they were caught. Faecal samples were collected from 196 individual Telfair's skinks (identified by their sex, size that were recorded and distinguishing markings and body deformations, which were photographed) on Round Island, and previously underwent DNA metabarcoding to identify the floral component of skink diet (Moorhouse-Gann 2018; Moorhouse-Gann et al. 2022). Due to funding constraints, I were only able to advance 82 samples to sequencing, which were randomly selected for the current study from both dry (40) and wet (42) seasons.

3.3.3 Primer selection

Animal primers were tested *in silico* with a broad range of vertebrate and invertebrate taxa using PrimerMiner (Elbrecht and Leese 2017) and *in vitro* with DNA extracted from animals sampled on Round Island. BerenF-LuthienR (Cuff et al. 2021a) provided the most comprehensive coverage, amplifying all Round Island invertebrate DNA extracts tested. UniPlant general plant primers (Moorhouse-Gann et al. 2018) were used to amplify the ITS2 DNA barcode in plants and successfully amplify almost all plant species found on Round Island.

3.3.4 DNA extraction, PCR amplification, and sequencing

DNA extraction from Telfair's skink faecal samples and preparation of plant DNA for 250 bp paired-end Illumina MiSeq high-throughput sequencing followed Moorhouse-Gann *et al.* 2022 (also see Chapter 3 Appendix S3.1 and Table S3.1).

I used the following procedure to identify animal prey in the diet of Telfair's skinks. Polymerase Chain Reactions (PCR) used 25 µL reaction volumes containing 5 µL DNA template, 12.5 µL of multiplex PCR mix (Qiagen, Manchester, UK), 2.5 µL of both forward and reverse primers (0.2 µM each), and 2.5 µL of nuclease-free water (Qiagen, Manchester, UK). Polymerase chain reaction conditions went as follows: 95 °C for 15 minutes, 35 cycles of 95 °C for 30 s, 54 °C for 90 s, and 72 °C for 90 s, and 72 °C for 10 minutes, as instructed by the manufacturer (Qiagen, Manchester, UK). Each sample incorporated a unique combination of molecular identification (MID) tags (Binladen et al. 2007) that allowed for each skink to be identified after pooling and sequencing as per Brown *et al*. (2014). These 10-bp fragments were added to both the forward and reverse primers for each sample and thus dietary taxon sequences could be assigned to individuals. Products were then run through a 2 % agarose gel stained with SYBR®Safe (ThermoFisher Scientific, Paisley, UK). Twelve negatives were included in each PCR run, 10 PCR negatives and two extraction negatives. Additionally, two positive controls consisting of a standardised DNA concentration (4 ng / µL) of known invertebrate species likely absent from the study site (Chapter 3 Appendix S3.2) were used to control for tag-jumping between samples in the filtering steps detailed below. Products were run in a Qiagen QIAxcel Advanced System (Qiagen, Manchester, UK) to measure relative DNA concentrations and later measured individually using a Qubit Fluorometer (ThermoFisher Scientific, Paisley, UK) for more accurate determination of DNA concentrations. Each sample was then pooled based on the relative DNA concentrations of the amplicon of interest as measured by the QIAxcel Advanced System. Negative controls were pooled based on the average volume pooled for the skink samples. The pooling process involved adding a volume from each sample as a proportion of the sample with the highest concentration of DNA, to ensure approximate equimolarity of DNA from each sample. Each pool was cleaned using SPRIselect beads (Beckman Coulter, Brea, USA), with a left-side size selection using a 1:1 ratio. After final elution, the pool was run on a Qubit Fluorometer, to measure DNA concentration (=49.6 ng / µL), as well as an Agilent 2200 TapeStation with D1000 ScreenTape (Agilent Technologies, Waldbronn) to check for significant levels of primer dimer, which were not found. This pool of MID-tagged samples was then used for library preparation using the NEXTflex™ Rapid DNA-Seq Kit following the manufacturer's instructions (Bioo Scientific Corp, Austin, TX, United States), which is suitable for pools with DNA concentrations of 1 ng – 1 µg. A final DNA concentration was measured for the prepared library using a Qubit Fluorometer (=11.7 ng / µL) and was then sequenced on an Illumina MiSeq desktop sequencer (Illumina, San Diego, CA, United States) with a Nano cartridge using 2 x 250 bp paired reads (expected reads ≤ 1,000,000).

3.3.5 Bioinformatics

The Illumina Nano cartridge run generated 750,645 reads, giving an average per sample read depth of 7,819. High-throughput sequencing data for the animal component of Telfair's skink diet followed the bioinformatic process of Drake *et al.* (2022): FastP (Chen et al. 2018) was used to check the quality of reads, discard poor quality reads (<Q30, <125bp long or too many unqualified bases, denoted by "N"), trim reads to a minimum length of 300 bp and merge read pairs from Miseq files (R1 and R2). Read pairs were assigned to samples and demultiplexed using Mothur v1.39.5 (Schloss et al. 2009), after which MID-tag and primer ends were removed. Unoise3 (Edgar 2010) was used to remove replicates, denoise the sequences, and group identical sequences into zero-radius operational taxonomic units (ZOTUs, which are clustered without % identity to avoid multiple species being nested within an OTU). Processed sequences were given taxonomic information from GenBank using BLASTn v2.7.1 (Camacho et al. 2009) with a 93 % identity threshold. This threshold was chosen to capture the wide variety of invertebrates on Round Island to genus- or family-level, most of which have not been barcoded or formally described. When more than one taxon was assigned to a sequence, I manually checked the feasibility for the presence of each taxon on Round Island by searching published articles, unpublished reports, and personal observations of species accounts. If these manual checks were inconclusive, I assigned the sequence to a higher taxonomic level (genus, family, order, etc.). MEGAN Community Edition v6.18.9 (Huson et al. 2016) was used to analyse the BLAST output and assign taxonomic identities to each ZOTU. Using the lowest e-value (a value estimating the number of hits "expected" by chance when searching a database of a given size - in this instance anything less than 0.00001) the top hit was assigned to each sequence. Where top hits were taxonomic levels higher than species, these were manually checked and assigned to a feasible taxon or deleted from the analysis if erroneous. ZOTUs that were assigned to the same taxon were aggregated.

Data were cleaned for statistical analysis following the methods set out by Drake *et al*. (2022): the combined removal of the maximum read count in blanks and negative controls, and reads not meeting a pre-defined per sample threshold, removes both erroneous reads (laboratory contaminants and sequencing errors) that are likely to occur in low abundances mitigates tag-jumping and bleeding of over-represented taxa into other samples, whilst utilising a per sample threshold and those arising through tag-jumping and bleeding of over-represented taxa into other samples removes erroneous reads (laboratory contaminants and sequencing errors) that are likely to occur in low abundances. The maximum read count of known contaminants and other obviously erroneous ZOTUs across the dataset was calculated as a percentage of their respective total sample read count, and any read counts less than this were removed. For this, a threshold of 0.3 % was applied, removing low-frequency laboratory contaminants and sequencing errors. Following this, the highest read count within a blank or negative per ZOTU was calculated and any ZOTU reads below this value were removed. In addition, I established an extra per-ZOTU filtering step, which removed remaining erroneous taxa. The per-ZOTU threshold was set to 0.74%. After these filters were applied, read counts were converted to presence-absence data for each sample. Nine samples were removed due to the absence of any dietary detections, leaving 73 samples to be taken forward for statistical analyses. Bioinformatic analysis for plant sequencing data followed Moorhouse-Gann *et al*. (2022) (Chapter 3 Appendix S3.1).

After animal ZOTUs were given taxonomic information, status of each taxon relative to Round Island was determined for each by manually searching for relevant data in published articles, unpublished reports, and personal species accounts, and then classified as "cryptogenic", "non-native" or "native". Cryptogenic species were defined as species that had no clear status, either because of poor taxonomic resolution, or because they may be known natives of the Indian Ocean islands but their history on Round Island is unknown. Plant status was taken from Moorhouse-Gann (2018; 2022).

3.3.6 Statistical analyses

Statistical analyses were conducted in R Statistical Software v4.1.0 (R Core Team 2021) after data were converted to presence/absence within each sample. Basic characteristics of the diet were quantified by measuring frequency of occurrence. I aimed to reveal whether there were significant differences in the mean frequency of occurrence of dietary taxa from different taxonomic kingdoms (animals, plants) or status relative to Round Island (cryptogenic, non-native, native), hereafter "status". Data were not normally distributed (Shapiro-Wilk test for normality: $W = 0.64$, $p = <0.001$), and I therefore used two non-parametric Kruskal-Wallis tests, one each for kingdom and status, to determine whether there were significant differences in average consumption between categories of each variable.

I also wanted to quantify dietary diversity and show whether our samples could be used to sufficiently represent the broad dietary patterns of Telfair's skinks. Sample-size and effort-based standardisation poorly represent the true diversity of communities because they fail to account for the species-abundance distribution of the community being sampled (Cao et al. 2007; Roswell et al. 2021). I therefore used coverage-based rarefaction and extrapolation rather than asymptotic species-accumulation curves (Chao and Jost 2012; Roswell et al. 2021), and robustly estimated species diversity using Hill diversity (Hill 1973; Roswell et al. 2021). I define Hill diversity with Equation 3.1:

$$D = \left(\sum_{i=1}^{S} p_i \, (r_i)^{\iota}\right)^{\frac{1}{\iota}} \tag{3.1}$$

where D is diversity, S is number of species, p_i is the proportion of all individuals that belong to species i, r_i is the rarity of species i, defined as $1/p_i$, and ι is the exponent determining the rarity scale on which the mean is taken (Hill 1973; Bullen 2003; Roswell et al. 2021). Hill diversity is the generalised mean species rarity, and the exponent ι determines the sensitivity of the equation to rare species. ι of 1 uses the arithmetic mean rarity, or species richness (Hill-richness), and is very sensitive to the rarest species; ι of 0 uses the geometric mean rarity, or the exponential of Shannon's entropy (Hill-Shannon), and responds to both high and low rarity species; and ι of -1 uses the harmonic mean rarity, or the inverse of Simpson's index (Hill-Simpson), and is most sensitive to the relative abundance of common species (Roswell et al. 2021). Coverage is a measure of how completely a community has been sampled and is an estimated proportion of the sampled individuals in the community that belong to species already detected (Chao and Jost 2012). For example, a coverage of 0.85 denotes that 15 % of the individuals in the community being sampled belong to species that have not been found. I computed these metrics in R package "iNEXT" (Hsieh et al. 2016). Variation in diet composition was visualised with non-metric multidimensional scaling (NMDS) in the "vegan" package (Oksanen et al. 2019) using the "metaMDS" function on a matrix of Jaccard distances, where I extracted three dimensions. Data were plotted using package "ggplot2" (Valero-Mora 2010). To illuminate whether sex, season, or their interaction affects Telfair's skink diet, R package "mvabund" was used (Wang et al. 2012). Multivariate generalised linear models (MGLMs) were run using the "manyglm" function with a Monte Carlo resampling method and "binomial" error family.

3.4 Results

There were 389 dietary presence counts belonging to 77 dietary taxa found across the 73 Telfair's skinks samples. Of these, 37 of 38 plant taxa were resolved to species due to extensive barcoding of the Round Island flora. The invertebrates of Round Island have not been described as extensively, and of the 39 dietary taxa detected, 20 were resolved to species, nine to genus, nine to family, and one to order. The invasive ant *P. megacephala* and a cryptogenic braconid wasp, *Heterospilus* sp., were the most frequently detected taxa, present in almost 40 % of all Telfair's skink samples (Table 3.1; Chapter 3 Appendix Table S3.2). Our Kruskal-Wallis tests showed that mean number of detections per dietary taxon was not significantly affected by status relative to Round Island ($\chi^2(3) = 1.51$, $p = 0.68$), but taxonomic kingdom did show a significant effect ($\chi^2(1) = 6.33$, $p = 0.012$), where plants were consumed more frequently on average per taxon than animals (mean consumption per dietary taxon (±SE): animals = 4.26 (± 1.2), plants = 5.87 (± 1.03); Figure 3.3). Non-native taxa were cumulatively consumed more frequently than all other status categories, accounting for 49.4 % of all detections, whilst cryptogenic and native taxa accounted for 20.6 % and 30%, respectively (Figure 3.4). I computed dietary diversity (Figure 3.5) and found that Hill-richness ($\iota = 1$) provided the highest diversity estimate in contrast to both Hill-Shannon ($\iota = 0$) and Hill-Simpson ($\iota = -1$) (Figure 3.5, left plot).

Table 3.1. Taxonomic information, frequency of occurrence F_O (%), and status relative to Round Island (cryptogenic, non-native, native) for all dietary taxa occurring in two or more Telfair's skink faecal samples. Cryptogenic taxa had no clear status, either because of poor taxonomic resolution, or because they may be known natives of the Indian Ocean islands but their history on Round Island is unknown.

Kingdom	Phylum	Class	Order	Family	Dietary taxon	F_O (%)	Status
Animalia	Arthropoda	Arachnida	Araneae	Thomisidae	*Ozyptila claveata*	5.48	non-native
		Crustacea	Isopoda	Porcellionidae	Porcellionidae sp.	34.25	cryptogenic
		Insecta	Blattodea	Blaberidae	Blaberidae sp.	2.74	cryptogenic
			Coleoptera	Coccinellidae	*Harmonia yedoensis*	20.55	non-native
			Diptera	Drosophilidae	*Drosophila melanogaster*	2.74	cryptogenic
					Zaprionus indianus	5.48	non-native
				Tachinidae	*Chetogena* sp.	2.74	cryptogenic
			Embioptera	Oligotomidae	*Oligotoma saundersii*	2.74	non-native
			Hemiptera	Aleyrodidae	*Dialeurodes hongkongensis*	2.74	non-native
				Rhyparochromidae	Rhyparochromidae	8.22	cryptogenic
			Hymenoptera	Apidae	*Inquilina* sp.	2.74	native
				Braconidae	*Heterospilus* sp.	39.73	cryptogenic
				Formicidae	*Brachymyrmex cordemoyi*	19.18	non-native
					Formicidae sp.	2.74	cryptogenic
					Monomorium floricola	2.74	non-native
					Pheidole megacephala	39.73	non-native
				Platygastridae	Platygastridae sp.	2.74	cryptogenic
Plantae	Angiosperms	Eudicots	Asterales	Asteraceae	*Erigeron bonariensis*	5.48	non-native
					Tridax procumbens	10.96	non-native
				Goodeniaceae	*Scaevola taccada*	10.96	native
			Caryophyllales	Amaranthaceae	*Achyranthes aspera*	19.18	non-native
					Amaranthus viridis	2.74	non-native
				Nyctaginaceae	*Boerhavia* sp.	19.18	native
			Celastrales	Celastraceae	*Gymnosporia pyria*	2.74	native
			Fabales	Fabaceae	*Desmodium incanum*	6.85	non-native
					Gagnebina pterocarpa	10.96	native
			Gentianales	Apocynaceae	*Vincetoxicum confusum*	12.33	native
			Lamiales	Lamiaceae	*Premna serratifolia*	5.48	native
			Malpighiales	Euphorbiaceae	*Euphorbia thymifolia*	4.11	cryptogenic
				Passifloraceae	*Passiflora suberosa*	15.07	non-native
				Phyllanthaceae	*Margaritaria anomala*	4.11	native
			Malvales	Malvaceae	*Abutilon indicum*	35.62	non-native
					Hibiscus tiliaceus	5.48	native
					Hilsenbergia petiolaris	4.11	native
			Myrtales	Myrtaceae	*Eugenia lucida*	4.11	native
			Solanales	Convolvulaceae	*Ipomoea pes-caprae*	21.92	native
				Solanaceae	*Solanum lycopersicum*	4.11	non-native
					Solanum nigrum	17.81	non-native
		Monocots	Arecales	Arecaceae	*Latania loddigesii*	32.88	native
			Poales	Poaceae	*Cenchrus echinatus*	13.70	non-native
					Chloris barbata	2.74	non-native
					Dactyloctenium ctenoides	4.11	native
					Digitaria horizontalis	12.33	non-native

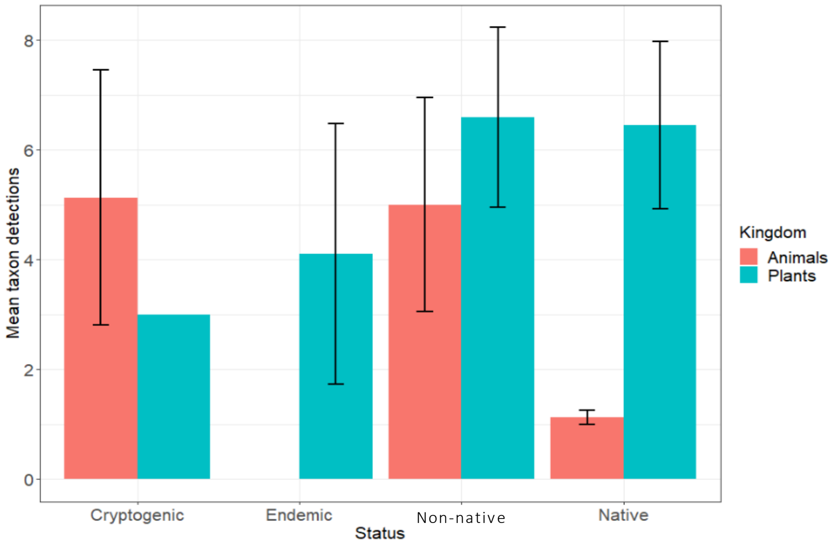


Figure 3.3. Mean (± SE) dietary taxon detections of Telfair's skinks (*Leiolopisma telfairii*) by dietary taxon status relative to Round Island (cryptogenic, endemic, non-native, or native) and taxonomic kingdom. Means within each category were calculated by dividing total detections by the number of dietary taxa detected. Note: there were no endemic animal taxa recorded in the diet of the skinks and only one cryptogenic plant was detected (no SE bar).

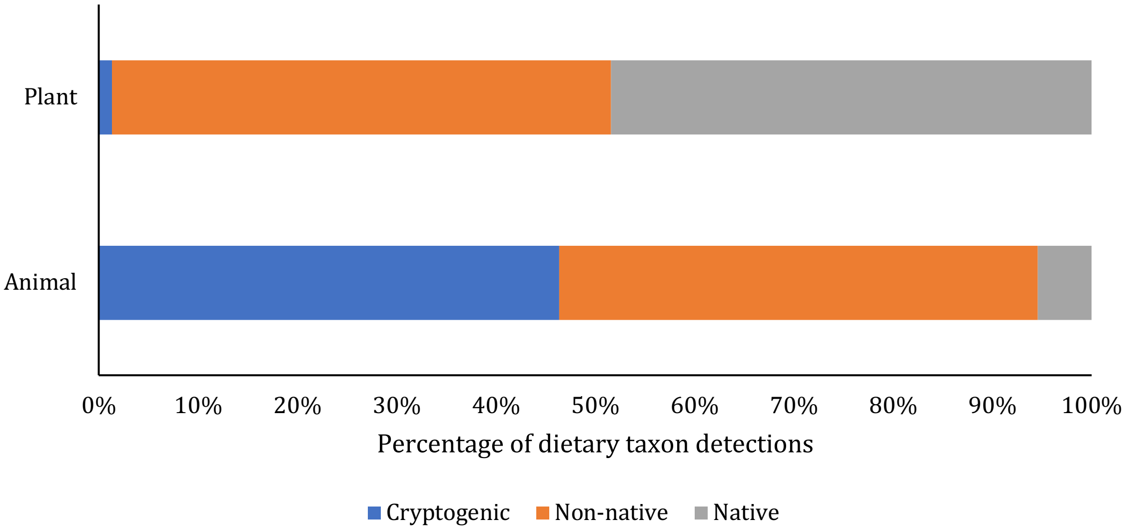


Figure 3.4. Plant and animal dietary taxon detections as a percentage of total detections by status relative to Round Island (non-native, cryptogenic, native).

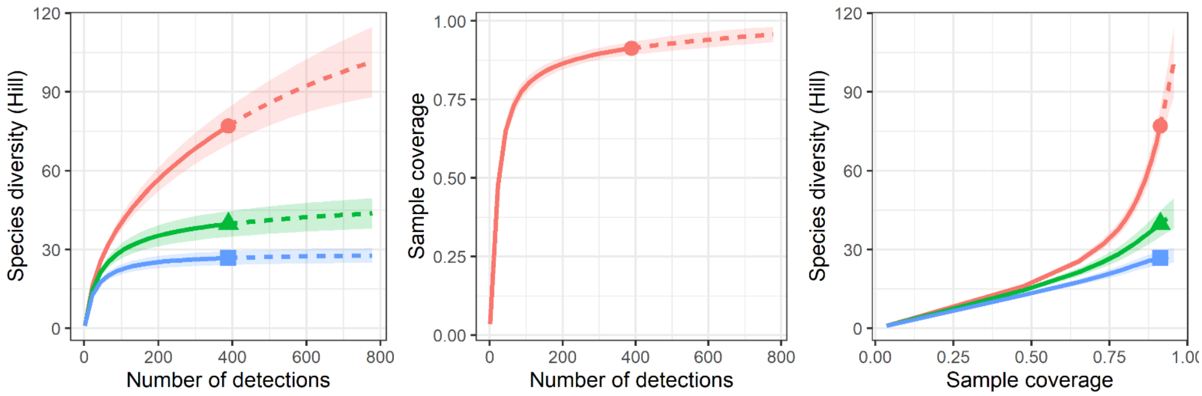


Figure 3.5. Dietary species diversity found in Telfair's skink faecal samples and the level of community coverage provided. Line colours denote values of the exponent ι that determines the rarity scale of different diversity estimates: Hill-richness, $\iota = 1$, red line with terminal circle; Hill-Shannon, $\iota = 0$, green line with terminal triangle; Hill-Simpson, $\iota = -1$, blue line with terminal square. Solid lines = observed, dashed lines = extrapolated. Confidence intervals (95 %) are denoted by shading around the line. Left: species diversity by number of dietary detections. Centre: sample coverage by number of dietary detections. Right: species diversity by sample coverage.

Together, these diversity estimates suggest Telfair's skinks consume many rarely eaten individual species instead of evenly consuming dietary taxa or just a few commonly eaten species. I estimated that our sampling provided 95.7 % (± 95% CI: 2.6 %) coverage of the dietary community (Figure 3.5, centre and right plots), estimating that 4.3 % of individuals in the theoretical complete diet belonged to species I did not detect.

MGLMs showed that diet composition differed significantly between season (LRT = 259.88, *p* = <0.001), sex (LRT = 226.22, *p* = <0.001), and their interaction (LRT = 30.54, *p* = 0.027). Diet composition between seasons was visualised using NMDS (Figure 3.6) (stress = 0.161). Three species showed at least one significant GLM result: *Abutilon indicum* (season * sex: LRT = 9.035, *p* = 0.031), *A. aspera* (season: LRT = 31.097, *p* = <0.001), and *L. loddigesii* (sex: LRT = 25.161, *p* = 0.002; season: LRT = 31.213, *p* = <0.001).

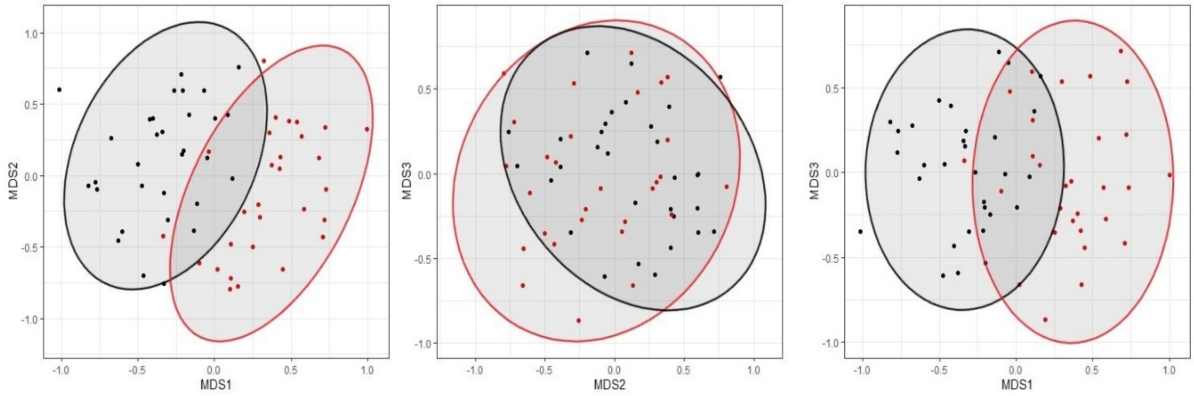


Figure 3.6. Pairwise biplots from non-metric multidimensional scaling (NMDS) analysis in three dimensions (stress = 0.161). Point and ellipse colours denote seasons: black = dry; red = wet. Ellipses show 95% of data rotated to the direction of maximum spread.

3.5 Discussion

3.5.1 Key trophic interactions and dietary diversity

These findings corroborate previous analyses of diet suggesting Telfair's skinks are generalist omnivores that consume a wide range of animal and plant taxa (Pernetta et al. 2005; Brown et al. 2014; Cole et al. 2018a; Moorhouse-Gann et al. 2022). Moreover, our diversity estimates suggest Telfair's skinks consume many species infrequently instead of consuming taxa evenly. This study achieved a greater taxonomic resolution compared to previous molecular analyses of Telfair's skink diet (Pernetta et al. 2005; Brown et al. 2014), resolving almost all plant taxa and nearly half of the invertebrate taxa to species-level. In contrast, previous analyses could not resolve dietary invertebrate taxa to species level at all.

Dietary taxa consumed once or twice form a large component of Telfair's skink diet, suggesting that they may opportunistically consume many rare species, but rely on a few other species for more consistent nutrition, which may also be seasonal. For example, *L. loddigesii* was the most frequently consumed native dietary taxon, being present in 32.9 % of samples, and all detections occurred in the dry season. These trees form the dominant native habitat type on Round Island and produce fruits, pollen, and nectar that skinks are known to readily consume (Cole et al. 2018a; Cole et al. 2018c). Since all detections of *L. loddigesii* occurred in the dry season, when fruits are produced, this suggests they are a seasonal nutritional resource for Telfair's skinks. Moreover, female skinks accounted for 60 % of all *L. loddigesii* detections and fruits may therefore be disproportionately important or attractive to female skinks. Telfair's skinks typically mate throughout the dry season (Cole et al. 2018a) and *L. loddigesii* consumption may provide essential nutrition or minerals for growth and/or egg-production in females. In contrast, 34 dietary taxa were detected only once, 12 taxa were detected twice, and six taxa were detected three times, cumulatively representing almost a fifth of total detections. Our diversity estimates suggest Telfair's skinks consume a few species regularly but supplement their diet by opportunistically consuming a much greater diversity of other animals and plants at low frequencies.

There are an estimated 46,000 Telfair's skinks on Round Island (Cole et al. 2018c), with an estimated 210 skinks per ha island-wide. This represents a major component of total animal biomass. Given the abundance and size of Telfair's skinks, they may exert some top-down effects across the wider ecological community of Round Island. Our results suggest these results are most likely to be seen by consuming invertebrates and by eating seeds, possibly participating in seed dispersal.

3.5.2 Prevalence of non-native taxa

Overall, non-native taxa formed the primary component of Telfair's skink diet as measured by frequency of occurrence. The majority of dietary detections and richness were of non-native taxa, accounting for almost half in both cases. Therefore, this study illuminates that non-native taxa have become a large part of the diet of a globally threatened endemic species. However, for some taxa it is unclear whether skinks rely on them for nutrition, and this is a broader issue in dietary metabarcoding studies because sequencing data cannot convey nutritional information (Alberdi et al. 2019; Lamb et al. 2019). For example, the non-native ant *P. megacephala* is present in 39.7 % of samples but may be a distasteful meal for Telfair's skinks. On Round Island, *P. megacephala* is hyperabundant and found in every habitat type in this study. Predation may not provide a cost-effective nutritional reward to an unspecialised ant-eating vertebrate given that the ant is very small compared to Telfair's skinks and ants typically possess unpleasant and/or harmful compounds (Schmidt 2009). If these ants truly are deleterious to skinks, their high frequency of occurrence in the diet could be explained by accidental consumption. Accidental consumption may occur when skinks consume food items that have been colonised by ants, which typically occurs rapidly on Round Island. Another explanation is through secondary predation, which entails detection of food items in the digestive system of primary skink prey. Both accidental consumption and secondary predation may complicate interpretation of dietary analyses using HTS (Robeson et al. 2018; da Silva et al. 2020; Tercel et al. 2021). Nevertheless, even accidental ingestion of some species could provide nutritional benefits to skinks.

With roughly half of all dietary detections originating from non-native species, non-native taxa appear to be a dominant part of Telfair's skink diet. It may be that the original components of the diet have been lost after Round Island suffered severe habitat destruction and have been subsequently replaced by non-native species. Equally, the availability or nutritional value of non-native species may be relatively higher than existing native food.

Cryptogenic invertebrates represented almost 20 % of all dietary detections and therefore likely represent an important component of Telfair's skink diet. In reality, cryptogenic species are either non-native or native, but this information is lost without adequate taxonomic information. Unfortunately, many of the invertebrates on Round Island have not been studied and are absent from barcode reference libraries, which presents a problem when assigning an origin to ZOTUs that do not resolve to species-level. Therefore, invertebrate species categorised as cryptogenic may be endemic and globally threatened. The use of a 93 % identity threshold in this study permits assignment of sequences to a higher taxonomic level for species absent from barcode libraries, i.e. to genus or family, but this does not solve how to assign a dietary taxon a status. Our study deliberately took a conservative approach to assigning a status category to taxa, but it may be more likely for cryptogenic species to be native than non-native. This is because many non-native species are globally common and have been barcoded, whereas endemic species have not. Work to formally describe, identify and barcode Round Island invertebrate species is therefore essential to disentangling this problem and to more fully describing the ecology of Round Island.

3.5.3 Seasonal and sex differences

The presence of plant species in the diet of the skinks solely or primarily in one season, such as *L. loddigesii* (all 24 detections in the dry season) and *A. aspera* (all 14 detections in the wet season), partly explains the strong difference in diet composition between seasons. Broad seasonal differences in diet are further explained by animal taxa being a much greater component of skink diet in the dry season, where 61.4 % of animal prey detections occurred. Despite this, most animal taxa were consumed across both seasons, whilst most plant taxa were consumed primarily in only one season. This confirms that Telfair's skinks rely on different dietary taxa at different times of the year at a broad scale, with only modest overlap in composition (Figure 3.7). Seasonal differences in diet composition very likely arise because of changes in the availability of food sources between the markedly different seasons in Mauritius (Senapathi et al. 2009).

Two dietary taxa were consumed at different rates between male and female skinks: *L. loddigesii*, as discussed above, and *A. indicum*, which was the most frequently consumed plant, present in 35.6 % of samples. *Abutilon indicum* is native to tropical and subtropical Asia but has been widely transported across the global tropics and is locally abundant over much of Round Island. It produces flowers and seeds that may be attractive to skinks year-round. Male skinks consumed *A. indicum* more frequently in the wet season (71.4 % of detections) than the dry season (28.6 %), but the inverse was true of female skinks (100 % of detections in the dry season). A possible explanation is that plant tissues of *A. indicum* (e.g., flower, nectar, seeds) are consumed differently between sexes. Because the availability of these varies throughout the year, it may mean that males and females consume *A. indicum* differently between seasons. Exactly how the tissue types of *A. indicum* may differentially benefit male and female skinks requires further study.

Understanding the nutritional requirements between sexes could be an important factor governing the success of skink translocations. Though I broadly see that male and female skinks consume the same species, I show that female skinks might rely more on certain species during the breeding season, which is a pivotal period in any reintroduction program.

3.5.4 Limitations

The general limitations of dietary metabarcoding have been reviewed extensively by other authors (Nielsen et al. 2018; Taberlet et al. 2018; Alberdi et al. 2019; Lamb et al. 2019), but I also identified some study-specific limitations. This study converts sequence data to presence/absence and subsequently frequency of occurrence. I believe this is the most robust interpretation of sequencing data, because sequencing output only very weakly correlates to biomass in a sample (Deagle et al. 2019; Lamb et al. 2019). Nevertheless, frequency of occurrence therefore also omits how much biomass is consumed in each sample and, thus, a dietary taxon may appear frequently between samples but not contribute proportionately to the nutrition of the consumer.

As discussed above, the very high prevalence of non-native ants in Telfair's skink diet is difficult to explain ecologically with any certainty. These have not been observed to be directly eaten by the skinks, but are ubiquitous over Round Island, and colonise food resources rapidly. Moreover, a very frequently found tiny (<2 mm) cryptogenic braconid wasp, *Heterospilus* sp., seems unlikely to be actively preyed upon by adult Telfair's skinks. Accidental consumption or secondary predation might explain these detections, as has been seen in other dietary metabarcoding studies (Silva *et al.* 2019), and have been identified as a potential source of error that may disproportionately complicate the interpretation of dietary analyses of omnivores (Tercel et al. 2021). With an aim to tease apart some of these issues, I conducted a co-occurrence analysis (Chapter 3 Appendix S3.3 and Figure S3.1) but found no clear ecological patterns that explain these detections. Indeed, co-occurrence analyses may be used as an exploratory element in ecological studies but cannot provide strong evidence to support ecological hypotheses in this context (Blanchet et al. 2020), and may not facilitate interpretation (Tercel et al. 2021).

Omnivores can exert top-down effects across the breadth and depth of ecological networks, and studying their diet is therefore valuable to the field of ecology. However, omnivorous diets require extra caution when inferring ecological conclusions from sequencing results given that some detections may not be ecologically meaningful. In this study, it may be that Telfair's skinks *are* directly consuming both *P. megacephala* and *Heterospilus* sp., but it remains unclear whether this is true from conflicting behavioural observations of Telfair's skinks and our inconclusive co-occurrence analysis that does not provide alternative ecological explanations.

3.4.5 Concluding remarks

Our study represents one of the few complete dietary analyses of an omnivore using DNA metabarcoding (though see de Barba et al. 2014; Robeson et al. 2018; da Silva et al. 2020; Ducotterd et al. 2021) and the first study examining the omnivorous diet of a threatened endemic reptile. I found that Telfair's skinks consume a few species regularly and many species rarely. I also found that Telfair's skinks rely on *L. loddigesii* fruits during the dry season on Round Island, coinciding with when breeding takes place. Though restored habitat on Round Island does not cover the whole island, extensive habitat regeneration efforts since 2002 have led to the continued recovery of the forests on Round Island, and this bodes well for the future of the skinks. Nevertheless, almost half of all dietary detections were of non-native species and it is increasingly clear that the ecological impacts of non-native species on Round Island are multi-faceted, with some species acting as *de facto* ecological replacements. Telfair's skinks have probably incorporated non-native species into their diet as a replacement for the many species that were presumably lost by severe habitat destruction on Round Island. Further studies showing the mechanisms by which Telfair's skinks consume certain species would help to provide more confident ecological explanations for some of these interactions.

Although many non-native species damage native ecosystems, some species may benefit regenerating habitats and their inhabitants, particularly in the context of island restoration. This study shows that many non-native species of animal and plant contribute positively to providing nutritional subsidies to a globally threatened endemic omnivore. Positive effects of non-native species must therefore be weighed up against potential negative consequences of colonisation for the ecosystem. This is pertinent for conservation managers to consider when restoring native habitats and controlling non-native species, especially when threatened animal species may be consuming non-native taxa in the absence of lost native food resources.

A view of the “crater” area of Round Island from the south-east ridge; Serpent Island can be seen in the distance.

Chapter 4: Just the Twelve of Us: the Trophic Ecology of Ants on Round Island, Mauritius

Dolichoderus sp. tending aphids, Cuc Phuong National Park, Vietnam.

4.1 Abstract

Non-native ants are implicated in the demise of native ants worldwide, though the trophic ecology of non-native ant communities is poorly understood. Identifying the trophic interactions of non-native ant communities might therefore improve our understanding of how non-native ants survive in their new range, as well as how ant-ant interactions could structure ant communities. Here I present a community-wide dietary analysis using DNA metabarcoding across twelve of the most abundant ant species in a non-native ant community on Round Island, Mauritius. Using three PCR primer pairs amplifying animal and plant DNA in the gasters of 755 individual ants sampled across Round Island, I revealed 1,947 dietary detections belonging to 156 dietary taxa of both non-native and native origin. All ants showed generalist diet profiles and approximately half of all dietary detections were of ants consuming other ants (911). Dietary composition significantly varied primarily between ant species and seasons, and the ant community showed a degree of dietary niche separation. I also measured prey choice, showing a range of species-specific diet preferences, and conducted co-occurrence analyses, both of which suggest all other ant species are avoiding the hyper-abundant *Pheidole megacephala* disproportionately in terms of nesting habits and diet. Our results corroborate the hypothesis that *P. megacephala* dominates the ant community on Round Island and may be significantly influencing community dynamics. This study is the first to successfully measure the species-level trophic interactions of hundreds of individual ants across multiple species at the community-scale. This is the first evidence of its kind showing that coexisting non-native ant species that share no evolutionary history have dissimilar diets despite broad generalist dietary profiles, that non-native ants consume other ants very frequently, and that there may be a link between 'dominance' and trophic interactions within ant communities.

4.2 Introduction

Over the course of their ca. 140-million-year evolution, ants have become the most speciose and ecologically diverse group of social insects (Grimaldi and Agosti 2000) and often numerically dominate invertebrate communities (Hölldobler and Wilson 1990; Lach et al. 2010). In canopy fogging experiments, for example, up to 86 % of the total average biomass of samples can consist of ants (Tobin 1995; Davidson et al. 2003). Moreover, ants are widely considered to be ecologically dominant insects, filling a broad range of functional roles within ecosystems, including as parasites, predators, herbivores, decomposers, soil turners, and mutualists. Interactions between different species of ants have been termed the "hallmark of ant ecology" (Hölldobler and Wilson 1990), and a large body of research suggests that ant-ant interactions help shape the spatial distributions of ant species at the site- and plot-level, e.g., between 1-100 m distances (Greenslade 1971; Vepsalainen and Pisarki 1982; Savolainen and Vepsäläinen 1988; Andersen 1992; Andersen and Patel 1994), and small-scale interaction outcomes at food resources (Savolainen and Vepsäläinen 1988; Bestelmeyer 2000; LeBrun 2005). Broad dominance hierarchies exist in ant communities where some species are behaviourally, numerically, or ecologically dominant (see Chapter 1 for definitions of these types of dominance and Lach, Parr and Abbott, 2010), though these hierarchies can be mediated by additional factors, such as temperature (Bestelmeyer 2000; Blight et al. 2014) or the presence of parasitoids (LeBrun 2005). Intraguild interactions, such as predatory aggression or territorial conflict, appear to strongly structure most ant communities (Mabelis 1983; Rosengren 1986; Ryti and Case 1988; Hoenle et al. 2019) but weakly in others (Sanders et al. 2007; Stuble et al. 2013b). Thus, native ant communities are probably structured by a series of ant-ant interactions, hierarchies, and other relationships, alongside non-ant species interactions and abiotic variables.

Non-native ants may be able to outcompete native ants and deconstruct native communities by, for example, having a very broad dietary range, high level of behavioural dominance, finding food resources more rapidly than native species, and/or remaining highly active across a range of temperatures (Holway 1999; Holway et al. 2002; Thomas and Holway 2005; Arnan et al. 2018b). This may partly explain why native ant communities invaded by non-native ants tend to decrease in diversity substantially (see Chapter 2). Ant-ant predation can similarly contribute to the decline of native species in invaded areas through nest raiding and opportunistic predation along territorial boundaries by non-native species, especially where ant species have no overlap in diet. Nest raiding is recognised as an important ecological event in both native and invaded communities (Zee and Holway 2006; Dejean et al. 2008; Lach et al. 2010; Dejean et al. 2014), though the contribution of nest raiding towards overall ant diet is not known.

The ecology of communities consisting almost entirely of non-native species is not well studied in any animal taxon. Few studies have attempted to identify how non-native species interact with one another and it is not clear whether patterns of interaction follow those of native species. Non-native ant communities presumably reach some sort of dynamic equilibrium once the rate of new colonisations decreases (MacArthur and Wilson 1963) and may be structured by the same processes as those of native ants. However, certain factors could be uniquely important in structuring non-native ant communities, such as the contribution of nest raiding to overall diet, interactions with native non-ant species, and, given the extreme dietary generalism of most non-native ant species (Holway et al. 2002), how different foods are selected by different species.

Round Island's ant community consists of 18 recorded species, 17 of which are non-native, and the remaining species requires taxonomic identification to confirm whether it is native. The colonisation history of ants on Round Island is not known, with only a few species having any estimate of their arrival. Several of these species have been implicated in dramatic declines of native ants, invertebrates, and vertebrates across the tropics, most notably *Pheidole megacephala*, which is included on the IUCN's "100 of the World's Worst Invasive Alien Species List" (Luque et al. 2014). These ants may therefore represent a major threat to the unique ecosystem and biodiversity found on Round Island. Despite ongoing habitat regeneration, Round Island's endemic invertebrate fauna likely represents one of the most threatened animal communities on Earth given the extremely small area of occupancy (2.19 km^2), a poor knowledge base of the natural history of most species, and the presence of invasive ants known to severely reduce native invertebrate diversity elsewhere.

No studies to date have identified the species-level food resources used by individual ants of either native or non-native origin at the community-scale. Revealing these interactions will provide valuable insights into how ant species within a community might be able to coexist, and which processes may drive dietary change. The aim of this Chapter is two-fold: 1) to examine the trophic ecology of the non-native ant community on Round Island, with an emphasis on ant-ant interactions and to identify the processes driving their dynamics, and 2) to determine which food resources non-native ants use on Round Island and to what extent they are consuming native species. To do this I will use dietary DNA metabarcoding to reveal the trophic interactions of twelve ant species across habitats and seasons, and invertebrate community samples to describe the potential food resources available to the ants.

4.3 Methods

4.3.1 Study site

See Chapter 1 (Section 1.5.1) for a description of the geography and habitats of Round Island and Chapter 3 (Section 3.3.1) for a description of vertebrate community.

Several non-native invertebrate species are established over the island, such as the webspinner *Oligotoma saundersii* and the ladybird *Harmonia axyridis*. Non-native mealybugs (Pseudococcidae), including the citrus mealybug, *Planococcus minor*, are locally abundant on various native and non-native herbaceous plants, though their impact currently appears minimal on the established plant communities across the island. They do, however, reach high densities on young plants in the Round Island nursery and can cause significant rates of seedling death if left unchecked. Non-native mealybugs and scale insects present on Round Island have formed invader-invader mutualisms with multiple species of ants, which protect them from natural enemies in return for honeydew (pers. obs.). The impact of non-native invertebrates on Round Island is poorly understood, though several species, are ubiquitous, hyper-abundant, and may constitute a significant proportion of biomass of the Round Island ecosystem. Nevertheless, as seen in Chapter 3, non-native invertebrates may act as valuable nutritional resources for a range of native animals.

The ant fauna of Round Island consists almost entirely of non-native species (Table 4.1). One of the 18 species of ants (*Hypoponera mu03*) recorded on Round Island is potentially native, though this remains uncertain. The remaining 17 species are all pantropically distributed non-native ants commonly found on both mainland and island systems (McGlynn 1999; Holway et al. 2002). The invasion history of ants on Round Island is not well understood. Based on records from mainland Mauritius dating back to the late 18th century, it is possible some of the Mauritian islets, such as Round Island, were colonised by non-native ants soon after Mauritius was visited by European settlers. For example, the holotype for *P. megacephala* was collected in Mauritius (Fabricius 1793), suggesting it was probably already common enough to be readily found by that time. Because of this, it was originally thought that *P. megacephala* was native to Mauritius, but specimens collected many years earlier (but not identified) suggest it originates either from mainland Africa or Madagascar (Wetterer 2012). It is uncertain whether other non-native ants had colonised Mauritius by that time, though non-native African or Asian species may be more likely to have reached Mauritius than Neotropical species. Similarly, *Nylanderia bourbonica* was originally described from a specimen collected in Réunion but is thought to be native to south-east Asia (Deyrup 2017). Likewise, *Tetramorium simillimum* has been present on Round Island since at least 1975 (collected by D. Bullock). Non-native ants present in Mauritius may have faced delays in subsequently colonising Round Island due to its relative inaccessibility. Indeed, there are at least 53 recorded species of ants found on mainland Mauritius, most of which are non-native (unpubl. data), that have not colonised Round Island.

Table 4.1. Ant species recorded on Round Island. Life history traits, the year of first confirmed record, and whether they have been taken forward for dietary metabarcoding in this study are noted for each species. Note: the first confirmed record does not indicate when an ant colonised Round Island, but rather when a species was formally confirmed to be present on Round Island by a taxonomic expert.

Species	Subfamily	Native range	Assumed diet	Colony structure	First confirmed record on Round Island	Taken forward for dietary metabarcoding?
Brachymyrmex cordemoyi	Formicinae	Neotropics	Generalist omnivore	Unknown	2005	Yes
Camponotus maculatus	Formicinae	Afrotropics + possibly Mascarenes	Generalist omnivore	Monogyny + monodomy	2001	No
Cardiocondyla emeryi	Myrmicinae	Africa	Generalist omnivore	Polygyny + monodomy	2005	Yes
Hypoponera mu03	Ponerinae	Unknown	Predator	Unknown	2005	Yes
Monomorium floricola	Myrmicinae	SE Asia	Generalist omnivore	Polygyny + polydomy	2005	Yes
Nylanderia bourbonica	Formicinae	SE Asia	Generalist omnivore	Polygyny + polydomy	1975	Yes
Pheidole indica	Myrmicinae	Unknown (possibly SE Asia)	Generalist omnivore	Polygyny + polydomy	2004	Yes
Pheidole megacephala	Myrmicinae	Afrotropics	Generalist omnivore	Polygyny + unicolonial	2005	Yes
Pheidole parva	Myrmicinae	SE Asia	Generalist omnivore	Unknown	2019 (this study)	Yes
Strumigenys simoni	Myrmicinae	Afrotropics	Specialist predator	Unknown	Unknown	Yes
Syllophopsis sechellensis	Myrmicinae	Unknown	Unknown	Unknown	2019 (this study)	No
Tapinoma melanocephalum	Dolichoderinae	Unknown	Generalist omnivore	Polygyny + unicolonial	Unknown	No
Tapinoma subtile	Dolichoderinae	Afrotropics + possibly Mascarenes	Unknown	Unknown	Unknown	Yes
Technomyrmex albipes	Dolichoderinae	Possibly SE Asia	Generalist omnivore	Polygyny	2005	No
Technomyrmex pallipes	Dolichoderinae	Afrotropics	Generalist omnivore	Unknown	1978	No
Technomyrmex vitiensis	Dolichoderinae	Unknown	Generalist omnivore	Unknown	2005	Yes
Tetramorium bicarinatum	Myrmicinae	SE Asia	Generalist omnivore	Polygyny + unicolonial	2019 (this study)	No
Tetramorium simillimum	Myrmicinae	Afrotropics	Generalist omnivore	Polygyny	1975	Yes

The native entomofauna of mainland Mauritius is reasonably well-developed given its size and biogeographic history, with ~2,000 species across 22 orders recorded by 2007, 744 of which are endemics (Motala et al. 2007b), though many native species remain undescribed. On Round Island, invertebrates are more severely understudied (Motala et al. 2007b), with only a few species receiving any form of scientific investigation, and many invertebrates, particularly insects and spiders, requiring formal taxonomic description. These undescribed species constitute a very large proportion of invertebrates on Round Island and are likely to be either native to Mauritius or endemic to Round Island. It is feasible many endemic species survived the severe habitat destruction of the last ~350 years on Round Island in small populations. These probably started to recover once mammals were eradicated in 1986 with the regeneration of the palm habitat. This period also included a marked increase in the number of exotic plants, which may have assisted both native and non-native invertebrates in terms of food and habitat. Restoration efforts further intensified post-2002. Nevertheless, some endemic invertebrates may have already become extinct before being taxonomically described because of habitat destruction, as well as the high prevalence of invasive ants, such as *P. megacephala* (see Chapter 2 for the effects of non-native ant invasion), and other non-native species.

Endemic species of arthropod that have been described include the Round Island stick insect, *Apterograeffea marshallae*, a herbivore of *L. loddigesii* (Moldowan et al. 2016), the Serpent Island centipede, *Scolopendra abnormis*, a large invertebrate predator endemic to Round Island and Serpent Island (Lewis et al. 2010), and the scorpion *Lychas serratus*, found on Gunner's Quoin and Round Island that was presumed to be extinct for 150 years until it was rediscovered in 2013. Some other undescribed common invertebrates are presumed to be native, such as an abundant cockroach species (Blaberidae), a leaf-cutter bee (Megachilidae), and a tree cricket (Gryllidae: Oecanthinae).

4.3.2 Invertebrate community collection

Invertebrate community samples were collected on Round Island between August 2019 and March 2020 using pitfall traps in 69 randomly generated 4 m^2 quadrats (Figure 4.1). I wanted to compare invertebrate community composition and dietary differences of the ants between dry and wet seasons, and thus aimed to sample quadrats twice. Of the 69 quadrats that were visited in the 2019 dry season, 42 were revisited in the 2020 wet season. Unfortunately, I were unable to revisit the remaining 27 quadrats because of a cyclone and the coronavirus pandemic.

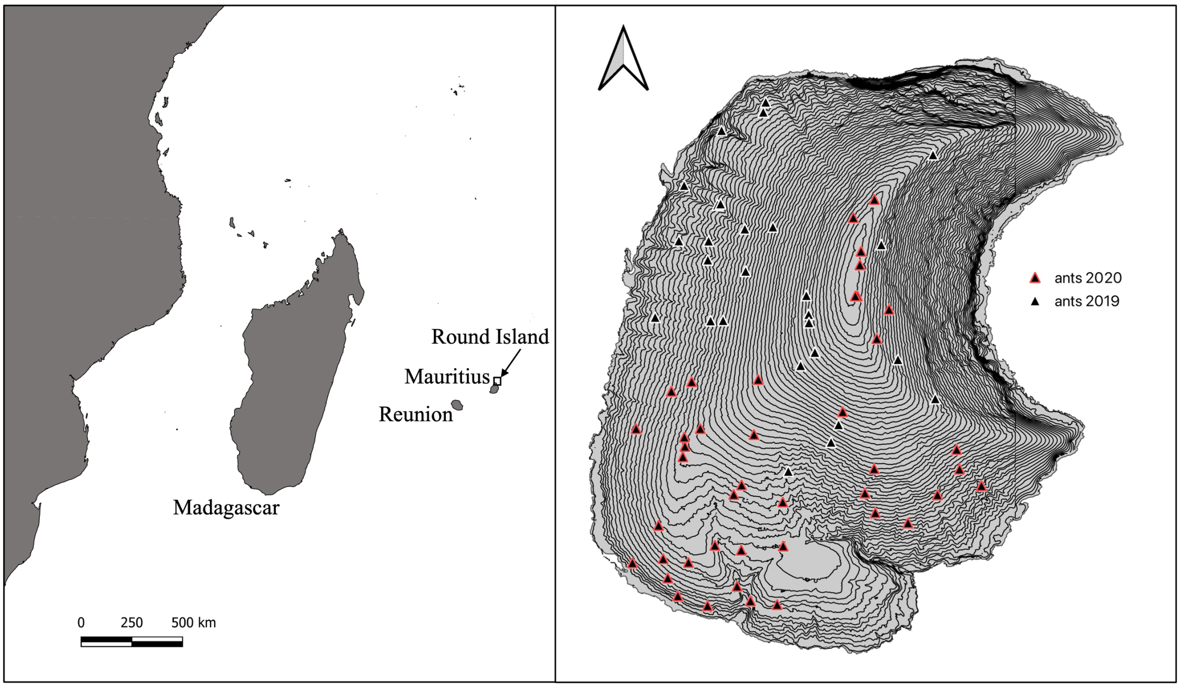


Figure 4.1. Location of the study. The left map shows the location of Round Island in the Indian Ocean. The right map shows the topography of Round Island (5 m contour lines) and the sampling locations for ants in 2019 (white border) and 2020 (red border). Note: all ant quadrats sampled in 2020 were also sampled in 2019.

To sample epigeal invertebrates, a 150 mL pitfall trap was placed in the centre of each quadrat, ensuring the rim of the container was flush with the surface of the soil, and half-filled with 50% ethanol. Traps were collected after approximately 48 h and the contents were decanted into 15 mL universal collection tubes. A large proportion of the ground surface area on Round Island is bare rock, where traditional pitfall trapping is impossible. For quadrats that were generated in these areas, I designed pitfall traps with 12 cm canvas skirting radiating from the rim that could be fixed to the substrate using masonry nails (Figure 4.2). I found that different trap types (canvas vs. ground) approached a significant difference in the average number of invertebrates captured (t-test: t = -1.94, df = 74, p = 0.056), though the effect size of this result was very small (Hedges' g = -0.012). The composition significantly differed between trap types (MGLM in mvabund: LRT = 44.84, p = 0.006), but our preliminary analysis revealed that these differences were driven by *P. megacephala* abundance between trap types (Wilcoxon rank sum: W = 427.5, p = 0.002, median difference between all pairs = -0.4). Once *P. megacephala* was removed from the analysis, trap type did not significantly affect the number (W = 627, p = 0.346, median difference between pairs = -0.25) or composition (MGLM: Dev = 30.01, p = 0.107) of invertebrates captured and I therefore treated different trap types identically. All pitfall trap samples were stored at room temperature until they could be refrigerated at -20 °C on the Mauritius mainland and transported to Cardiff University.

Figure 4.2. Skirted canvas pitfall trap suitable for rocky substrates.

4.3.3 Ant collection for dietary analysis

Ants for dietary analysis were collected between August 2019 and March 2020 over Round Island in each randomly generated 4 m^2 quadrat (Figure 4.1) after invertebrate community samples were collected. The area was scoured primarily for ant nests by hand searching, e.g., flipping/breaking rocks, digging into soil, checking vegetation, and disturbing and sifting through leaf litter. Once a nest was found, a pooter was used to collect and transfer ants into 15 mL collection tubes. Ants were identified to species (or morphospecies) in the field and each ant species was collected separately. Each collection tube contained only a single ant species from a single colony and the quadrat was scoured until no new species could be found. Because I aimed to compare the diet of ants from different castes and developmental stages, an effort was made to collect ant individuals from these different categories.

Ants were killed by freezing and were preserved in 100 % ethanol. Samples were stored at -5 °C before being moved to -20 °C storage on mainland Mauritius and subsequently to -80 °C at Cardiff University.

I aimed to collect individuals from all ant species recorded on Round Island. During sample collection, several ant species were newly recorded on Round Island (*Pheidole parva*, *Syllophobsis seychellensis*, and *Tetramorium bicarinatum*), which increased the number of known ant species on Round Island to 18. However, I were only able to take 12 of 18 species forward for dietary analysis (Table 4.2) because of a lack of specimens of several species. Our community-level dietary analysis of 12 ant species includes all highly abundant ant species present on Round

Island as of 2020. Together, these species represent >95 % of all ant individuals on Round Island. The number of ants taken forward for dietary metabarcoding was roughly proportional to their abundance in the community. However, I also aimed to include as many species as possible in the dietary analysis and therefore uncommon ants are better represented whilst *P. megacephala* was sampled less (though is still the most comprehensively sampled species in the dietary analysis). One limitation of the sampling design is that ants of the same species collected in the same quadrat come from the same nests. Ants from the same nests are therefore non-independent in terms of diet. This is because ants will feed one another food foraged from the surrounding area via trophallaxis. Thus, ants collected in the same quadrat may have more similar diets to one another regardless of species. This potential limitation is discussed further in section 4.5.5.

Table 4.2. Numbers of ants taken forward for DNA extraction for each primer pair categorised by species and demography. Four species were not screened with plant primers; *H. mu03* and *S. simoni* are assumed to be carnivorous, whilst *M. floricola* and *T. simillimum* were not sequenced because of insufficient space going forward for sequencing.

Ant species	*Brachymyrmex cordemoyi*	*Cardiocondyla emeryi*	*Hypoponera mu03*	*Monomorium floricola*	*Nylanderia bourbonica*	*Pheidole indica*	*Pheidole megacephala*	*Pheidole parva*	*Strumigenys simoni*	*Tapinoma subtile*	*Technomyrmex vitiensis*	*Tetramorium simillimum*	**All ant species**
AntEx													
Minor	159	39	9	20	67	36	270	18	51	47	15	49	**780**
Major	-	-	-	-	-	6	102	1	-	-	-	-	**109**
Larva	7	4	0	0	0	0	35	0	23	0	0	17	**86**
Queen	17	1	4	0	0	0	6	0	2	0	0	3	**33**
Drone	15	0	0	0	1	0	11	0	0	0	0	0	**27**
Total	**198**	**44**	**13**	**20**	**68**	**42**	**424**	**19**	**76**	**47**	**15**	**69**	**1035**
Beren-Luthien													
Minor	159	39	9	20	67	36	270	18	51	47	15	49	**780**
Major	-	-	-	-	-	6	102	1	-	-	-	-	**109**
Larva	7	4	0	0	0	0	35	0	23	0	0	17	**86**
Queen	17	1	4	0	0	0	6	0	2	0	0	3	**33**
Drone	15	0	0	0	1	0	11	0	0	0	0	0	**27**
Total	**198**	**44**	**13**	**20**	**68**	**42**	**424**	**19**	**76**	**47**	**15**	**69**	**1035**
UniPlant													
Minor	157	39	0	0	66	36	119	18	0	37	15	0	**487**
Major	-	-	-	-	-	6	70	1	-	-	-	-	**77**
Larva	7	4	0	0	0	0	34	0	0	0	0	0	**45**
Queen	17	1	0	0	0	0	6	0	0	0	0	0	**24**
Drone	15	0	0	0	1	0	11	0	0	0	0	0	**27**
Total	**196**	**44**	**0**	**0**	**67**	**42**	**240**	**19**	**0**	**37**	**15**	**0**	**660**

4.3.4 Identification

Ants were identified to genus using Bolton (1994) and Fisher and Bolton (2016), and to species-level using Bolton (1980; 1987; 2007), Seifert (2002), Heterick (2006), LaPolla *et al*. (2011), Sarnat *et al*. (2015), Fisher and Bolton (2016), and the websites AntWiki (*AntWiki* 2022) and

AntWeb (*AntWeb* 2022), which include updated versions of dichotomous keys for species identification by geographical region.

Invertebrates in community samples were identified to order level.

4.3.5 Sample preparation for dietary analysis

Several adult worker ant specimens were removed from each tube for identification to ensure tubes solely contained a single species. Identified reference specimens were used to confirm the identity of each ant used in dietary analysis. To ensure adequate sub-group sample size, individual ants from each caste and developmental category in each species were selected where possible. Selected ants were grasped in forceps and surface-cleaned by washing them with 1 mL of 10 % bleach once and 1 mL of 100 % ethanol five times. The gaster of the ant was then removed with sterile forceps and placed in a 1.5 mL centrifuge tube filled with 100% ethanol. A total of 1,035 ants were taken forward for DNA extraction.

4.3.6 Primer selection, DNA extraction, and high-throughput sequencing

I used three primer pairs to reveal the diet of the Round Island ant community (Table 4.3). For all twelve ant species, I aimed to identify animal prey in the diet. Several primer pairs amplifying animals were tested *in vitro* with DNA extracts from Round Island animals to gauge their efficacy. BerenF-LuthienR (Cuff et al. 2021a), targeting a 314bp fragment of the mitochondrial COI barcoding region (Folmer et al., 1994a), provided the most comprehensive coverage, amplifying all Round Island animal DNA extracts tested. To overcome the problem associated with amplifying host DNA with BerenF-LuthienR universal primers (Cuff et al. 2022), I also developed new animal primers that excluded ant DNA. Termed AntEx, targeting a 214bp fragment of the mitochondrial COI barcoding region, these primers amplified all non-ant DNA extracts tested from Round Island invertebrates, with the exception of some spider extracts (Chapter 4 Appendix S4.1). Most of the ant species are presumed to be omnivorous. I therefore aimed to reveal the plants and animals consumed. To detect dietary plant DNA, I used the universal plant primers, UniPlant (Moorhouse-Gann et al. 2018), which were designed specifically to amplify the DNA of Round Island plants. See Table 4.2 for sample number and identity information for each primer pair.

Table 4.3. Primers used in the current study. AntEx primers were designed for the current study, targeting a 214bp amplicon of the mitochondrial COI gene. BerenF-LuthienR and UniPlant primers amplify 314bp and 250bp amplicons from the COI and ITS2 markers, respectively.

Primer	Sequence (5'-3')	Source	Direction	Base pairs
AntExF (ant exclusion)	TAATTGGDGGHTTYGGWAAYTG	This study	Forward	21
AntExR (ant exclusion)	CCTAAAATTGADGADAYHCCWGC	This study	Reverse	22
BerenF (general animal)	CAGGWTGAACWGTWTAYCCYCC	(Cuff *et al.*, 2021)	Forward	22
LuthienR (general animal)	ACTTCWGGRTGWCCAAARAAYCA	(Folmer *et al.*, 1994)	Reverse	23
UniPlantF (general plant)	TGTGAATTGCARRATYCMG	(Moorhouse-Gann *et al.*, 2018)	Forward	19
UniPlantR (general plant)	CCCGHYTGAYYTGRGGTCDC	(Moorhouse-Gann *et al.*, 2018)	Reverse	20

Extraction of DNA followed the Qiagen DNeasy Blood & Tissue Kit protocol (Qiagen, Hilden, Germany) with the following modifications: each ant gaster was placed in 180 µL of buffer ATL using sterile forceps and disintegrated to release food items in the crop and gut using a sterile micropestle in 1.5 mL centrifuge tubes. Extractions thereafter followed the DNeasy Blood & Tissue Kit manufacturer recommendations, but with an extended lysis time of approximately 14 hours to release any dietary DNA from the robust crop of the ants. I used one negative control per seven ant samples.

Primers were uniquely labelled using 8bp molecular identification tags (MID-tags) to allow each sample to be identified bioinformatically post-sequencing. Polymerase chain reactions (PCR) of 25 µL reaction volumes contained 12.5 µL Qiagen Multiplex Kit, 0.2 µmol (2.5 µL of 2 µM) of each primer and 5 µL of template DNA. Reaction cycles went as follows: 15 minutes initial denaturation at 95 °C, 35 cycles of 94 °C for 30 seconds, primer-specific annealing temperature for 90 seconds, and 72 °C for 90 seconds, and final elongation at 72 °C for 10 minutes. The annealing temperatures for each primer pair were: AntEx = 44 °C, BerenF-LuthienR = 52 °C, UniPlant = 56 °C.

Each 96-well PCR plate included 80 samples, 12 negative controls (DNA extraction and PCR), two blank controls, and two positive controls. Positive controls consisted of a standardised concentration (2 ng/µL) of an approximately equimolar mixture of DNA from amplifiable taxa that are not present on Round Island (Chapter 4 Appendix S4.2). Negative PCR controls were treated identically to samples, with 5 µL of DNase-free water added instead of DNA. Polymerase chain reaction products were viewed on 2% agarose gel stained with SYBR®Safe (ThermoFisher Scientific, Paisley, UK), or viewed on a Qiagen QIAxcel Advanced System (Qiagen, Machester, UK) to detect contamination. Polymerase chain reactions were re-run and new samples were re-extracted if significant contamination was found (e.g., presence of bands in negative controls on agarose gel or QIAxcel). All PCR products were eventually run on a QIAxcel Advanced System to measure relative DNA concentration. Each sample was then pooled according to the relative DNA concentrations of the target amplicon as a proportion of the sample with the highest concentration of DNA in the plate to ensure approximate equimolarity. Negative controls were pooled based on the average volume pooled for samples in a plate. Each pool was cleaned using SPRIselect beads (Beckman Coulter, Brea, USA), with a left-side size selection using a 1:1 ratio. After final elution, each pool was run on an Agilent 4200 TapeStation with D1000 ScreenTape (Agilent Technologies, Waldbronn) to check for significant levels of primer dimer, which were not found, and to confirm amplicon size. These pools of MID-tagged samples were then used for library preparation using the NEXTflex™ Rapid DNA-Seq Kit following the manufacturer's instructions (Bioo Scientific Corp, Austin, TX, United States), which is suitable for pools with DNA concentrations of 1 ng – 1 µg. PCR products from each primer pair were sequenced separately using an Illumina MiSeq. AntEx was sequenced with a V2 cartridge using 2 x 250bp reads, BerenF-LuthienR with a V3 cartridge using 2 x 250 bp reads, and UniPlant with a V2 cartridge using 2 x 250 bp reads.

4.3.7 Bioinformatics and data clean-up

The Illumina sequencing runs generated a total of 41,958,920 reads using the three cartridges (AntEx, V2 = 10,116,224; Beren-Luthien, V3 = 16,124,326; UniPlant, V2 = 15,718,370) across 1,241 samples (including positives and negatives) with AntEx and Beren-Luthien, giving an average sequencing depth per sample of 8,151 and 12,993, respectively. For UniPlant, 811 samples were taken forward, giving a per sample read depth of 19,381. High-throughput sequencing data processing followed the methods outlined in Chapter 3 and, broadly, that of Drake *et al*. (2022) with the following exceptions: I did not use MEGAN (Huson et al. 2016) to assign taxonomic identities to each zOTU and instead did this directly using BLASTn with an up-to-date BLAST database alongside the same parameters and justification as in Chapter 3.

Data were cleaned for statistical analysis broadly following the same methods as Chapter 3, whereby I removed the maximum read count found in blanks and negative controls for each taxon from all samples. I wanted to detect whether the introduced ants were consuming threatened native species, even if rarely. This is because, even if a species is consumed relatively rarely by a predator, it may still have a significant impact on the population of a species, especially if the predator is hyper-abundant. The philosophical underpinnings of how conservative to be during data clean-up are important to consider with the ecological context in mind (Tercel and Cuff 2022) and, in this scenario, I argue that false negatives may be more problematic than false positives given the sensitive conservation context on Round Island (Littleford-Colquhoun et al.

2022; Tercel and Cuff 2022). For this reason, I took a less conservative approach to data-cleaning relative to the methods found in Chapter 3 and I therefore omitted the percentage-based minimum sequence copy threshold. After data clean-up, 755 ant samples were taken forward for statistical analysis.

4.3.8 Statistical analyses

All statistical analyses were conducted in R version 4.2.0 (R Core Team 2021) after data were converted to presence/absence from read counts. Basic characteristics of the non-native ant community's diet were quantified by measuring frequency of occurrence (the proportion of individual ants testing positive for a particular dietary taxon). I aimed to measure dietary diversity for the entire ant community, as well as each ant species separately, and used coverage-based estimates to determine how effectively I captured the complete diet of consumers. I used Hill diversity to estimate these parameters as per Chapter 3 (Hill 1973; Roswell *et al.* 2021) and computed these metrics in R package "iNEXT" (Hsieh et al. 2016). I used the argument "endpoint = 1,250" in the iNEXT function to compute the extrapolated Hill-diversity values for each ant species to simulate results as if I had 1,250 dietary detections per ant species. This allows us to more robustly compare Hill-diversity results between species because I are able to predict dietary diversity at a normalised number of dietary detections (1,250) for each species, regardless of the number of actual observed detections.

I wanted to identify whether introduced ants on Round Island were feeding primarily on native or non-native resources. I first manually searched relevant literature in published scientific articles, unpublished reports, and online database repositories, as well as observational accounts, to discern whether dietary taxa were native or non-native. I then categorised them into "Endemic", "Native", "Likely native", "Unknown", "Likely non-native", and "Non-native" based on the weight of evidence for each taxon. I then aimed to quantify the total number of dietary detections across each status category and statistically test whether there were significant differences in average consumption rates between status categories.

I also wanted to determine whether there were any key drivers of the diet of the Round Island non-native ant community. R package "mvabund" (Wang et al. 2012) was used to test whether dietary composition differed between ant species (twelve ant species), season (dry and wet), habitat (crater, mixed weed, palm savannah, summit, rock slab), or ant caste (worker, drone, queen, larva, and, for polymorphic species, major or minor worker). To determine whether the diets of different ant species are disproportionately affected by season, habitat or ant caste, I included an interaction term between ant species and the three other variables, respectively, in our model. Multivariate generalised linear models (MGLMs) were run using the "manyglm" function with a Monte Carlo resampling method and “binomial” error family. Variation in the diet was visualised using non-metric multidimensional scaling analysis (NMDS) using the “metaMDS” function in the “vegan” (Oksanen et al. 2019) package and plotted using “ggplot2” (Valero-Mora 2010). Ant-ant interactions are thought to be particularly important drivers of ant community ecology and may follow more complex behavioural processes than predation of non-ant prey or consumption of plant matter. I therefore tested whether ant-ant trophic interactions were driven by the same processes as consumption of non-ant food. To do this, I ran identically structured MGLMs in “mvabund” across three separate analytical pathways: one including all dietary taxa, one excluding ant prey, and one solely dealing with ant-ant trophic interactions.

Different ant species are typically thought to be able to coexist with some ant species but not others (Savolainen and Vepsäläinen 1988; Hölldobler and Wilson 1990). I conducted a co-occurrence analysis in R package “cooccur” (Griffith et al. 2016) to test whether different ant species were found together significantly more or less than expected by chance in each of the 69 quadrats sampled across Round Island.

I tested whether ants showed preferences for dietary taxa using null network models in R package “econullnetr” (Vaughan et al. 2018) using the “generate_null_net” function averaged over 1,000 simulations (“sim = 1,000”). Dietary data were converted to order level, except for ants consumed by other ants which were analysed at species-level, and these were then compared to invertebrate count data captured in the pitfall traps associated with the dietary samples collected in the same quadrats. Plants were excluded from analyses because they were not sampled in quadrats. I then visualised the preferences for each prey taxon in each ant consumer species using the function “plot_preferences” to determine if prey were consumed disproportionately or as expected according to their abundance.

4.4. Results

4.4.1. Broad diet characteristics

Our dietary analysis revealed 1,947 dietary detections belonging to 156 taxa across 755 ant individuals from 12 ant species. Of these, 911 detections were of ants consuming other ants from 17 species. Of the plants, 20 of 22 taxa were resolved to species level and two to genus. Of the 117 non-ant animal prey taxa detected, 18 were resolved to species-level, 12 to genus, 58 to family and 29 to order-level or above. This discrepancy between plant and animal taxonomic resolution is a result of the barcoding gap between the two groups on Round Island. Nevertheless, taxa only given genus- or family-level taxonomic information were still treated at the equivalent of species-level by assigning unique sequences into different mOTUs (molecular operational taxonomic units). A staphylinid beetle was the most frequently consumed non-ant animal taxon, present in 99 samples, whilst *Boerhavia coccinea* was the most frequently consumed plant species, present in 37 samples (Table 4.4). Ants were consumed more frequently than any other family overall, with all ant prey taxa identified to species-level.

Table 4.4. Dietary taxa ordered by Kingdom, Class, Order, Family, and Taxon detected across the twelve species of ant consumer. Frequency of occurrence (FOO) % denotes the percentage of ants from the total number of ants with non-ant prey present (n = 524) where a given dietary taxon was detected. Ants consumed by other ants are excluded from this table. Ants solely eating other ants (n = 231) are excluded from the FOO calculation. Status relates to whether a dietary taxon is considered native or non-native to Round Island and follows a scale from "Endemic" to "Non-native", along with sources supporting the categorisation in the right-most column. "Endemic" relates to whether a species is endemic to Round Island or surrounding islands, excluding the Mauritian mainland.

Taxon	**Kingdom**	**Class**	**Order**	**Family**	**Number of detections**	**FOO (%)**	**Status**	**Source**
Acari sp. 1	Animal	Arachnida	Acari	Acari	4	0.763	Unknown	-
Acari sp. 3	Animal	Arachnida	Acari	Acari	1	0.191	Unknown	-
Acariformes sp.	Animal	Arachnida	Acari	Acari	1	0.191	Unknown	-
Agelenidae sp.	Animal	Arachnida	Araneae	Agelenidae	3	0.573	Unknown	-
Anyphenidae sp.	Animal	Arachnida	Araneae	Anyphaenidae	1	0.191	Unknown	-
Linyphiidae sp.	Animal	Arachnida	Araneae	Linyphiidae	6	1.145	Unknown	-
Philodromidae sp. 1	Animal	Arachnida	Araneae	Philodromidae	5	0.954	Unknown	-
Merizocera sp.	Animal	Arachnida	Araneae	Psilodercidae	2	0.382	Unknown	-
Orbatida sp.	Animal	Arachnida	Orbatida	Orbatida	8	1.527	Unknown	-
Damaeidae sp.	Animal	Arachnida	Oribatida	Damaeidae	3	0.573	Unknown	-
Lychas serratus	Animal	Arachnida	Scorpiones	Buthidae	6	1.145	Endemic	Expert knowledge; Field sampling
Crustacea sp. 2	Animal	Crustacea	Crustacea	Crustacea	5	0.954	Unknown	-
Crustacea sp. 1	Animal	Crustacea	Crustacea	Crustacea	2	0.382	Unknown	-
Geograpsus grayi	Animal	Crustacea	Decapoda	Grapsidae	1	0.191	Native	Expert knowledge; Field sampling; GBIF
Porcellionidae sp.	Animal	Crustacea	Isopoda	Porcellionidae	11	2.099	Likely native	Tercel et al 2022; Expert knowledge; Field sampling
Bourletiella sp.	Animal	Entognatha	Collembola	Bourletiellidae	10	1.908	Non-native	GBIF
Collembola sp. 1	Animal	Entognatha	Collembola	Collembola	3	0.573	Unknown	-
Coecobrya tenebricosa	Animal	Entognatha	Collembola	Entomobryidae	10	1.908	Non-native	GBIF; Internet searches
Entomobrya nivalis	Animal	Entognatha	Collembola	Entomobryidae	3	0.573	Non-native	GBIF
Homidia socia	Animal	Entognatha	Collembola	Entomobryidae	2	0.382	Non-native	BOLD
Willowsia nigromaculata	Animal	Entognatha	Collembola	Entomobryidae	2	0.382	Non-native	GBIF
Hypogastrura sp.	Animal	Entognatha	Collembola	Hypogastruridae	5	0.954	Unknown	-
Cryptopygus sp.	Animal	Entognatha	Collembola	Isotomidae	3	0.573	Unknown	-

Table 4.4. Dietary taxa ordered by Kingdom, Class, Order, Family, and Taxon detected across the twelve species of ant consumer. Frequency of occurrence (FOO) % denotes the percentage of ants from the total number of ants with non-ant prey present (n = 524) where a given dietary taxon was detected. Ants consumed by other ants are excluded from this table. Ants solely eating other ants (n = 231) are excluded from the FOO calculation. Status relates to whether a dietary taxon is considered native or non-native to Round Island and follows a scale from "Endemic" to "Non-native", along with sources supporting the categorisation in the right-most column. "Endemic" relates to whether a species is endemic to Round Island or surrounding islands, excluding the Mauritian mainland.

Taxon	**Kingdom**	**Class**	**Order**	**Family**	**Number of detections**	**FOO (%)**	**Status**	**Source**
Katiannidae sp.	Animal	Entognatha	Collembola	Katiannidae	2	0.382	Unknown	-
Tomoceridae sp.	Animal	Entognatha	Collembola	Tomoceridae	3	0.573	Unknown	-
Gastropoda sp. 2	Animal	Gastropoda	Gastropoda	Gastropoda	8	1.527	Unknown	-
Gastropoda sp. 1	Animal	Gastropoda	Gastropoda	Gastropoda	6	1.145	Unknown	-
Gastropoda sp. 4	Animal	Gastropoda	Gastropoda	Gastropoda	6	1.145	Unknown	-
Gastropoda sp. 5	Animal	Gastropoda	Gastropoda	Gastropoda	4	0.763	Unknown	-
Gastropoda sp. 6	Animal	Gastropoda	Gastropoda	Gastropoda	2	0.382	Unknown	-
Blaberidae sp.	Animal	Insecta	Blattodea	Blaberidae	7	1.336	Likely native	Expert knowledge; Field sampling
Blattella germanica	Animal	Insecta	Blattodea	Ectobiidae	13	2.481	Non-native	GBIF; Expert knowledge; Field sampling
Carabidae sp. 1	Animal	Insecta	Coleoptera	Carabidae	12	2.290	Likely native	Expert knowledge; Field sampling
Carabidae sp. 2	Animal	Insecta	Coleoptera	Carabidae	1	0.191	Unknown	-
Chrysomelidae sp.	Animal	Insecta	Coleoptera	Chrysomelidae	2	0.382	Unknown	-
Harmonia axyridis	Animal	Insecta	Coleoptera	Coccinellidae	8	1.527	Non-native	GBIF; Field sampling; Internet searches
Coleoptera sp. 2	Animal	Insecta	Coleoptera	Coleoptera	5	0.954	Unknown	-
Coleoptera sp. 1	Animal	Insecta	Coleoptera	Coleoptera	1	0.191	Unknown	-
Staphylinidae sp. 1	Animal	Insecta	Coleoptera	Staphylinidae	99	18.893	Likely native	Expert knowledge; Field sampling
Staphylinidae sp. 2	Animal	Insecta	Coleoptera	Staphylinidae	1	0.191	Likely native	Expert knowledge; Field sampling
Collembola sp. 2	Animal	Insecta	Collembola	Collembola	1	0.191	Unknown	-
Lucilia sp.	Animal	Insecta	Diptera	Calliphoridae	2	0.382	Likely native	Expert knowledge; Field sampling
Cecidomyiidae sp.	Animal	Insecta	Diptera	Cecidomyiidae	16	3.053	Unknown	-
Chironomidae sp. 1	Animal	Insecta	Diptera	Chironomidae	16	3.053	Likely non-native	Expert knowledge
Chironomidae sp. 2	Animal	Insecta	Diptera	Chironomidae	12	2.290	Likely non-native	Expert knowledge
Chironomidae sp. 3	Animal	Insecta	Diptera	Chironomidae	3	0.573	Likely non-native	Expert knowledge
Culex sp.	Animal	Insecta	Diptera	Culicidae	19	3.626	Non-native	Expert knowledge; Field sampling

Table 4.4. Dietary taxa ordered by Kingdom, Class, Order, Family, and Taxon detected across the twelve species of ant consumer. Frequency of occurrence (FOO) % denotes the percentage of ants from the total number of ants with non-ant prey present (n = 524) where a given dietary taxon was detected. Ants consumed by other ants are excluded from this table. Ants solely eating other ants (n = 231) are excluded from the FOO calculation. Status relates to whether a dietary taxon is considered native or non-native to Round Island and follows a scale from "Endemic" to "Non-native", along with sources supporting the categorisation in the right-most column. "Endemic" relates to whether a species is endemic to Round Island or surrounding islands, excluding the Mauritian mainland.

Taxon	Kingdom	Class	Order	Family	Number of detections	FOO (%)	Status	Source
Drosophila sp.	Animal	Insecta	Diptera	Drosophilidae	26	4.962	Unknown	-
Drosophilidae sp.	Animal	Insecta	Diptera	Drosophilidae	1	0.191	Unknown	-
Empididae sp.	Animal	Insecta	Diptera	Empididae	1	0.191	Unknown	-
Phoridae sp. 1	Animal	Insecta	Diptera	Phoridae	5	0.954	Unknown	-
Phoridae sp. 2	Animal	Insecta	Diptera	Phoridae	3	0.573	Likely non-native	Expert knowledge
Pollenia sp.	Animal	Insecta	Diptera	Polleniidae	1	0.191	Unknown	-
Psychodidae sp. 1	Animal	Insecta	Diptera	Psychodidae	16	3.053	Likely non-native	Expert knowledge
Psychodidae sp. 2	Animal	Insecta	Diptera	Psychodidae	5	0.954	Unknown	-
Clogmia albipunctata	Animal	Insecta	Diptera	Psychodidae	3	0.573	Non-native	GBIF; Expert knowledge
Scatopsidae sp.	Animal	Insecta	Diptera	Scatopsidae	1	0.191	Unknown	-
Corynoptera concinna	Animal	Insecta	Diptera	Sciaridae	3	0.573	Non-native	GBIF
Sciaridae sp. 2	Animal	Insecta	Diptera	Sciaridae	2	0.382	Unknown	-
Sciaridae sp. 3	Animal	Insecta	Diptera	Sciaridae	2	0.382	Unknown	-
Sciaridae sp. 1	Animal	Insecta	Diptera	Sciaridae	1	0.191	Unknown	-
Tachinidae sp.	Animal	Insecta	Diptera	Tachinidae	3	0.573	Unknown	-
Ceratitis sp.	Animal	Insecta	Diptera	Tephritidae	2	0.382	Likely native	Expert knowledge; Field sampling
Tipulidae sp.	Animal	Insecta	Diptera	Tipulidae	5	0.954	Unknown	-
Oligotoma saundersii	Animal	Insecta	Embioptera	Oligotomidae	58	11.069	Non-native	GBIF
Aphididae sp.	Animal	Insecta	Hemiptera	Aphididae	4	0.763	Unknown	-
Aphidinae sp.	Animal	Insecta	Hemiptera	Aphididae	3	0.573	Unknown	-
Aphis nasturtii	Animal	Insecta	Hemiptera	Aphididae	3	0.573	Non-native	GBIF, Expert knowledge
Forda sp.	Animal	Insecta	Hemiptera	Aphididae	3	0.573	Non-native	GBIF, Expert knowledge
Cicadellidae sp.	Animal	Insecta	Hemiptera	Cicadellidae	3	0.573	Unknown	-
Coccidae sp.	Animal	Insecta	Hemiptera	Coccidae	8	1.527	Unknown	-

Table 4.4. Dietary taxa ordered by Kingdom, Class, Order, Family, and Taxon detected across the twelve species of ant consumer. Frequency of occurrence (FOO) % denotes the percentage of ants from the total number of ants with non-ant prey present (n = 524) where a given dietary taxon was detected. Ants consumed by other ants are excluded from this table. Ants solely eating other ants (n = 231) are excluded from the FOO calculation. Status relates to whether a dietary taxon is considered native or non-native to Round Island and follows a scale from "Endemic" to "Non-native", along with sources supporting the categorisation in the right-most column. "Endemic" relates to whether a species is endemic to Round Island or surrounding islands, excluding the Mauritian mainland.

Taxon	**Kingdom**	**Class**	**Order**	**Family**	**Number of detections**	**FOO (%)**	**Status**	**Source**
Coreidae sp.	Animal	Insecta	Hemiptera	Coreidae	1	0.191	Unknown	-
Cydnidae sp.	Animal	Insecta	Hemiptera	Cydnidae	6	1.145	Likely native	Expert knowledge; Field sampling
Fromundus sp.	Animal	Insecta	Hemiptera	Cydnidae	6	1.145	Likely native	Expert knowledge; Field sampling
Pentatomidae sp.	Animal	Insecta	Hemiptera	Pentatomidae	6	1.145	Unknown	-
Dysmicoccus brevipes	Animal	Insecta	Hemiptera	Pseudococcidae	1	0.191	Non-native	Invasive Species Compendium (CABI)
Psyllidae sp.	Animal	Insecta	Hemiptera	Psyllidae	8	1.527	Unknown	-
Rhyparochromidae sp.	Animal	Insecta	Hemiptera	Rhyparochromidae	4	0.763	Unknown	-
Heterospilus sp.	Animal	Insecta	Hymenoptera	Braconidae	4	0.763	Unknown	-
Chalcididae sp. 1	Animal	Insecta	Hymenoptera	Chalcididae	70	13.359	Unknown	-
Crabronidae sp.	Animal	Insecta	Hymenoptera	Crabronidae	40	7.634	Unknown	-
Halticidae sp.	Animal	Insecta	Hymenoptera	Halictidae	4	0.763	Unknown	-
Apocrita sp.	Animal	Insecta	Hymenoptera	Hymenoptera	14	2.672	Likely native	Expert knowledge; Field sampling
Hymenoptera sp.	Animal	Insecta	Hymenoptera	Hymenoptera	3	0.573	Unknown	-
Ichneumonidae sp. 1	Animal	Insecta	Hymenoptera	Ichneumonidae	3	0.573	Unknown	-
Ichneumonidae sp. 2	Animal	Insecta	Hymenoptera	Ichneumonidae	1	0.191	Unknown	-
Megachilidae sp.	Animal	Insecta	Hymenoptera	Megachilidae	6	1.145	Native	Expert knowledge; Field sampling
Anagrus sp.	Animal	Insecta	Hymenoptera	Mymaridae	3	0.573	Unknown	-
Pompilidae sp.	Animal	Insecta	Hymenoptera	Pompilidae	24	4.580	Unknown	-
Symphyta sp.	Animal	Insecta	Hymenoptera	Symphyta	4	0.763	Unknown	-
Bucculatrix sp.	Animal	Insecta	Lepidoptera	Bucculatricidae	2	0.382	Unknown	-
Erebidae sp.	Animal	Insecta	Lepidoptera	Erebidae	2	0.382	Unknown	-
Geometridae sp.	Animal	Insecta	Lepidoptera	Geometridae	4	0.763	Likely native	Expert knowledge; Field sampling
Lasiocampidae	Animal	Insecta	Lepidoptera	Lasiocampidae	2	0.382	Unknown	-
Lepidoptera sp. 1	Animal	Insecta	Lepidoptera	Lepidoptera	10	1.908	Unknown	-

Table 4.4. Dietary taxa ordered by Kingdom, Class, Order, Family, and Taxon detected across the twelve species of ant consumer. Frequency of occurrence (FOO) % denotes the percentage of ants from the total number of ants with non-ant prey present (n = 524) where a given dietary taxon was detected. Ants consumed by other ants are excluded from this table. Ants solely eating other ants (n = 231) are excluded from the FOO calculation. Status relates to whether a dietary taxon is considered native or non-native to Round Island and follows a scale from "Endemic" to "Non-native", along with sources supporting the categorisation in the right-most column. "Endemic" relates to whether a species is endemic to Round Island or surrounding islands, excluding the Mauritian mainland.

Taxon	Kingdom	Class	Order	Family	Number of detections	FOO (%)	Status	Source
Lepidoptera sp. 3	Animal	Insecta	Lepidoptera	Lepidoptera	5	0.954	Unknown	-
Lepidoptera sp. 2	Animal	Insecta	Lepidoptera	Lepidoptera	1	0.191	Unknown	-
Lycaenidae sp. 1	Animal	Insecta	Lepidoptera	Lycaenidae	1	0.191	Unknown	-
Noctuidae sp. 1	Animal	Insecta	Lepidoptera	Noctuidae	16	3.053	Unknown	-
Noctuidae sp. 2	Animal	Insecta	Lepidoptera	Noctuidae	14	2.672	Unknown	-
Noctuidae sp. 3	Animal	Insecta	Lepidoptera	Noctuidae	2	0.382	Unknown	-
Papilionidae sp. 2	Animal	Insecta	Lepidoptera	Papilionidae	1	0.191	Likely native	Expert knowledge; Field sampling
Psychidae sp.	Animal	Insecta	Lepidoptera	Psychidae	6	1.145	Unknown	-
Pyralidae sp.	Animal	Insecta	Lepidoptera	Pyralidae	10	1.908	Unknown	-
Tortricidae sp. 2	Animal	Insecta	Lepidoptera	Torticidae	2	0.382	Unknown	-
Tortricidae sp. 1	Animal	Insecta	Lepidoptera	Torticidae	1	0.191	Unknown	-
Acrididae sp.	Animal	Insecta	Orthoptera	Acrididae	1	0.191	Likely native	Expert knowledge
Gryllodes sigillatus	Animal	Insecta	Orthoptera	Gryllidae	3	0.573	Non-native	Expert knowledge
Ornebius sp.	Animal	Insecta	Orthoptera	Mogoplistidae	7	1.336	Native	Expert knowledge
Ruspolia differens	Animal	Insecta	Orthoptera	Tettigoniidae	2	0.382	Native	Expert knowledge; Field sampling
Apterograeffea marshallae	Animal	Insecta	Phasmatodea	Phasmatidae	2	0.382	Endemic	Moldowan et al 2016
Ectopsocus	Animal	Insecta	Pscocoptera	Ectopsocidae	3	0.573	Unknown	-
Psocoptera sp.	Animal	Insecta	Psocoptera	Psocoptera	2	0.382	Unknown	-
Frankliniella occidentalis	Animal	Insecta	Thysanoptera	Thripidae	1	0.191	Non-native	Field sampling
Scolopendra abnormis	Animal	Myriapoda	Chilopoda	Scolopendridae	14	2.672	Endemic	Lewis and Daszak, 1996
Nematoda sp.	Animal	Nematoda	Nematoda	Nematoda	14	2.672	Unknown	-
Gongylomorphus bojerii	Animal	Reptilia	Squamata	Scincidae	5	0.954	Endemic	Cole and Payne, 2015
Polyscias maraisiana	Plant	Eudicots	Apiales	Araliaceae	5	0.954	Endemic	Moorhouse-Gann, 2017; Forestry Service Database

Table 4.4. Dietary taxa ordered by Kingdom, Class, Order, Family, and Taxon detected across the twelve species of ant consumer. Frequency of occurrence (FOO) % denotes the percentage of ants from the total number of ants with non-ant prey present (n = 524) where a given dietary taxon was detected. Ants consumed by other ants are excluded from this table. Ants solely eating other ants (n = 231) are excluded from the FOO calculation. Status relates to whether a dietary taxon is considered native or non-native to Round Island and follows a scale from "Endemic" to "Non-native", along with sources supporting the categorisation in the right-most column. "Endemic" relates to whether a species is endemic to Round Island or surrounding islands, excluding the Mauritian mainland.

Taxon	Kingdom	Class	Order	Family	Number of detections	FOO (%)	Status	Source
Tridax procumbens	Plant	Eudicots	Asterales	Aateraceae	7	1.336	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Ageratum conyzoides	Plant	Eudicots	Asterales	Asteraceae	5	0.954	Non-native	Lambdon, 2019
Erigeron canadensis	Plant	Eudicots	Asterales	Asteraceae	3	0.573	Non-native	Lambdon, 2019
Bourreria petiolaris	Plant	Eudicots	Boraginales	Boraginaceae	4	0.763	Native	Plants of the World Online (Kew)
Achyranthes aspera	Plant	Eudicots	Caryophyllales	Amaranthaceae	14	2.672	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Boerhavia coccinea	Plant	Eudicots	Caryophyllales	Nyctaginaceae	37	7.061	Native	Moorhouse-Gann, 2017; Lambdon, 2019
Gymnosporia pyria	Plant	Eudicots	Celastrales	Celastraceae	5	0.954	Native	GBIF; Internet searches
Elaeodendron orientale	Plant	Eudicots	Celastrales	Celastraceae	2	0.382	Native	Plants of the World Online (Kew)
Desmodium incanum	Plant	Eudicots	Fabales	Fabaceae	1	0.191	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Vincetoxicum confusum	Plant	Eudicots	Gentianales	Apocynaceae	1	0.191	Native	Plants of the World Online (Kew)
Passiflora pallida	Plant	Eudicots	Malpighiales	Passifloraceae	16	3.053	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Phyllanthus mauritianus	Plant	Eudicots	Malpighiales	Phyllanthaceae	5	0.954	Native	Moorhouse-Gann, 2017; GBIF
Combretum englerii	Plant	Eudicots	Myrtales	Combretaceae	1	0.191	Non-native	Plants of the World Online (Kew)
Terminalia bentzoe	Plant	Eudicots	Myrtales	Combretaceae	1	0.191	Native	Moorhouse-Gann, 2017; Lambdon, 2019
Syzgium sp.	Plant	Eudicots	Myrtales	Myrtaceae	3	0.573	Likely native	Forestry Service Database
Dodonaea viscosa	Plant	Eudicots	Sapindales	sapindaceae	11	2.099	Native	Moorhouse-Gann, 2017; Lambdon, 2019
Ipomoea pes-caprae	Plant	Eudicots	Solanales	Convolvulaceae	14	2.672	Native	Moorhouse-Gann, 2017; Lambdon, 2019
Solanum pinnatisectum	Plant	Eudicots	Solanales	Solanaceae	12	2.290	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Cenchrus echinatus	Plant	Monocots	Poales	Poaceae	20	3.817	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Aegilops sp.	Plant	Monocots	Poales	Poaceae	16	3.053	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019
Digitaria horizontalis	Plant	Monocots	Poales	Poaceae	4	0.763	Non-native	Moorhouse-Gann, 2017; Lambdon, 2019

4.4.2. Hill diversity and sampling completeness

I calculated the Hill diversity (see Chapter 3 for formulae and theory) of total ant community diet and for each ant species separately. I found that the ant community were consuming many species rarely, rather than relying on a small number of food species, thus indicating a high level of dietary generalism (Figure 4.3). Sampling coverage was estimated to be 98.9 %, meaning that 1.1 % of dietary detections would be from species I did not originally observe if sample size increased. I also calculated Hill diversity and sample coverage for each species of ant separately (Figure 4.4). Both the highest observed Hill-richness and sample coverage were found in *P. megacephala*, at 135 and 96.3 %, respectively, though this is probably a function of the much greater sampling effort. In contrast, extrapolated Hill-richness diversity values at 1,250 simulated dietary detections between *P. megacephala, B. cordemoyi,* and *S. simoni* are similar. *Nylanderia bourbonica* exceed those of all other species (>200 at 1,250 samples) with observed coverage being 73.6 %, indicating the highest predicted level of dietary diversity amongst all ant consumers in this study. The lowest extrapolated Hill-richness value was found in *T. subtile* at 51 and sample coverage for this species was relatively high at 91.9 %, indicating a relatively high level of specialism compared to the other ant consumers.

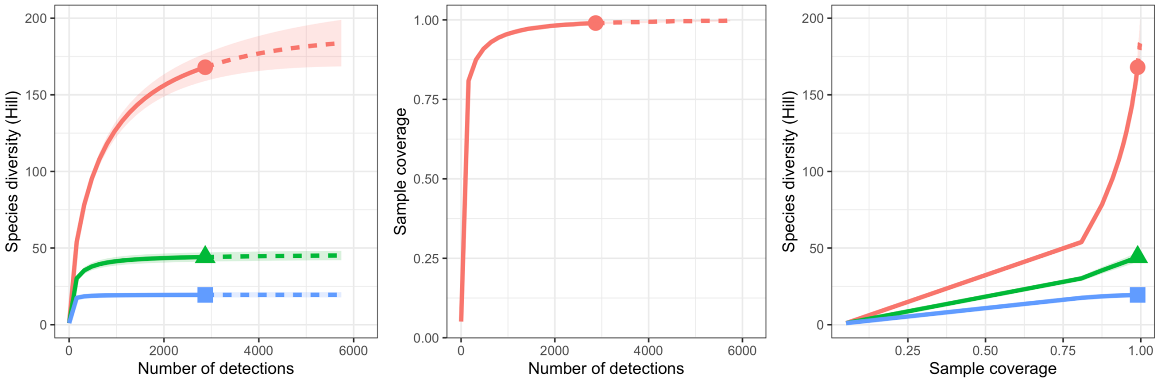


Figure 4.3. Hill diversity estimates of ant diet and level of sampling coverage provided. Line colours denote values of the exponent ι that determines the rarity scale of different diversity estimates: Hill-richness, ι = 1, red line with terminal circle; Hill-Shannon, ι = 0, green line with terminal triangle; Hill-Simpson, ι = -1, blue line with terminal square. Solid lines = observed, dashed lines = extrapolated. Confidence intervals (95%) are denoted by shading around the line. Left: species diversity by number of dietary detections. Centre: sample coverage by number of dietary detections. Right: species diversity by sample coverage.

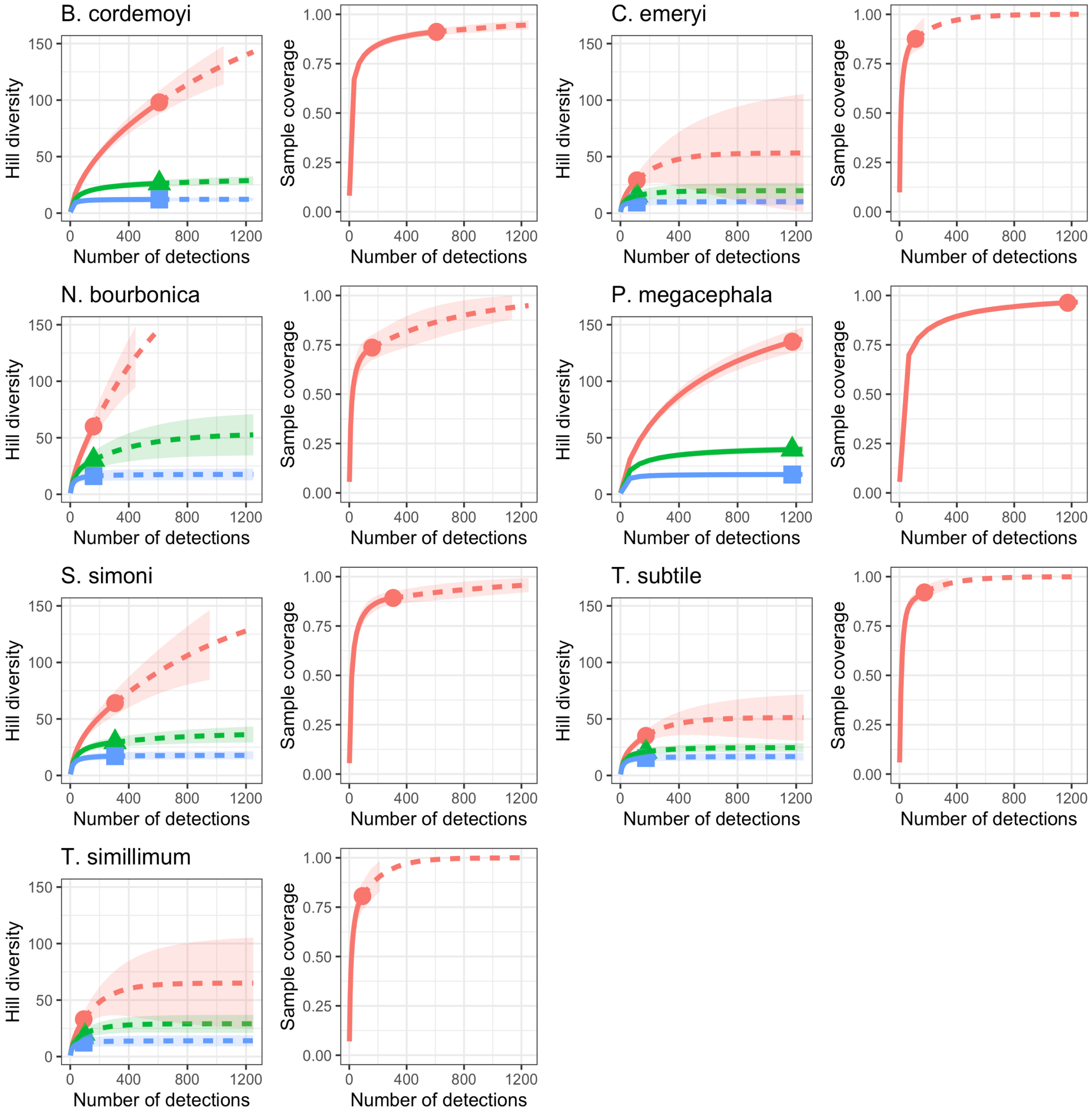


Figure 4.4. Hill diversity and sampling coverage estimates for each consumer ant species with more than 30 samples. Line colours denote values of the exponent ι that determines the rarity scale of different diversity estimates: Hill-richness, $\iota = 1$, red line with terminal circle; Hill-Shannon, $\iota = 0$, green line with terminal triangle; Hill-Simpson, $\iota = -1$, blue line with terminal square. Solid lines = observed, dashed lines = extrapolated. Confidence intervals (95%) are denoted by shading around the line. Left plots beneath species labels show Hill diversity by number of dietary detections, right plots show sample coverage by number of dietary detections.

4.4.3. Non-native vs. native prey consumption

I first used a range of online databases, literature, and expert accounts to assign a status category to each dietary taxon (Table 4.4). I found that the Round Island ant community consumed many more non-native taxa than any other category, comprising 59 % of all dietary detections (Figure 4.5). The majority of the 1,152 detections of non-native food taxa were ant-ant interactions (911) and, once removed, non-native taxa accounted for 23.26 % of the ant community's diet. Data did not conform to a normal distribution (Shapiro-Wilk: W = 0.43, p = <0.0001) and thus I ran a

Kruskal-Wallis test to determine whether the mean number of detections per dietary taxon between status categories were significantly different. I found that there were significant differences in average number of detections per taxon between at least two of the status categories (Kruskal-Wallis: $X^2 = 16.04$, $p = 0.006$). I therefore ran Dunn's test using a Bonferroni correction to identify the groups that were significantly different and found that only "non-native" and "unknown" categories differed significantly in the average number of detections per taxon (Dunn: $Z = -3.77$, adjusted $p = 0.002$; mean ±SD: introduced = 26.18 ± 44.29, unknown = 5.97 ± 9.79). However, once ant prey had been removed from the analysis, no groups showed significantly different rates of consumption on a per taxon basis (Kruskal-Wallis: $X^2 = 6.6$, $p = 0.25$).

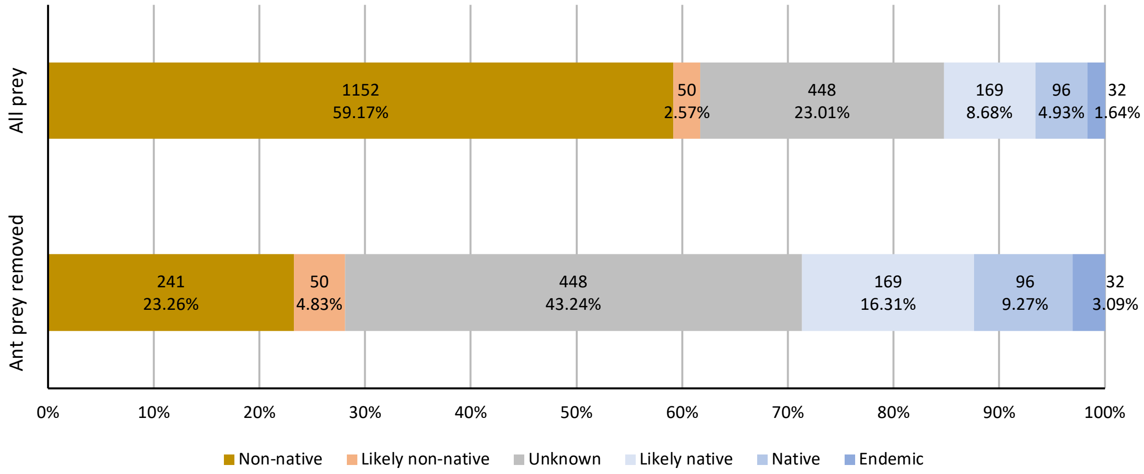


Figure 4.5. Ant diet by "nativeness" status of dietary taxa relative to Round Island presented as a proportion of total dietary detections. The upper bar includes all prey taxa, the lower bar excludes ant prey taxa. Data labels show the number of detections for each status category and the percentage contribution to total dietary detections.

4.4.4. Drivers of ant diet

MGLMs showed ant dietary composition to vary significantly between ant species, seasons, castes, habitats, and the interaction between ant species and the other three variables across datasets with or without ant prey (Table 4.5; Figure 4.6). When solely considering ant-ant trophic interactions, dietary composition differed significantly between ant species and seasons but no other variables. Our univariate tests indicated that consumption rate varied significantly between ant species, season and/or habitat for several prey taxa (Table 4.6).

Table 4.5. Significant MGLM results in 'mvabund', ordered by dataset and model term.

Dataset	Model term	LRT, p-value
All prey	Ant species	LRT = 1579.8, p = <0.001
	Season	LRT = 914.5, p = <0.001
	Caste	LRT = 459.3, p = <0.001
	Habitat	LRT = 694.5, p = <0.001
	Ant species * Season	LRT = 117.5, p = 0.002
	Ant species * Caste	LRT = 249.9, p = <0.001
	Ant species * Habitat	LRT = 418.8, p = <0.001
Ant prey removed	Ant species	LRT = 907.8, p = <0.001
	Season	LRT = 778.6, p = <0.001
	Caste	LRT = 358.2, p = <0.001
	Habitat	LRT = 504.5, p = <0.001
	Ant species * Season	LRT = 75.9, p = <0.001
	Ant species * Caste	LRT = 186.4, p = <0.001
	Ant species * Habitat	LRT = 255.2, p = <0.001
Only ant prey	Ant species	LRT = 577.7, p = <0.001
	Season	LRT = 110.9, p = <0.001

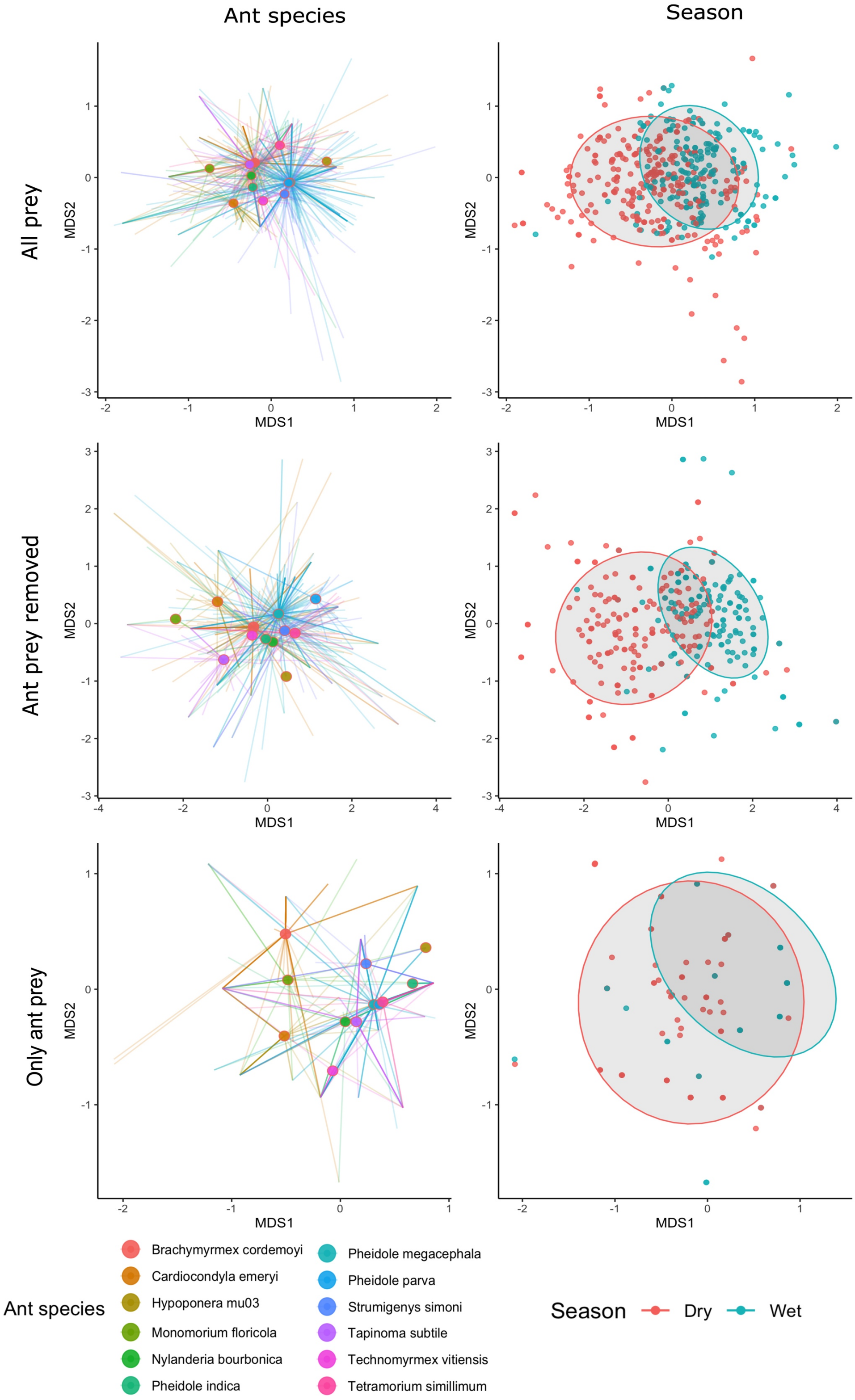


Figure 4.6. Ant community diet composition visualised using non-metric multidimensional scaling (NMDS). On the left plots, colours denote the species of ant, and the large points show the associated centroid (mean co-ordinates); terminal ends of lines represent individual ants and are connected to their associated centroid. On the right plots, points represent individual ants and colours denote the season the sample was collected in with 80% data ellipses. Rows correspond to the three MGLM models run using 'mvabund': top row = all prey, middle row = ant prey removed, bottom row = only ant prey.

Table 4.6. Dietary taxa with at least one significant result (p = ≤0.05) from the univariate species-level generalised linear models (GLMs).

Taxon	Taxonomy (Order: Family)	Model	Significant model terms (LRT, p-value)
Achyranthes aspera	Caryophyllales: Amaranthaceae	Ants as prey removed	Season (LRT = 10.72, p = 0.041)
Boerhavia coccinea	Caryophyllales: Nyctaginaceae	All prey	Ant species (LRT = 25.69, p = 0.032), Season (LRT = 18.41, p = 0.002)
		Ants as prey removed	Ant species (LRT = 28.84, p = 0.006), Season (LRT = 19.98, p = 0.002)
Bourletiella sp.	Collembola: Bourletiellidae	All prey	Season (LRT = 11.85, p = 0.03)
		Ants as prey removed	Season (LRT = 11.28, p = 0.031)
Brachymyrmex cordemoyi	Hymenoptera: Formicidae	All prey	Ant species (LRT = 107.71, p = 0.001), Season (LRT = 24.66, p = 0.001), Habitat (LRT = 21.1, p = 0.012)
		Only ant prey	Ant species (LRT = 164.33, p = 0.001), Season (LRT = 34.77, p = 0.001)
Camponotus maculatus	Hymenoptera: Formicidae	All prey	Ant species (LRT = 161.55, p = 0.001)
		Only ant prey	Ant species (LRT = 161.63, p = 0.001)
Carabidae sp. 1	Coleoptera: Carabidae	Ants as prey removed	Season (LRT = 11.21, p = 0.031)
Cecidomyiidae sp.	Diptera: Cecidomyiidae	All prey	Season (LRT = 16.96, p = 0.003)
		Ants as prey removed	Season (LRT = 17.85, p = 0.003)
Chalcididae sp.	Hymenoptera: Chalcididae	All prey	Ant species (LRT = 45.05, p = 0.001), Season (LRT = 86.33, p = 0.001), Habitat (LRT = 27.87, p = 0.001)
		Ants as prey removed	Ant species (LRT = 52.53, p = 0.001), Season (LRT = 90.38, p = 0.001), Habitat (LRT = 33.89, p = 0.001)
Chironomidae sp. 1	Diptera: Chironomidae	All prey	Season (LRT = 20.68, p = 0.001)
		Ants as prey removed	Season (LRT = 35.56, p = 0.001)
Chironomidae sp. 2	Diptera: Chironomidae	Ants as prey removed	Season (LRT = 18.57, p = 0.003)
Coecobrya tenebricosa	Collembola: Entomobryidae	All prey	Season (LRT = 16.69, p = 0.004)
		Ants as prey removed	Season (LRT = 17.69, p = 0.003)
Crabronidae sp.	Hymenoptera: Crabronidae	All prey	Ant species (LRT = 34.43, p = 0.003)
		Ants as prey removed	Ant species (LRT = 29.08, p = 0.006)
Culex sp.	Diptera: Culicidae	All prey	Season (LRT = 17.73, p = 0.003)
		Ants as prey removed	Season (LRT = 19.33, p = 0.003)
Drosophila sp.	Diptera: Drosophilidae	All prey	Season (LRT = 16.9, p = 0.004)
		Ants as prey removed	Season (LRT = 14.64, p = 0.007)
Monomorium floricola	Hymenoptera: Formicidae	All prey	Ant species (LRT = 71.5, p = 0.001)
Noctuidae sp. 1	Lepidoptera: Noctuidae	All prey	Season (LRT = 22.18, p = 0.001)
		Ants as prey removed	Season (LRT = 21.32, p = 0.001)
Noctuidae sp. 2	Lepidoptera: Noctuidae	All prey	Season (LRT = 12.04, p = 0.028)
		Ants as prey removed	Season (LRT = 13.35, p = 0.012)
Nylanderia bourbonica	Hymenoptera: Formicidae	All prey	Ant species (LRT = 58.4, p = 0.001), Habitat (LRT = 25.99, p = 0.001)
Oligotoma saundersii	Embioptera: Oligotomidae	All prey	Ant species (LRT = 25.85, p = 0.032), Season (LRT = 77.25, p = 0.001)
		Ants as prey removed	Season (LRT = 76.79, p = 0.001)
Pheidole megacephala	Hymenoptera: Formicidae	All prey	Ant species (LRT = 75.19, p = 0.001), Season (LRT = 19.38, p = 0.001)
		Only ant prey	Ant species (LRT = 57.59, p = 0.001), Season (LRT = 27.34, p = 0.001)
Pompilidae sp.	Hymenoptera: Pompilidae	All prey	Season (LRT= 14.92, p = 0.008)
Porcellionidae sp.	Isopoda: Porcellionidae	All prey	Ant species (LRT = 25.79, p = 0.032)
		Ants as prey removed	Ant species (LRT = 23.58, p = 0.036)
Psychodidae sp	Lepidoptera: Psychodidae	All prey	Season (LRT = 17.06, p = 0.003)
		Ants as prey removed	Season (LRT = 23.58, p = 0.036)
Pyralidae sp.	Lepidoptera: Pyralidae	Ants as prey removed	Season (LRT = 12.04, p = 0.023)

Scolopendra abnormis	Chilopoda: Scolopendridae	All prey Ants as prey removed	Season (LRT = 11.82, p = 0.03) Season (LRT = 13.37, p = 0.012)
Staphylinidae sp.	Coleoptera: Staphylinidae	All prey Ants as prey removed	Ant species (LRT = 152.34, p = 0.001), Season (LRT = 29.47, p = 0.001) Ant species (LRT = 145.93, p = 0.001), Season (LRT = 17.09, p = 0.003)
Strumigenys simoni	Hymenoptera: Formicidae	All prey Only ant prey	Ant species (LRT = 35.11, p = 0.002) Ant species (LRT = 42.53, p = 0.001)
Tapinoma melanocephalum	Hymenoptera: Formicidae	All prey Only ant prey	Ant species (LRT = 57.12, p = 0.001), Season (LRT = 27.37, p = 0.001) Ant species (LRT = 53.73, p = 0.001), Season (LRT = 27.34, p = 0.001)
Technomyrmex pallipes	Hymenoptera: Formicidae	Only ant prey	Ant species (LRT = 53.09, p = 0.001)
Tetramorium simillimum	Hymenoptera: Formicidae	Only ant prey	Ant species (LRT = 30.89, p = 0.002)

4.4.5. Ant-ant interactions

For each of the 12 ant consumer species, I calculated the proportion of the diet that consisted of non-ant and ant prey (Figure 4.7), and further compared which ant prey species were consumed by each consumer species (Figure 4.8).

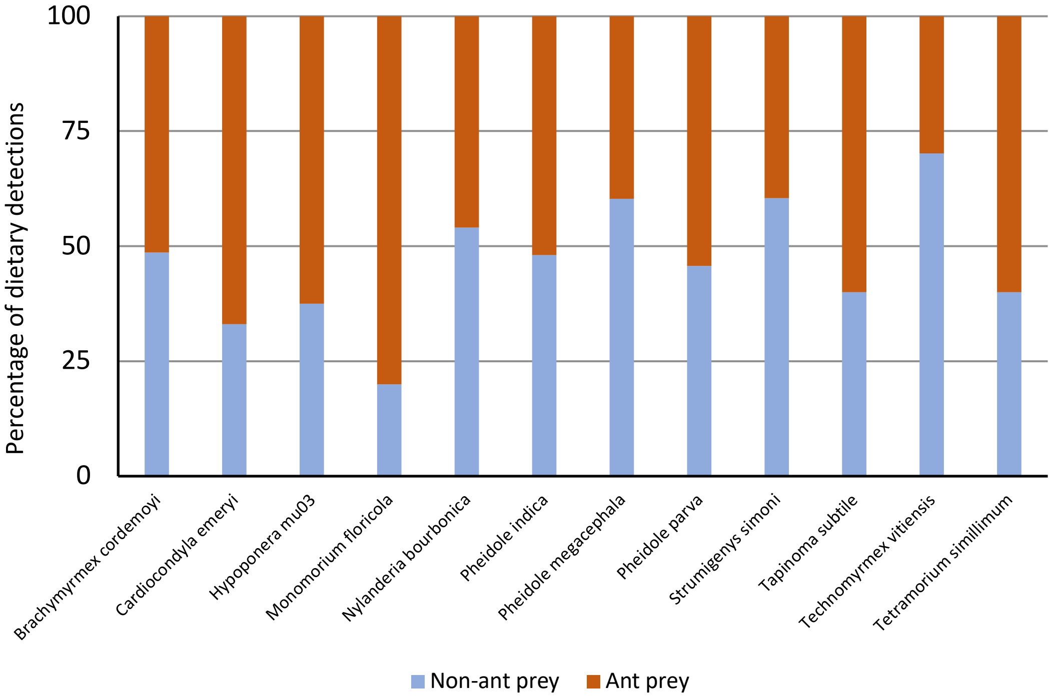


Figure 4.7. Dietary detections divided into non-ant and ant prey for each consumer species.

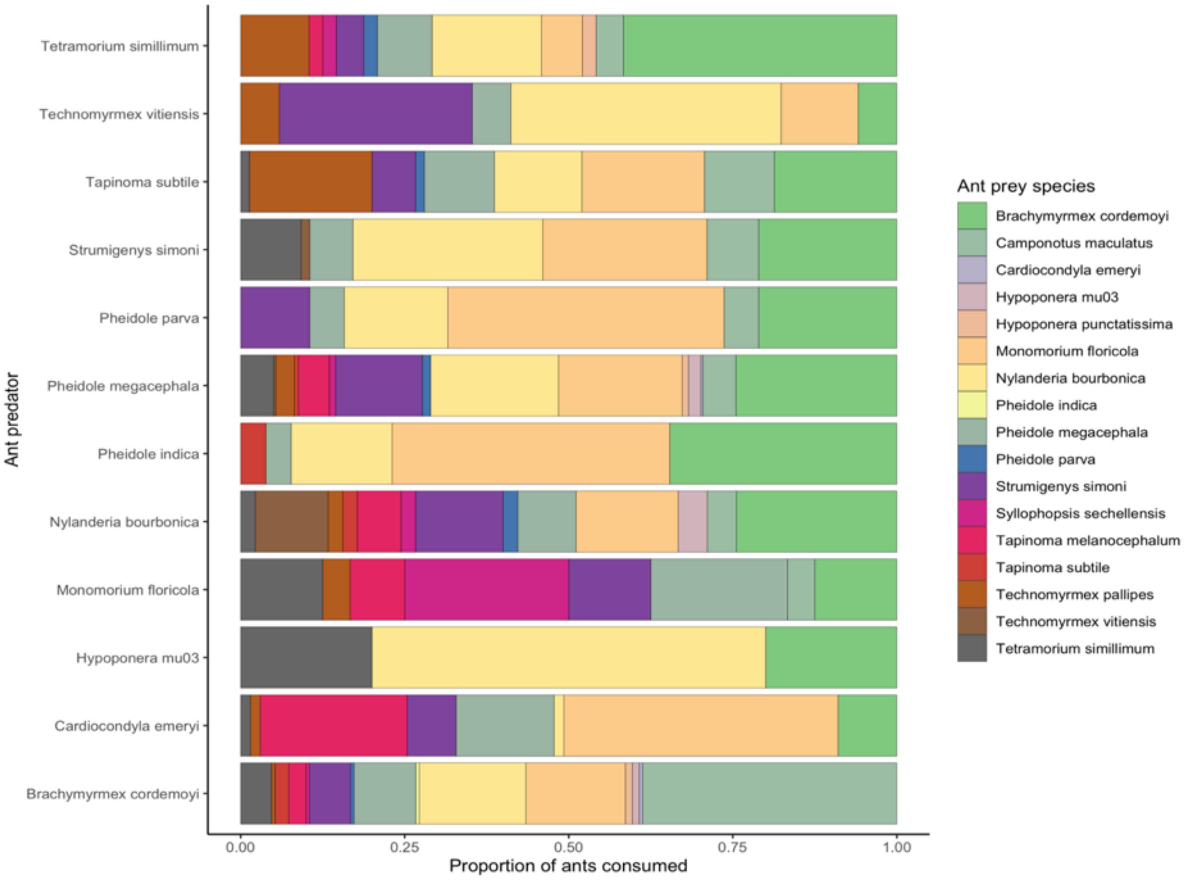


Figure 4.8. Ant-ant trophic interactions represented as a proportion of all ants consumed by each species.

I conducted separate co-occurrence analyses between dry and wet seasons using field samples to show whether ant species co-occurred more or less than expected by chance in each quadrat. The dry season analysis initially compared 66 species combinations across the 69 quadrats, though 56 pairs (84.85 %) were removed because expected co-occurrence was <1. Ten species pairs were therefore analysed, revealing seven random and three negative co-occurrence relationships. All negative co-occurrence relationships existed with *P. megacephala* (Figure 4.9). The wet season analysis initially compared 28 species combinations across the 42 quadrats sampled in the wet season, though 21 pairs (75 %) were removed because expected co-occurrence was <1. Seven species pairs were analysed, all showing random co-occurrence patterns.

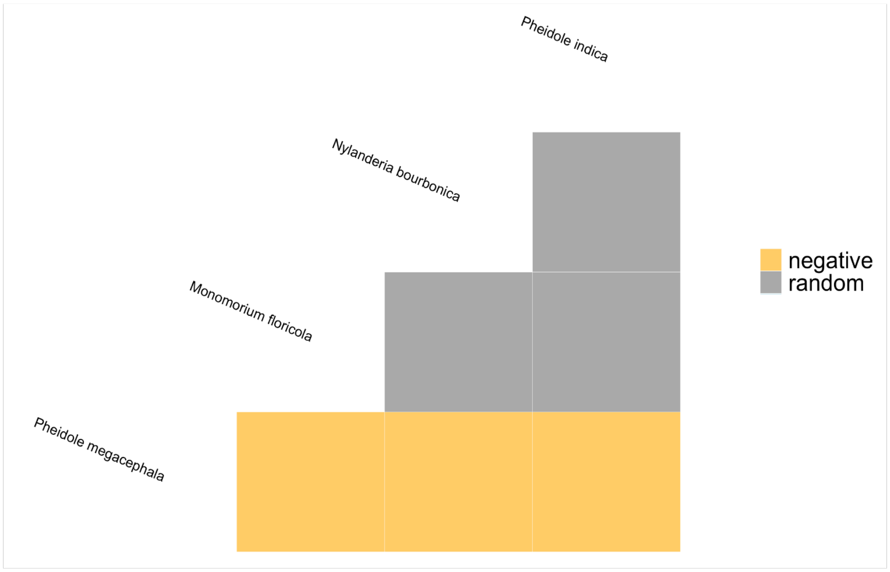


Figure 4.9. Co-occurrence relationships between ant species found in quadrats across Round Island in the dry season. Negative, random, and positive co-occurrence relationships denote presence less than, equal to, or more than expected by chance, respectively. Negative = yellow; random = grey.

4.4.6. Prey choice

Our prey choice analysis showed that ants selected certain prey groups and avoided others (Figure 4.10). All ant predator species showed an aversion to *P. megacephala,* and 6 of the 12 ant predator species avoided Acari. Ant predators positively selected many more prey taxa than they avoided. However, ant species showed some variability in their preference or avoidance of specific prey taxa.

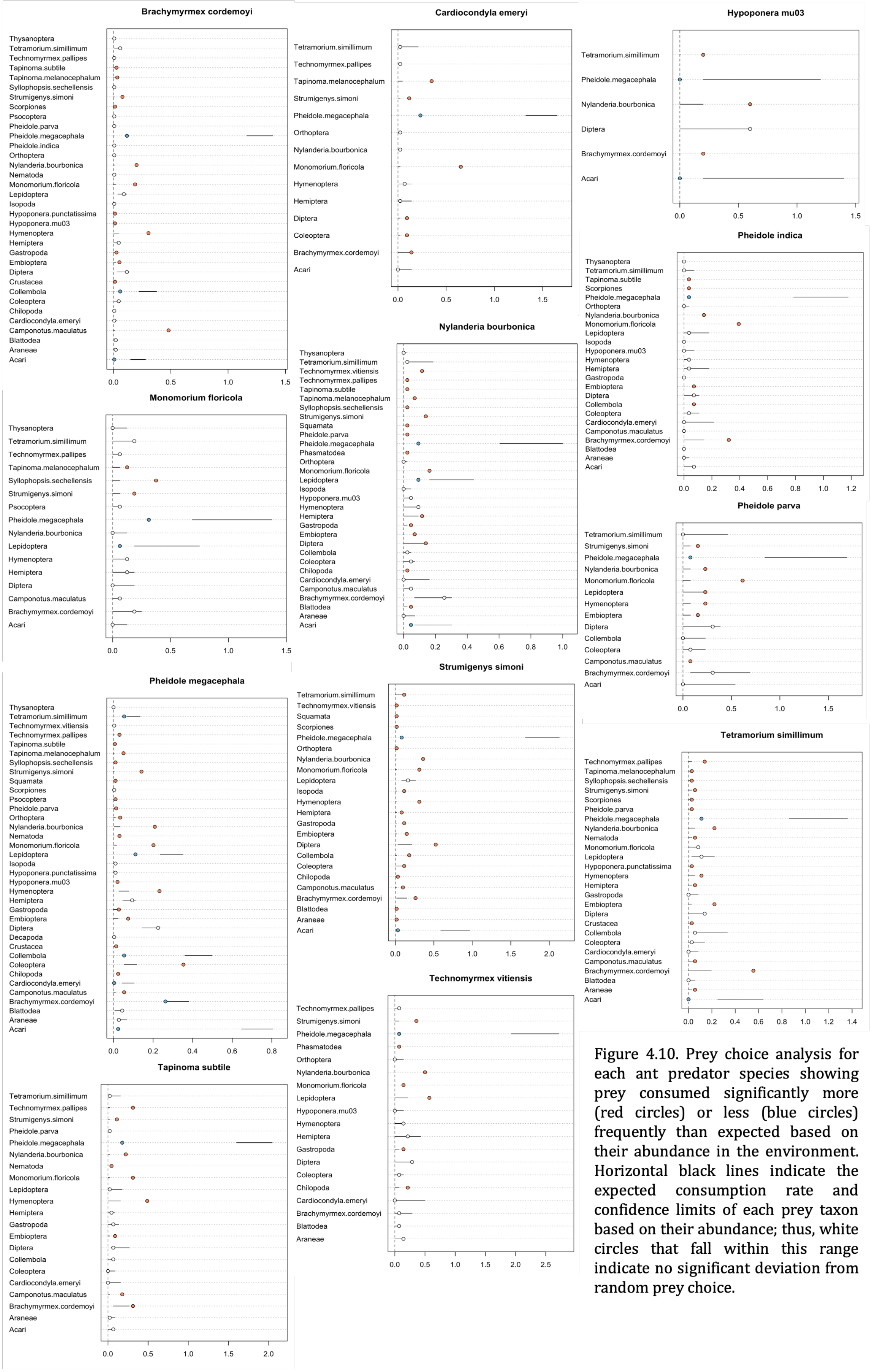


Figure 4.10. Prey choice analysis for each ant predator species showing prey consumed significantly more (red circles) or less (blue circles) frequently than expected based on their abundance in the environment. Horizontal black lines indicate the expected consumption rate and confidence limits of each prey taxon based on their abundance; thus, white circles that fall within this range indicate no significant deviation from random prey choice.

4.5 Discussion

This study is the first to successfully measure the species-level trophic interactions of hundreds of individual ants across multiple species at the community-scale. I were able to sample the majority of ant species found on Round Island and these species probably cumulatively contribute >95 % of total ant abundance. Ants were sampled across the entire landscape of Round Island, with over 1,000 individuals initially screened for dietary data. The resulting data from 755 individual ants provides highly detailed information relating to the trophic ecology of the non-native ant community on Round Island.

4.5.1 Diet characteristics and diversity

Our dietary metabarcoding methodology revealed the trophic interactions of the Round Island ant community with high taxonomic precision, at the equivalent of species-level. I detected a very wide diversity of food items, including invertebrates, vertebrates, and plants. Insects and other terrestrial arthropods made up the majority of dietary detections and this conforms to previous observations that non-native generalist ants will forage for a wide variety of invertebrates for nutrition, which is assumed to significantly contribute to their successful establishment globally (Holway et al. 2002).

The diets of ant species on Round Island vary by up to four-fold in diversity, as quantified by Hill-richness. The estimated dietary diversity of *Tapinoma subtile* is approximately a quarter of that of *Nylanderia bourbonica*, for example. Though all ants broadly showed a "generalist" dietary profile, consuming several taxonomic groups of invertebrates, plants, and/or vertebrates, some ant species showed a narrower base of dietary taxa than others. The most abundant and dominant ant on Round Island, *P. megacephala*, did not show a very diverse diet relative to other ant species. Instead, *P. megacephala* showed a dietary profile with modest, slightly above-average, diversity. This may be because *P. megacephala* does not need to have a very diverse diet profile, instead relying on its numerical and behavioural dominance to monopolise the most valuable food sources, rather than acquiring nutrition from diverse but sub-optimal sources, as many generalists do in the field (Symondson et al. 2002). Similarly, *S. simoni* showed an above-average dietary diversity, though this is more surprising given that *Strumigenys* are generally considered to be specialist predators (Hölldobler and Wilson 1990). It is possible that *S. simoni* does not show a particularly "specialist" dietary profile because it lacks the trap-jaws characteristic of some species in the genus that are assumed to be adapted for catching Collembola and other small invertebrates. This dietary diversification may have enabled its extremely widespread distribution and status as a "tramp" ant (Holway et al. 2002).

Ant species may be able to modulate their diets based on the community of other ants they coexist with. Ants that are behaviourally dominant in one community may not be dominant in a community with a different composition, for example, and this may ultimately affect the food resources available to them. Dietary breadth could be one factor that ant species are able to modulate based on the presence of competitors. This could be termed the "dominance-release hypothesis", whereby sub-dominant species are able to achieve dominant-like command of food resources in the absence of "true" dominant species they may compete with. However, predicting how diet may change based on this hypothesis is challenging because the dietary diversity could theoretically increase or decrease in response to the presence of more dominant species. For example, a sub-dominant ant species may diversify its diet in the presence of a true dominant to incorporate sub-optimal sources of nutrition. In contrast, its diet breadth might instead narrow because of fewer available resources, now primarily available to the true dominant. In the former case, the carrying capacity for the species may stay the same or slightly decrease because the resources are sub-optimal. In the latter case, the carrying capacity for the species would drop because of fewer available resources.

Non-native ants may be particularly good at dietary modulation and this may explain how varied suites of non-native ants are able to coexist with one another in different locations globally (Balzani et al. 2021). Nevertheless, some ant species appear unable to coexist because of heightened interspecific behavioural aggression (Wilson 1976; Parr and Gibb 2009) or, for example, an inability to resist nest raids (Zee and Holway 2006; Dejean et al. 2008).

This study finds that invasive ants are both highly abundant and that dietary diversity is high. It may be the large number of interactions between invasive ants and the wider community that may threaten certain native species, rather than a disproportionate effect of invasive ants. In other words, the ecological consequences of ant invasion are density-dependent, as has been tested directly across a range of ant species in different locations (Cooling et al. 2015). However, the shape (linear vs. non-linear), direction (positive vs. negative), and scale of these impacts may have been at their highest when invasive ants were initially invading Round Island (Bradley et al. 2019). Other invaded systems have empirically tested this, showing that the initial stage of ant invasion leads to the highest impact (Heller et al. 2008). This is probably the initial stage of invasion sees the loss of most species which are unable to coexist with invasive ants. Unfortunately, Round Island has minimal historical ecological data allowing us to explore this question further.

4.5.2 Consumption of non-native and native species

The Round Island ant community most frequently consumed non-native species, accounting for 59 % of all dietary detections. However, this majority was because of very frequent ant-ant predation. Once these detections were removed, non-native and native taxa were consumed at approximately the same frequency (sum of non-native categories = 28.09 %; sum of native categories = 28.67 %). Species-level identifications of Round Island invertebrates were not possible from community samples. This is because virtually no identification resources exist and the invertebrate community is poorly described taxonomically. Therefore, whether native and non-native species were consumed as expected based on their availability, or whether they were consumed disproportionately, cannot be verified from this analysis.

Two native species listed on the IUCN Red List were consumed: the Serpent Island centipede, *Scolopendra abnormis*, which is listed as Vulnerable (Pearce-Kelly 1996), and Bojer's skink, *Gongylomorphus bojerii*, which is listed as Critically Endangered (Cole and Payne 2015; Cole and Payne 2022). Of Round Island's invertebrate fauna, only *S. abnormis* has received a Red List assessment to estimate extinction risk. Several other native or endemic invertebrate species are likely to be considered threatened if Red List assessments are conducted. For example, the Round Island stick insect, *Apterograeffea marshallae*, is endemic and may be considered threatened with extinction, especially given that it is also consumed by non-native ants. Moreover, it is possible that many of the detections listed in the "unknown" category, comprising 43.24 % of detections once ant-ant interactions are removed are from unbarcoded species native to Round Island.

Even infrequent predation of a native species by a non-native predator may have an impact on the population size of the native species. Given the extremely high density of non-native ants on Round Island, especially *P. megacephala*, it may be that predation events are rare relative to ant population size, but significant in relation to the population size of their native prey. For example, *S. abnormis* centipedes were consumed by 14 of 755 ants in our study, but this may nevertheless have an impact on centipede populations when scaled up across Round Island given the presumed millions of ants present on the island. It is, however, impossible to estimate or measure population-level impacts of predation based on these metabarcoding data.

Greater knowledge of the extinction risk and population health of the Round Island native invertebrate community is urgently needed to quantify and address the potential impacts of non-native ants. Given the importance of insects and other terrestrial invertebrates to the functioning of ecosystems, threats to the native invertebrate community likely have significant consequences to the rest of the unique Round Island ecosystem.

4.5.3 Drivers of ant diet and ant-ant interactions

Diets varied most notably between ant species and seasons, though caste and habitat also significantly affected diet. Moreover, the contribution of season, caste, and habitat towards dietary variation significantly differed between ant species. This was seen in datasets including all taxa and when ant prey were removed. In contrast, when ant prey were considered exclusively, diets differed only between ant species and season. These results suggest that different ant species have different diets that are mediated most strongly by season. The invertebrate community composition varied between wet and dry seasons (Chapter 4 Appendix S4.3, Figure S4.2), and this explains the strong seasonal variation in diet across ant species.

Different ant species had distinct diets, though the degree of difference between any two species varied considerably, and there was a high degree of dietary overlap between several species. Nevertheless, even in a community consisting almost entirely of non-native ants, which have not shared habitats or distributions over long evolutionary timescales, I see that ant species have somewhat dissimilar diets. These results are the first evidence to date measuring species-level trophic interactions across an ant community at the level of individual ants, and they suggest that moderate dietary niche partitioning is taking place between ant species. Ant species may be modulating their diets in the context of competitors to avoid competition for food resources. Alternatively, differences in diet may not show high levels of dietary modulation to avoid competition, but rather a diet range intrinsic to each species. The ability of these ants to coexist on Round Island may be a combination of these explanations, whereby ant species possess an intrinsic but plastic diet that can be modulated based on the community context. The high level of dietary generalism observed in non-native ants here and elsewhere supports this hypothesis (Holway et al. 2002; Tillberg et al. 2007; Probert et al. 2021). Other aspects of the niches of these ants may minimise observed differences in diet composition whilst still reducing competition for food resources. For example, two different ant species may respectively forage for the same prey taxa during the day and night, thus having the same dietary composition but reducing the level of direct competition at food resources. However, this scenario may still lead to exploitation competition even if ant individuals from these two species never meet. See section 4.5.5 for a further discussion of these dietary patterns.

Ants were consumed very frequently by other ants, comprising about 50 % of total dietary detections. Ant-ant predation is thought to be an important factor in the ecology of some ant species (Zee and Holway 2006; Dejean et al. 2008; Lach et al. 2010; Dejean et al. 2014), though the results presented here show that almost all ants studied consume other ants at a relatively high frequency. These unexpected results suggest that ant-ant predation is particularly important in communities of non-native ants, which are typically ecologically important generalist species.

However, it is not possible to confirm whether this holds for primarily native communities of ants. The frequency at which ants were consumed varied between species, ranging from 29.8 % of detections in *T. vitiensis* to 80 % in *M. floricola*. Different ant species consumed a different composition of ant prey, though several similarities are common to all ants studied. For example, though *P. megacephala* is the most abundant ant on Round Island, it was not eaten especially frequently by any ant. In contrast, the rate at which *N. bourbonica* was consumed varied significantly between ant species, with some species avoiding it entirely whilst it made up most of the ant component of the diet for other species.

It is not possible to reliably identify from these data the mode by which ants were consumed, e.g., nest raiding, opportunistic predation, scavenging etc. However, known natural history traits of several species suggest which modes may be most likely. For example, *C. emeryi* is a subordinate inconspicuous ant species able to coexist with dominant species such as *Solenopsis geminata* and *Pheidole dentata* and produces an effective repellent pheromone when another species approaches it (Creighton and Snelling 1974). Moreover, *C. emeryi* have small colonies of dozens to a few hundred workers and are probably unable to raid the nests of behaviourally or numerically dominant ants (Heinze et al. 2006). On Round Island, *C. emeryi* may take advantage of the very high abundance of other ants and scavenge dead individuals or exploit post-battle nest raids conducted by other species.

I observed direct nest raids by *P. megacephala*, *N. bourbonica*, and *B. cordemoyi* on Round Island. Though these species did not consume other ants at a particularly high level, they are the three most widely distributed and conspicuous ant species on Round Island. Nest raiding success is well known in *P. megacephala* (Dejean et al. 2008) and it may partly explain its successful establishment globally. However, nest raiding may confer significant nutritional and ecological rewards as well as costs for a colony. For instance, though raids can lead to a glut of high energy ant larvae for the raiding colony, raiders may also lose a much greater number of workers during a raid than if those workers were instead foraging on non-ant prey. Raiding a competing species' nest also reduces their abundance, potentially reducing subsequent conflict at food resources. In *P. megacephala*, I see approximately 40 % of total dietary detections are from consuming ants, though this is one of the lowest rates in our study. Though other ants are highly abundant on Round Island, consuming non-ant prey and plant food may be more efficient than highly frequent nest raids for *P. megacephala*. More generally, ants conform to Lanchester's laws of combat (Franks and Partridge 1993; McGlynn 2000), whereby numerical advantages, as well as size and combat prowess, determine the outcome of conflicts. Infrequent facultative nest raiding by the most ecologically dominant species might amplify the benefits of nest raiding (access to larvae of other ant species) whilst minimising the costs (loss of workers due to combat). If raids were conducted very frequently, the cumulative loss of workers may reach a threshold at which further raids become less efficient, as well as imperilling colony defence. This may partly explain why the three most abundant ant species on Round Island consume ants at a below-average rate despite conducting nest raids.

The behavioural dominance, combat prowess, and numerical advantages of *P. megacephala* affords it ecological dominance on Round Island, and similar patterns have been observed elsewhere (Heterick 1997a; Hoffmann 1998; Hoffmann et al. 1999; Wetterer 2007; Dejean et al. 2008). Our co-occurrence analysis shows that *P. indica, M. floricola*, and *N. bourbonica* all co-occur with *P. megacephala* in quadrats significantly less than expected by chance. These co-occurrence patterns may arise because of a mismatch in habitat/nesting preferences. Alternatively, it may be that these species are unable to coexist with *P. megacephala* due to competitive dynamics and/or nest raiding. It is not possible to confidently draw conclusions from co-occurrence analyses alone (Blanchet et al. 2020), though our observations of nest raiding, dietary analyses, and prey choice analyses corroborate the latter explanation.

4.5.4 Prey choice

Ants showed clear density independent preferences for certain prey taxa, though the taxa which species preferred broadly varied. However, there are a few commonalities. For example, webspinners (Embioptera) are preferred disproportionately by almost all consumer species where they are found. Likewise, centipedes (Chilopoda) are preferred where present. These are both primarily ground-dwelling organisms, and thus vulnerable to attack by ants. Lepidoptera are consumed approximately proportionately to their abundance, though some species weakly prefer or avoid them. Consumption of Lepidoptera and other volant insects on Round Island may be related to developmental patterns of each species, whereby ants have access to larvae but may not have easy access to adults. However, there may be many dead volant insects towards the end of the season that ants are able to easily forage.

Some taxa were also avoided by ants. The most consistently avoided taxon was *P. megacephala*, which was avoided by all other species. Because of the combative abilities and numerical dominance of *P. megacephala*, it is unlikely to represent a cost-effective nutritional reward for other ant species. Mites (Acari) were also eaten less frequently than expected based on their abundance. However, this may be because of the massive abundances in pitfall traps and their very small size rather than ants actively avoiding them. Mites are often found in locations inaccessible to the ants of Round Island, hiding in minute crevices or between particles of soil. These results suggest that ants do consume mites, but perhaps opportunistically on larger individuals. A final commonality is that ants are generally consumed more frequently than expected based on their abundance. However, as above, this pattern is reversed for *P. megacephala*.

4.5.5 Limitations

Limitations of metabarcoding generally and those relating to dietary analyses of omnivores are outlined in Chapter 3 and apply here (Nielsen et al. 2018; Alberdi et al. 2019; Lamb et al. 2019; Tercel et al. 2021; Cuff et al. 2022; Tercel and Cuff 2022; Tercel et al. 2022). However, there are several additional limitations specific to this study.

Firstly, it was not possible to identify how taxa were consumed. For certain taxa in the study, this has ecological consequences, e.g., if other ants were consumed via nest raiding or scavenging. The mode of consumption may have important implications for the community dynamics of ants on Round Island, and perhaps ants more generally. Considering ants were consumed at such high frequencies, it may point to non-native ants being particularly adapted to consuming other ants. More empirical studies exploring this elsewhere are required to confirm whether non-native ants consume other ants so commonly and by what means.

There were very uneven sample sizes between ant species. Though diversity analyses attempt to correct for this, a greater sample size may have nevertheless revealed different patterns of dietary diversity for those with <50 samples. This is because ant species with only few samples were typically only found in a few colonies and quadrats. Ants collected in the same quadrats are likely to have similar diets regardless of species or other factors, ultimately confounding the interpretation of species-level analyses, because ants will only be able to forage from what is available in the immediate vicinity of their nests. Thus, it is possible the observed and extrapolated dietary diversity of these species may be affected by the low habitat, and presumably food resource, diversity where they were found. This is also an alternative explanation for the clustering we see for species with a low sample size. Rather than differences because of how a species forages, observed dietary differences between ant species sampled from only a few quadrats may simply be due to the availability of food items.

Finally, the invertebrate community samples for each quadrat were originally planned to be collected as pitfall traps and suction samples. However, due to a cyclone and the coronavirus pandemic, the suction samples were discarded. Incorporating invertebrates collected using a different sampling method may have shown different results in the prey choice analysis. This also raises the broader point that different sampling methods are taxonomically biased and may not measure invertebrate abundance in the same way. For example, pitfall traps are generally considered to measure the activity density of ground-dwelling animals, whilst suction samples are snapshots of the abundance of invertebrates on a range of different substrate types, and they therefore collect some taxa which are not common between the two methods. For highly generalist taxa like non-native ants, using multiple sampling methods to capture and represent the prey available to them in the environment increases the robustness of the analyses that rely on those data. Volant or arboreal taxa that may not be particularly active on the ground, but may have been collected in suction samples, might therefore be under-represented in our prey choice analysis and subsequently shown to be "preferred" by ants. These taxa may or may not be preferred by ants, but rather their actual abundance may be underestimated.

4.5.6 Conclusions

This study identified the trophic interactions of the majority of species of ants on Round Island. This was at the equivalent of species level, revealing both the animals and plants consumed. I see that these non-native ants are highly generalist, consuming a wide diversity of plant and animal taxa, though the dietary diversity varies between species. Some species-specific results were surprising, where presumed "specialist" species show high dietary diversity (*S. simoni*) and several "generalist" species show low dietary diversity (*C. emeryi*, *T. subtile*). It is difficult to predict dietary diversity based on abundance or level of assumed behavioural dominance; studies explicitly linking dominance to dietary diversity in different contexts might help reveal these patterns more lucidly. The main drivers of the diet appear to be intrinsic to each species, i.e., diets differ primarily between ant species, whilst seasonality affects the food resources available to ants and thus strongly affects dietary composition. Caste and habitat also affect the diet, though the scale of this varies between ant species. This suggests that these ant species generally have an intrinsic but plastic diet that can be modified based on the ecological context. On Round Island, perhaps ants are taking advantage of the very high abundances of other ants; they are nevertheless disproportionately consuming them, which is corroborated by our prey choice models. It may be that non-native ants tend to consume other ants frequently regardless of the community composition. Further studies in varied locations with different communities of ants will help to support this hypothesis. Ant diet may also vary based on the presence of other ants in the immediate area whereby behaviourally or numerically dominant competitors force a species to change its diet. Predicting this change, however, is challenging and may be species- and context-dependent.

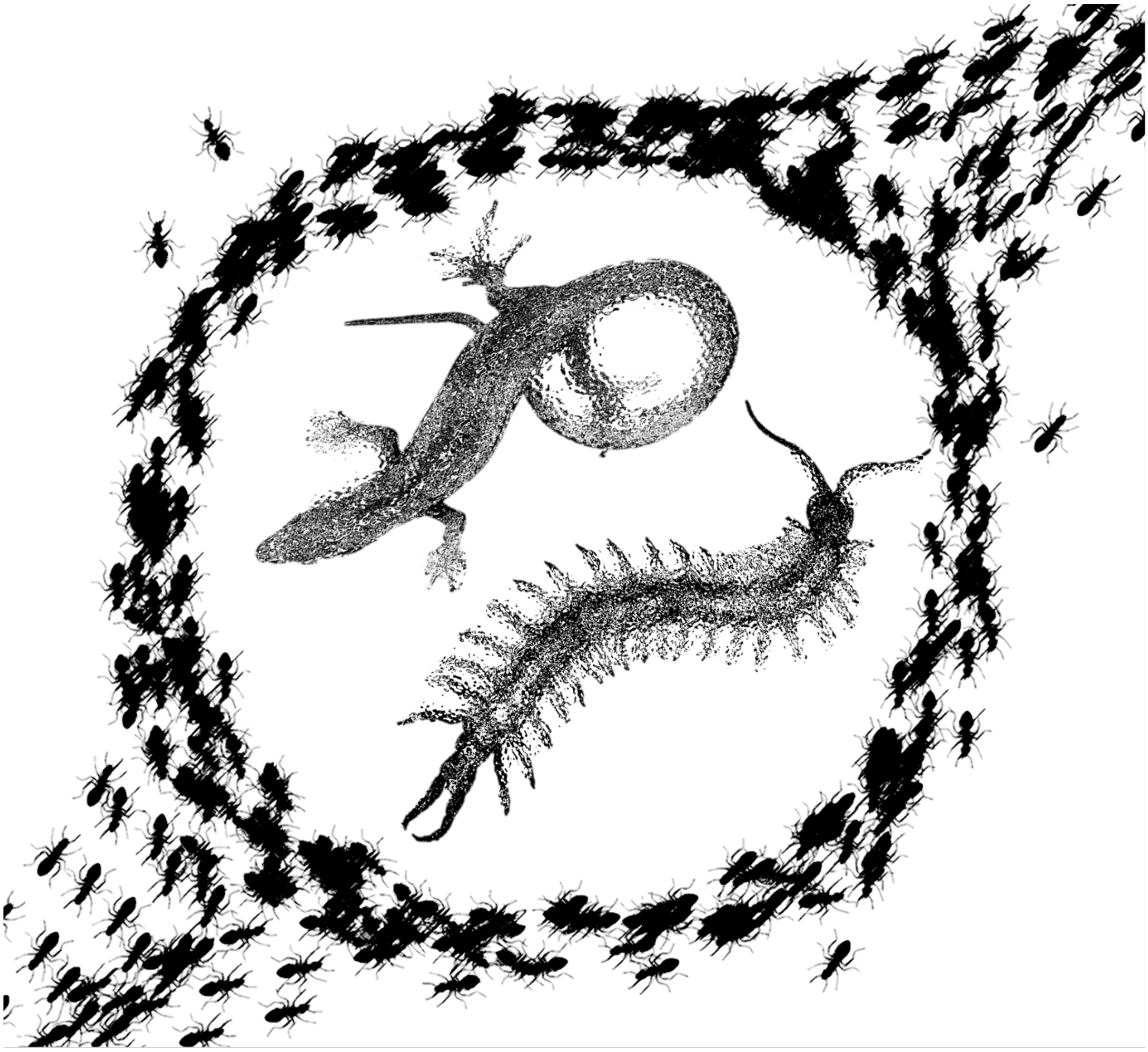
Across our co-occurrence and prey choice analyses I see that other ants are avoiding *P. megacephala*. Based on the wealth of literature examining the behaviour of this species, it is very likely that other ants avoid *P. megacephala* because of its ecological dominance. Indeed, the vast abundance of ants on Round Island consists mostly of *P. megacephala*, comprising approximately 90% of all ant individuals. Given its abundance, behavioural aggression at food resources, and successful nest raiding abilities, *P. megacephala* probably significantly affects the population dynamics of the rest of the ant community on Round Island. Despite this, I see *P. megacephala* consume other ants at a low level relative to other ant species. Instead, it seems to rely on non-ant prey. This is a major cause for concern for the fragile native Round Island invertebrate community given the sheer abundance of *P. megacephala*.

This study is one of the most comprehensive dietary analyses conducted on a community of generalist omnivore species, yet it raises many more questions than it answers. Though I see broad dietary diversity patterns, prey choice, ant-ant interactions, and reliance on native vs. non-native food resources, replicate studies in other locations globally are required to confirm some of the tentative conclusions and hypotheses presented based on the current results. Most notably, determining the mode by which ants consume other ants, and the exact roles that dominance and community dynamics play into diet are questions left unanswered. I present several points to incorporate into future studies that would help to reveal the dietary dynamics of ant communities more fully:

1) Explicitly linking dominance with diet. For example, conducting extensive baiting trials to help build a dominance hierarchy would present powerful evidence that could be used to explore the "dominance-release" hypothesis, as well as whether diet and dominance are correlated.
2) Behavioural observations of mode of consumption. Providing evidence of nest raiding, for example, by conducting behavioural observations at nest entrances to determine the probable modes of consumption in ant-ant interactions.
3) Utilise multiple sampling methods to represent the wider invertebrate community, rather than relying on one method, e.g., pitfall traps. This will improve the robustness of prey choice analyses.

A shaded spot under a *Pandanus vandermeschii* tree on the path leading to the summit. This is a prime location to find Serpent Island centipedes.

Chapter 5: Has a Shadow Fallen over Paradise? Ant Invasion and the Trophic Ecology of Native Centipedes and Skinks



5.1 Abstract

Competition between non-native and native species is thought to be a key driver of native diversity declines post-invasion, though evidence for this in animal communities is minimal. Round Island is home to several consumer species that may be competing with the hyper-abundant non-native ants for food resources. It is also not known to what degree there may be cross-predation between some of these consumers. Here I present a comparative dietary analysis between Telfair's skink, *Leiolopisma telfairii*, the Serpent Island centipede, *Scolopendra abnormis*, and the non-native ant community to determine to what degree the consumers are preying upon and competing with one another. Results from 73 skinks, 43 centipedes, and 383 ants suggest that both skinks and centipedes consume ants frequently, though this does not present a significant top-down pressure on ant population size, and the mode by which the consumers eat ants is unknown. Our results, in contrast, show non-native ants consuming centipedes. Once this is scaled-up to the population size of the ants over Round Island, this may have significant implications for the endemic centipede population. Moreover, our results suggest centipedes nest in areas of low ant activity, where only four of 43 centipedes were found in close proximity to an ant nest or foraging trail. This is contrasted with our random sampling of ants, where all sample sites contained at least one ant species. Furthermore, ants are consuming 100 % of the dietary taxa that centipedes consume, and thus may be competing with them for food resources. Our results therefore suggest that non-native ants may be affecting the wider community through top-down pressures as hyper-abundant generalist consumers, and through predation and competition with native invertebrates.

5.2 Introduction

The ways in which non-native species affect colonised ecosystems are numerous and, though non-native species are typically viewed negatively (Gurevitch and Padilla 2004; Simberloff et al. 2013), their effects on the wider native ecosystem can be positive or negative in different scenarios and from different perspectives. Non-native species may, for example, consume native species to the point of extinction (Doherty et al. 2016), participate in functional roles such as pollination (Baldock et al. 2015), or provide valuable resources for native species (Schlaepfer et al. 2011; Ando et al. 2013; Tercel et al. 2022). There may also be indirect effects cascading across trophic levels with both positive (Schlaepfer et al. 2011; Russo et al. 2014) and negative (O'Dowd et al. 2003; Davis et al. 2010) outcomes for native species. The trophic interactions of non-native species, and how they fit into food-webs, can partly describe how these impacts come about. Non-native species may, for instance, compete with native species for food (Thomas and Holway 2005), be consumed by native species (Parker and Hay 2005; Tercel et al. 2022), or prey upon native species (Clavero and Garcia-Berthou 2005; Ware et al. 2009; Lach et al. 2016), and any combination of these. The scale of these effects depends on the interacting species, their relative trophic positions, and the abundance of the non-native species (Bradley et al. 2019). Identifying the trophic ecology of non-native species is therefore vital in understanding how they fit into and modify colonised ecosystems.

Competition between non-native and native species for food resources is thought to be a key driver of native diversity declines (Gurevitch and Padilla 2004). Despite this, there are few examples of complete extinction of a native species due to exploitation competition with an invasive species (Gurevitch and Padilla 2004), i.e., when one consumer reduces the fitness of another consumer because it is better able to exploit a resource. One possible explanation for this is that native species use some resources which are not accessed by non-native invaders. Most non-native species are typically generalists and the chance they are competitively dominant on every resource consumed by the native species is quite low. Exclusive trophic links between food resources and native consumers may provide a "niche refuge", allowing native species to co-exist with ecologically dominant non-native species (David et al. 2017). Therefore, a native species may have a severely reduced dietary range through exploitation competition with a non-native species, and may decrease dramatically in abundance, but is still able to survive. Specialist native species that rely on one or a few resources are therefore predicted to be at greater risk of extinction through exploitation competition than generalists. Interference competition, the direct aggression of one consumer over another to obtain a greater share of resources, is far more likely to lead to extinction than exploitation competition because attack-and-defence mechanisms are important during resource use, similar to direct predation (Holway et al. 2002; David et al. 2017; Bradley et al. 2019).

Despite the assumed importance of competition in the context of biological invasions, relatively few studies identify the species-level trophic interactions of potentially competing native and non-native species. This probably reflects the difficulty in empirically identifying diets, rather than the rarity of competition between native and non-native species for food resources. Historically, gathering species-level trophic interaction data is fraught with numerous methodological issues. For example, morphological methods to determine the diet of consumers are taxonomically coarse, they fail to detect soft-bodied prey, and cannot identify the diets of the majority of small consumers and fluid-feeding organisms, i.e., most life on earth (Symondson 2002; Pompanon et al. 2012). Nevertheless, several studies have successfully identified species-level trophic interactions of native and non-native species and have calculated degrees of dietary overlap, e.g., by using molecular tools (McCrary et al. 2007; Gebremedhin et al. 2016; Minder et al. 2021; Moorhouse-Gann et al. 2022). Results vary between little overlap to almost complete overlap and are highly context dependent.

Round Island is home to several native consumers that may be competing with the hyper-abundant non-native ants for food resources. Some of these are listed as threatened on the IUCN Red List, such as the Serpent Island centipede, *Scolopendra abnormis*, and Telfair's skink, *Leiolopisma telfairii,* among others. Many of these native consumers are, nevertheless, highly abundant on Round Island, the last sizeable area of native Mauritian lowland palm forest in the Mascarene archipelago. Both *S. abnormis* and *L. telfairii* are thought to be integral to the functioning of the Round Island ecosystem (Cheke and Hume 2008; Lewis et al. 2010; Cole et al. 2018c). The centipedes are the dominant native invertebrate predators on Round Island, whilst Telfair's skinks are highly abundant large omnivorous predators, preyed on only by the keel-scaled boa, *Casarea dussumieri* (Cole et al. 2018b), and Günther's gecko, *Phelsuma guentheri* (Cole et al. 2018d). However, it is not known to what extent *S. abnormis* or *L. telfairii* are affected by the hyper-abundant non-native ants. Comparing their diets to one another and to that of the non-native ant community will build a bank of evidence describing how non-native ants may be affecting the native consumers of Round Island. Competitive dynamics and predation between consumers may play important roles, for example.

The aim of this Chapter is to identify and compare the diets of the native centipedes and skinks with that of the non-native ants using dietary metabarcoding. In doing so I hope to reveal the effects of the non-native ants by answering two main questions: 1) Do ants, centipedes, and skinks prey upon one another? 2) Do ants, centipedes, and skinks compete for food? By answering these questions I can begin to reveal the role of the non-native ant community on Round Island and to what extent they may be adversely affecting threatened native consumers.

5.3 Methods

5.3.1 Site and species description

See Chapter 3 (section 3.3.1) for a description of Round Island's flora, vertebrate fauna, geology, and history, and Chapter 4 (section 4.3.1) for a description of Round Island's invertebrate fauna. Chapters 3 and 4 outline the trophic ecology of Telfair's skinks and the non-native ant community, respectively, on Round Island.

The Serpent Island centipede, *Scolopendra abnormis*, is a relatively large (~13 cm maximum length) abundant predator found over the entirety of Round Island, though is found in greater densities in thickets of *Pandanus vandermeeschii* and *Latania loddigesii* trees. It typically nests under rocks or other debris in the root network of these trees and are therefore found more regularly here than in exposed rock stacks. Individuals are not commonly found in areas with good soil cover and dense herbaceous vegetation (Lewis et al. 2010), though they are present under trees and in rock stacks in these areas, including in regeneration zones. They are nocturnal hunters and have been observed consuming cockroaches on Round Island, as well as scavenging on dead terns on Serpent Island (Lewis et al. 2010). Due to their large size and high abundance, *S. abnormis* may directly and indirectly influence many other species in the Round Island ecosystem.

The mating season of *S. abnormis* takes place in the wet season. Females nurse clutches of eggs in their nests (Figure 5.1) from at least late-February to late-March (recorded for the first time during this study), though the breeding season may extend from December through to April. No females tending eggs have been seen in the dry season. Centipedes of similar size do not seem to show immediate aggression to one another upon meeting (Lewis et al. 2010) and several individuals can be found nesting in close proximity, though cannibalism of similarly sized adult centipedes has been seen on Serpent Island (Nik Cole, pers. obvs.).

There are several predators of *S. abnormis* on Round Island. Of the island's lizards, Telfair's skinks will readily attack centipedes should they be revealed in the Round Island field station, for example, and adult Bojer's skinks have been seen opportunistically biting off legs of active centipede individuals if they are uncovered in the day. However, *S. abnormis* is nocturnal, whilst the skinks are primarily diurnal (though they may be active during moonlit nights), and it is therefore unlikely that Telfair's skinks or Bojer's skinks present a significant predation pressure on *S. abnormis*. Likewise, Günther's gecko, a large arboreal species, is unlikely to frequently prey upon the ground-dwelling centipedes, though have been seen on the ground and are active during both day and night (Carpenter et al. 2003; Cole et al. 2018c; Roesch et al. 2021). Occasional predation of *S. abnormis* by *P. guentheri* is therefore probable. The ornate day gecko, *Phelsuma ornata,* is unlikely to present a major threat to adult *S. abnormis* individuals because it is probably too small to take large individuals. Nevertheless, *P. ornata* has been seen to occasionally feed on smaller centipedes and is active on rocks during both day and night (Cole and Harris 2011). It may present a predation pressure on juvenile *S. abnormis* centipedes. Durrell's night gecko, *Nactus durrellorum*, a nocturnal predator growing to an average length of 9 cm (Cole and Jones 2018), is hypothesised to prey upon juvenile centipedes but has never been recorded doing so. It is therefore unlikely *N. durrellorum* presents a predation pressure for *S. abnormis centipedes.*

Figure 5.1. A female Serpent Island centipede, *Scolopendra abnormis*, found tending eggs beneath a rock under a *Pandanus vandermeeschii* tree. Photographed in the wet season, early March, 2020.

As seen in Chapter 4, non-native ants consume *S. abnormis* at a relatively low frequency, though the effect this has on the population is uncertain from metabarcoding data. It is possible that the ants, given their extremely high abundance, do cause problems for the Round Island centipede population despite infrequent consumption.

5.3.2 Sample collection and preparation for dietary metabarcoding

See Chapters 3 and 4 describing sample collection for skinks and ants, respectively. After DNA extraction, 1,035 ants and 80 skink faecal samples were taken forward for sequencing, respectively.

Centipedes were collected by searching in soil, under rocks, and in leaf litter. It quickly became apparent that individuals were far more abundant under rocks beneath *P. vandermeeschii* and *L. loddigesii* trees and, due to a limited timeframe to conduct fieldwork, sample collection focussed around the base of these tree species. Once a centipede was found, it was apprehended using forceps and transferred into a collection tube. Centipedes were killed by freezing and stored in 100 % ethanol at -25 °C until they could be transferred to -80 °C at Cardiff University. A total of 43 centipedes were taken forward for dietary metabarcoding, 27 from the dry season and 16 from the wet season (Figure 5.2). To determine if ants are possibly involved in interference competition with centipedes, the surrounding area was also searched for nearby ant foraging trails within an approximately 5 m radius around the point a centipede was found, recording both the ant species and distance from the centipede nest.

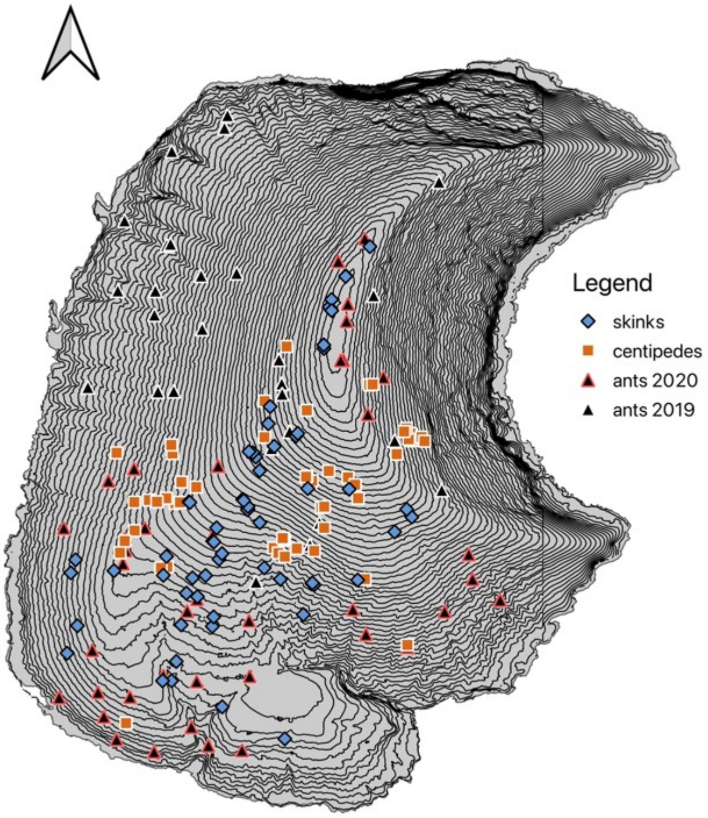


Figure 5.2. Sampling locations on Round Island for ant quadrats (triangles), centipedes (squares), and skinks (diamonds). The topography of Round Island is shown by 5 m contour lines. All ant quadrats sampled in 2020 were also sampled in 2019.

Centipedes were dissected to remove the gut. This was done in fresh 100 % ethanol in a sterile petri dish using sterile scissors and forceps under a dissection microscope. Once a gut had been removed, it was placed in a 1.5 mL microcentrifuge tube and homogenised using a Qiagen TissueLyser (Qiagen, Manchester, UK) with sterile beads at 60 Hz for 30 seconds.

5.3.3 Primer selection, DNA extraction, high-throughput sequencing, and bioinformatics

Primer selection, DNA extraction, and high-throughput sequencing followed the processes outlined in Chapter 4. Ants and centipedes were sequenced together, whilst skink samples were sequenced separately.

The ants and centipedes were screened with three primer pairs, AntEx, Beren-Luthien, and UniPlant, whilst the skinks were screened only with the latter two primer pairs. Because I wanted to compare the diet of the ants, centipedes, and skinks, I removed dietary data arising from the AntEx primers for the ants and centipedes. Almost all centipede species known to science are exclusively carnivorous, and I thus removed plant detections from centipede dietary data as these almost certainly represented secondary consumption (Tercel et al. 2021). These edits to the dataset were chosen to best represent the ecology of each consumer group whilst allowing for fair comparisons between them. This may nevertheless present a biased image because the proportion of host reads arising from Beren-Luthien primers was far lower in skinks and, to a lesser extent, centipedes than it was in ants. See Section 5.5.2 for more details.

The two Illumina cartridges generated 31,842,696 reads for the ants and centipedes (Beren-Luthien, V3 = 16,124,326; UniPlant, V2 = 15,718,370). For Beren-Luthien, 1,284 samples (including positives and negatives) were taken forward, giving an average per sample read depth of 12,558. For UniPlant, 811 samples were taken forward, giving an average per sample read depth of 19,381. The Illumina Nano cartridge run for skink samples amplified using Beren-Luthien primers generated 750,645 reads across 96 samples (including positives and negatives), giving an average per sample read depth of 7,819.

Bioinformatics for skinks followed Chapter 3 and Moorhouse-Gann *et al.* (2022), whilst bioinformatics for ants and centipedes followed Chapter 4.

5.3.4 Statistical analyses

All statistical analyses were conducted in R version 4.2.0 (R Core Team 2021) after data were converted to presence/absence. I visualised trophic interactions in a bipartite network using R package "bipartite" (Dormann et al. 2008). I also examined the structure of the trophic network using commonly used metrics calculated with the "networklevel" function of "bipartite". I computed linkage density (diversity of interactions per species weighted by the sum of column and row totals), which characterises the level of generalism in the network by determining the average number of links per species; a high number indicates high generalism and low specialism. Equally, it can be thought of as the average number of links added to the network for each additional species. I also calculated nestedness (NODF; the degree to which sections of the network are subsets of each other (Almeida-Neto et al. 2008; Ulrich et al. 2009)), which describes whether the trophic network is compartmentalised by different interaction patterns; for example, whether trophic interactions of centipedes are a subset of the interactions of ants. Furthermore, I calculated the normalised degree centrality of the consumers using the "ND" function, which is the number of links divided by the maximum possible number expressed as a percentage for each consumer, to determine to what extent each consumer group may influence the wider community through their trophic interactions.

I compared the diets of the main native consumers on Round Island with the non-native ants and used Hill numbers to estimate dietary diversity as per Chapters 3 and 4 (Hill 1973; Chao et al. 2014; Roswell et al. 2021) in R package "iNEXT" (Hsieh et al. 2016). As per Chapter 4, I used the argument "endpoint = 1,250" to extrapolate Hill diversity to the value predicted at 1,250 dietary detections for ants, centipedes, and skinks. This allows us to compare Hill-diversity results between the groups because I can predict dietary diversity at a normalised number of dietary detections (1,250) for each species, regardless of the number of actual observed detections and sample number.

I used R package "mvabund" (Wang et al. 2012) to test whether dietary composition differed significantly between ants, centipedes, and skinks, as well as between seasons (wet and dry). I added an interaction term to determine whether seasonal changes in diet affected ants, centipedes, or skinks differently. Multivariate generalised linear models (MGLMs) were run using the "manyglm" function with a Monte Carlo resampling method and "binomial" error family. Variation in the diet was visualised using non-metric multidimensional scaling analysis (NMDS) using the "metaMDS" function in the "vegan" R package (Oksanen et al. 2019) and was plotted using "ggplot2" (Valero-Mora 2010). Furthermore, I statistically tested whether the diets of the three consumers overlapped significantly more or less than expected by chance. I did this by comparing our observed data to a null model of resource use using Pianka's niche overlap index (Pianka 1973) in R package "EcoSimR" (Gotelli et al. 2015) with the "niche_null_model" function ('ra3' algorithm) over 10,000 replications. I also ran identically structured pairwise comparisons between the three consumers to determine niche overlap between each consumer pair.

5.4 Results

The dietary analysis revealed 1,281 dietary detections across 129 dietary taxa. Of these detections, 752 were from 383 individual ants, 143 from 42 centipedes, and 386 from 73 skink faecal samples (Figure 5.3). Insects featured heavily in the ten most frequently consumed dietary taxa for all three consumers (ants = 8/10, centipedes = 8/10, skinks = 4/10; Table 5.1). I calculated a linkage density of 18.45 for the trophic network, suggesting a high level of overall generalism (MacDonald et al. 2020). The network tended towards a relatively low level of nestedness (NODF = 27.01), where most empirical studies of food webs fall within the 40-60 range (Almeida-Neto et al. 2008; Ulrich et al. 2009), suggesting the patterns of trophic interaction between ants, centipedes, and skinks are broadly dissimilar but with some overlap. Normalised degree centrality was high for ants (53.1 %) and skinks (58.6 %), and relatively low for centipedes (18 %).

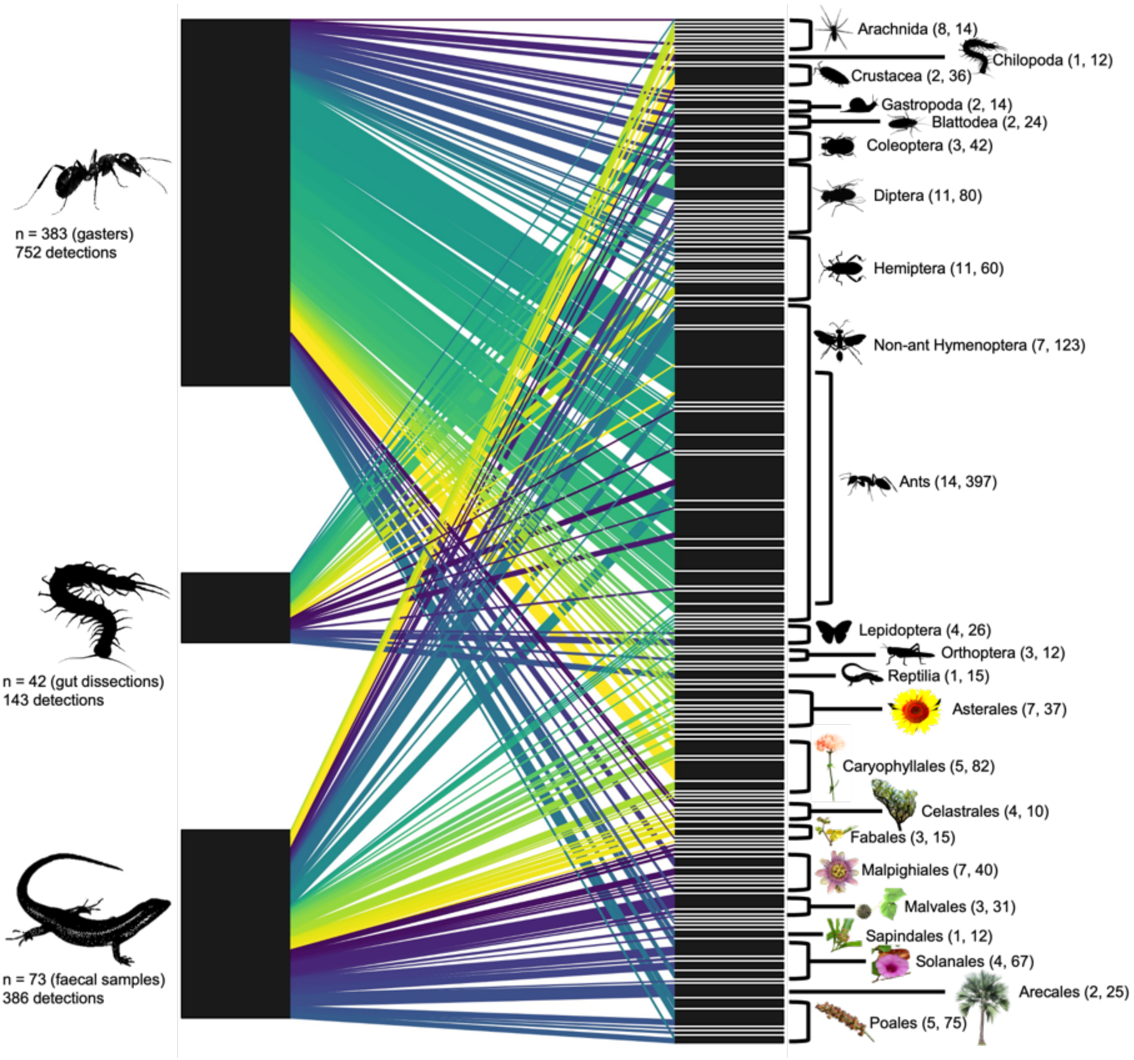


Figure 5.3. A bipartite food web showing consumers (left) and dietary taxa (right) on Round Island. The text below each consumer group denotes the sample size, sample type in parentheses, and total number of dietary detections. The height of black rectangles, and the width of coloured links between them, is proportional to the number of detections associated to them. Links are arbitrarily coloured to aid visualisation. Notable dietary taxa are labelled with higher taxonomic information; because of their high diversity, insects and plants are labelled by order, whilst non-insect animals are labelled by class. The numbers in parentheses right of taxonomic labels denote the number of species-level taxa (first number) and detections (second number) in each taxon.

Table 5.1. The ten most frequently consumed dietary taxa for ants, centipedes, and skinks. Frequency of occurrence was calculated as the number of detections for a dietary taxon divided by the total number of samples for the relevant consumer group (ants = 383, centipedes = 42, skinks = 73).

Consumer	Dietary taxon	Taxonomy	Number of detections	Frequency of occurrence
Ants	Chalcididae sp. 1	(Insecta: Hymenoptera: Chalcidae)	70	18.27%
	Brachymyrmex cordemoyi	(Insecta: Hymenoptera: Formicidae)	50	13.05%
	Pheidole megacephala	(Insecta: Hymenoptera: Formicidae)	49	12.79%
	Strumigenys simoni	(Insecta: Hymenoptera: Formicidae)	45	11.74%
	Tapinoma subtile	(Insecta: Hymenoptera: Formicidae)	41	10.7%
	Monomorium floricola	(Insecta: Hymenoptera: Formicidae)	40	10.44%
	Boerhavia coccinea	(Eudicots: Caryophyllales: Nyctaginaceae)	37	9.66%
	Nylanderia bourbonica	(Insecta: Hymenoptera: Formicidae)	25	6.52%
	Technomyrmex pallipes	(Insecta: Hymenoptera: Formicidae)	25	6.52%
	Cenchrus echinatus	(Monocots: Poales: Poaceae)	20	5.22%
Centipedes	Diptera sp. 1	(Insecta: Diptera)	27	64.28%
	Psyllidae sp. 1	(Insecta: Hemiptera: Psyllidae)	14	33.33%
	Blaberidae sp. 1	(Insecta: Blattodea: Blaberidae)	12	28.75%
	Pyralidae sp. 1	(Insecta: Lepidoptera: Pyralidae)	11	26.19%
	Pheidole megacephala	(Insecta: Hymenoptera: Formicidae)	10	23.81%
	Gongylomorphus bojerii	(Reptilia: Squamata: Scincidae)	10	23.81%
	Strumigenys simoni	(Insecta: Hymenoptera: Formicidae)	9	21.43%
	Fromundus sp. 1	(Insecta: Hemiptera: Cydnidae)	8	19.04%
	Gastropoda sp. 1	(Gastropoda)	8	19.04%
	Coleoptera sp. 1	(Insecta: Coleoptera)	8	19.04%
Skinks	*Heterospilus* sp. 1	(Insecta: Hymenoptera: Braconidae)	29	39.73%
	Pheidole megacephala	(Insecta: Hymenoptera: Formicidae)	29	39.73%
	Abutilon indicum	(Eudicots: Malvales: Malvaceae)	26	35.62%
	Porcellionidae sp. 1	(Crustacea: Isopoda: Porcellionidae)	25	34.24%
	Latania loddigesii	(Monocots: Arecales: Arecaceae)	24	32.88%
	Ipomoea pes-caprae	(Eudicots: Solanales: Convolvulaceae)	16	21.92%
	Harmonia yedoensis	(Insecta: Coleoptera: Coccinellidae)	15	20.55%
	Brachymyrmex cordemoyi	(Insecta: Hymenoptera: Formicidae)	14	19.18%
	Achyranthes aspera	(Eudicots: Caryophyllales: Amaranthaceae)	14	19.18%
	Boerhavia sp. 1	(Eudicots: Caryophyllales: Nyctaginaceae)	14	19.18%

Telfair’s skinks showed the highest extrapolated dietary diversity of the three consumers, as measured using Hill numbers, with an estimated Hill richness of approximately 120 at 1,250 dietary detections (Figure 5.4). Estimated dietary diversity for ants and centipedes was significantly lower than that of skinks. Extrapolated Hill richness at 1,250 dietary detections was 73 and 27 for ants and centipedes, respectively. Sample coverage was high for all groups, at 90% or above.

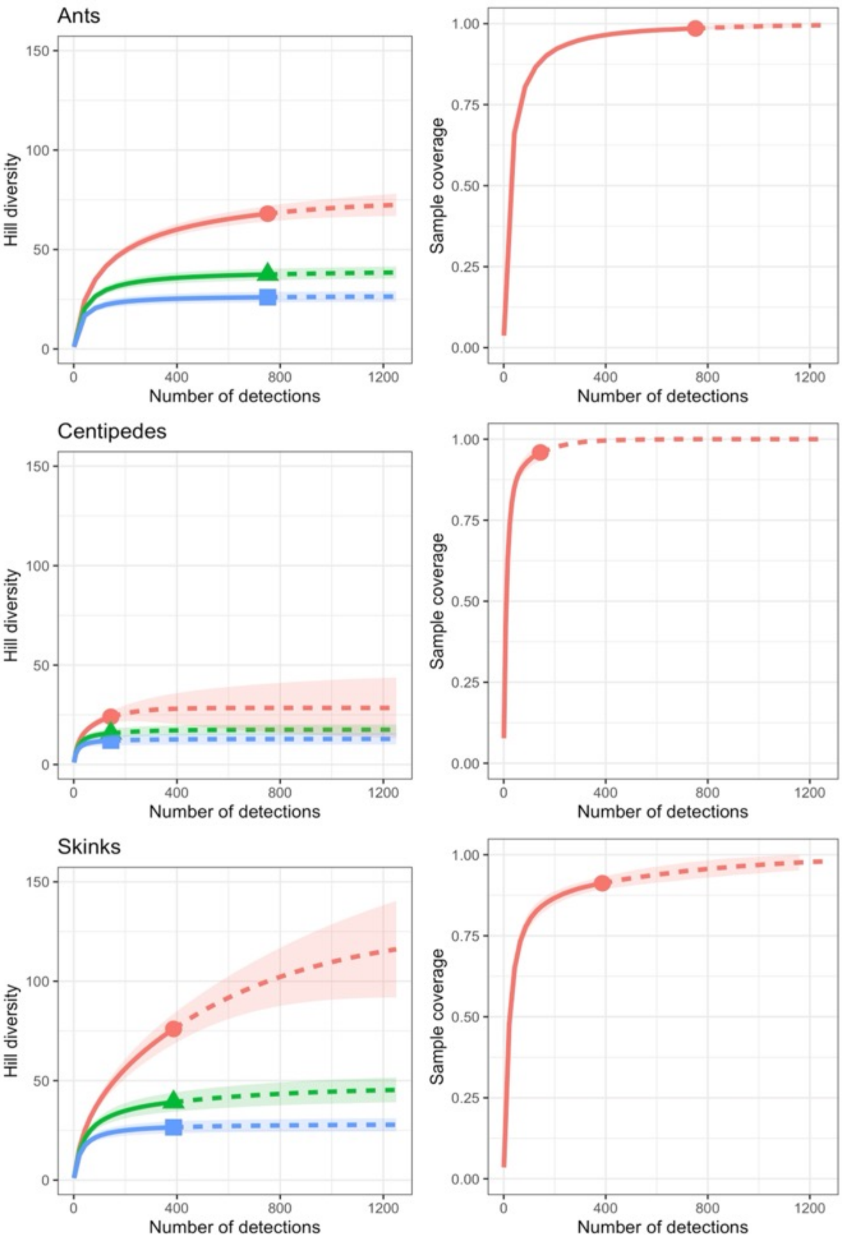


Figure 5.4. Hill diversity and sampling coverage estimates for ants, centipedes, and skinks. Left plots beneath species labels show Hill diversity by number of dietary detections, right plots show sample coverage by number of dietary detections. Line colours denote values of the exponent ι that determines the rarity scale of different diversity estimates: Hill-richness, ι = 1, red line with terminal circle; Hill-Shannon, ι = 0, green line with terminal triangle; Hill-Simpson, ι = -1, blue line with terminal square. Solid lines = observed, dashed lines = extrapolated. Confidence intervals (95%) are denoted by shading around the line.

MGLMs in mvabund revealed that dietary composition varied significantly between consumers (LRT = 1262.1, p = <0.001; Figure 5.5a), showing that different consumers had broadly dissimilar diets overall. Season also significantly affected dietary composition (LRT = 332.3, p = <0.001; Figure 5.5b) with many taxa being consumed slightly more or less in a given season, though only *L. loddigessi* and *Aegilops* sp. 1 were consumed at significantly different rates between seasons (for both, all detections were in the dry season). The slight variations in the availability of food between seasons may explain the overall shift in dietary composition of the consumers (see Chapter 4 Appendix 4.3; Figure S4.2). I also found that the interaction term between consumer type and season was significant (LRT = 109.5, p = <0.001) suggesting that ants, centipedes, and skinks react differently to seasonal shifts in diet.

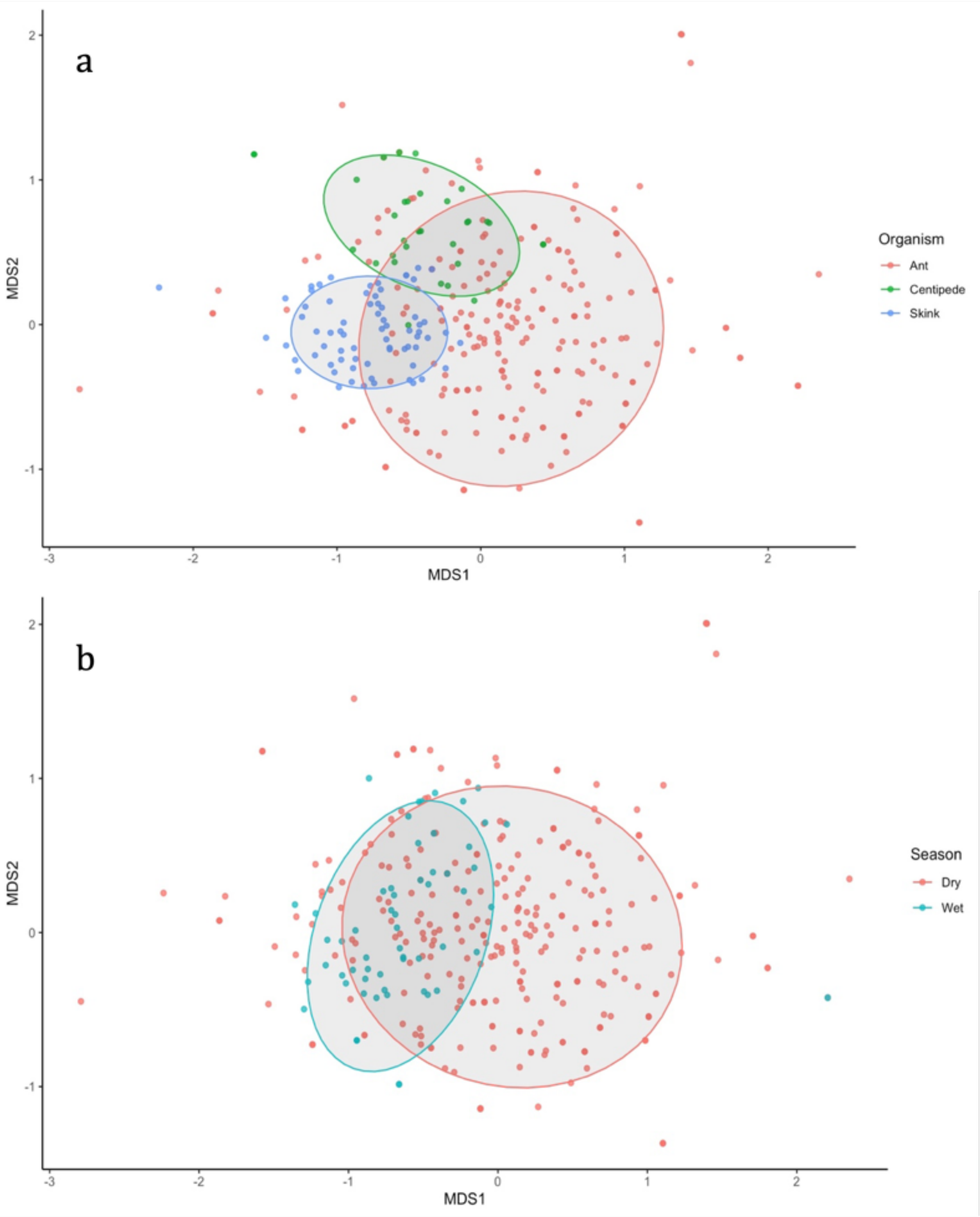


Figure 5.5. Ant, centipede, and skink diet composition visualised using non-metric multidimensional scaling. The upper plot "a" shows dietary composition between ants, centipedes, and skinks, where different colours denote the consumer type. The lower plot "b" shows dietary composition between wet and dry seasons. Points represent individual samples. Ellipses are 80% data circles.

Despite the broad differences in diet, food resource use between the three consumers overlapped significantly more than expected by chance (p = 0.0142, standardised effect size (SES) = 2.585), though no pairwise comparisons between consumer pairs were statistically significant (ants and centipedes: p = 0.145, SES = 1.05; ants and skinks: p = 0.128, SES = 1.169; centipedes and skinks: p = 0.51, SES = -0.186). Whilst these results appear to contradict one another, using two complementary statistical measures of niche overlap/difference identifies the niche space more fully than relying solely on one measure. The mvabund analysis and Pianka's niche overlap measure dietary differences using different mathematical methods. The former runs GLMs for each dietary species to determine whether consumption rate differs between factors. It then runs all individual GLMs together, enhancing its statistical power because the test accounts for correlated abundance in each sample and corrects the p-value accordingly for species that have a correlated abundance structure (Wang et al. 2012). This multivariate analysis uses the likelihood ratio test statistic. Pianka's niche overlap instead looks at the frequency with which each consumer uses a resource and is therefore more similar to a network analysis that measures nestedness and whether dietary resources overlap, rather than measuring the composition of a community. Ultimately, these are two different methods of measuring the dietary niche space. For example, though the dietary communities identified by the mvabund analysis are broadly distinct from one another, Pianka's niche overlap shows that the identity of dietary taxa and the frequency with which they are consumed overlaps between the three consumer types, though no pairwise comparisons appeared significant.

5.5 Discussion

5.5.1 Predation, competition, and the wider impact of non-native ants on Round Island

Three of the most abundant and dominant consumers on Round Island, ants, centipedes, and skinks, have distinct diets with some shared resources. Our network analyses suggest the trophic network is highly generalist, with low levels of specialism, as many interactions between consumers and prey are shared. Ants and skinks are shown to be central to the Round Island trophic network, and roughly comparable in their level of centrality. Many centipede and skink individuals show a dietary composition similar to that of some non-native ants, and dietary composition significantly overlaps between the three consumer groups. I sampled these consumers at the island-scale and between seasons, and our study provides the first substantial ecological information relating to *S. abnormis* and the first study to the author's knowledge comparing the trophic interactions of dominant invertebrate and vertebrate consumers using dietary metabarcoding. As seen in Chapters 3 and 4, seasonal changes in the availability of different species probably explains the significant overall seasonal variation in dietary composition for consumers (see Chapter 4 and Chapter 4 Appendix 4.3; Figure S4.2), though few individual dietary taxa were consumed at significantly different frequencies between wet and dry seasons.

One of the main aims of this study was to determine whether the consumer groups were consuming one another. I found, unsurprisingly, that Telfair's skinks are not consumed by either centipedes or ants based on our metabarcoding data. Telfair's skinks are large lizards, growing to an average length of approximately 30 cm (Cole et al. 2018a) and even juveniles are probably too large for adult centipedes to overpower. Equally, ants would not be able to efficiently consume active Telfair's skinks, though it is likely they scavenge the remains of dead individuals when possible. *Pheidole megacephala* major workers have been seen attacking the toes of skinks; majors latch onto the toes with their powerful mandibles and remain attached even after death. This eventually leads to restricted blood circulation and ultimately loss of the attacked toe (Nik Cole, pers. comm.). Despite this, I found no evidence of ants consuming Telfair's skinks in our dietary data. Similarly, Telfair's skinks showed no predation of centipedes, probably because they are active at different times.

In contrast, centipedes were consumed by ants, albeit at a low frequency (3.1 % of samples). As discussed in Chapter 4, this may nevertheless have an overall negative effect on the population size of the centipedes. The non-native ant community primarily consists of ground-nesting species that forage epigeally and these probably encounter centipedes in certain areas. However, of the 43 centipedes I collected, only four were in close proximity to an ant foraging trail (from three ant species: *Pheidole megacephala, Nylanderia bourbonica,* and *Tetramorium simillimum*). Ant nests are sparse in areas where centipedes are abundant, suggesting that ants and centipedes are using different nest sites in broadly different habitats. This may reduce the chances that ants will be able to find a nesting centipede that they can attack, swarm, and overpower more easily. It is possible the current spatial distribution and nesting habits of centipedes is a result of the high abundance of ants on Round Island. Areas with low ant activity may effectively act as refuges for the centipedes. Foraging ant workers presumably encounter centipedes either when they are nesting nearby, i.e., when centipede and ant nesting preferences align, or when centipedes are foraging for food at night. Nevertheless, Serpent Island centipedes are large fast-moving active predators and are likely to be capable of escaping ants when they encounter them in the open. The effect of the non-native ants may therefore be primarily sub-lethal, whereby centipedes abandon nest sites near to ant colonies.

Centipedes appear to consume the ants *P. megacephala* and *S. simoni* relatively regularly. The population sizes of these ant species on Round Island are very large, and the centipedes are unlikely to present a major top-down pressure on the ant fauna. Overall, our data suggest that predation between consumer groups is unlikely to be a major factor governing the population dynamics of these dominant consumers on Round Island, with the possible exception of ants preying on centipedes, which is discussed in Chapter 4 and could represent a threat to the centipedes long-term.

Whilst ants, centipedes, and skinks show distinct diets, there are also many shared resources between them. Our dietary data show that ants consume multiple resources used by both centipedes and skinks. For example, ants consumed all taxa that centipedes consumed, and 14 of 75 taxa consumed by skinks. The high abundance and activity of ants on Round Island may ultimately reduce some food resources available to centipedes. This is difficult to empirically prove as population dynamic data for ants, centipedes, and their dietary taxa, are needed to adequately test whether exploitation competition between ants and centipedes is occurring. Nevertheless, with such a large population of non-native ants consuming what is presumably a large biomass of food suitable for centipedes, it is plausible that ants are directly influencing the availability of centipede food resources. Skinks appear less likely to be affected by potential exploitation competition with ants. Ants consume a far lower proportion of the total dietary diversity of skinks than they do centipedes (skinks = 18.66%, centipedes = 100%), and several important dietary taxa for skinks, such as *Latania loddigesii*, are not consumed by ants.

Centipedes and skinks show a low level of dietary overlap, with only 5 of 93 shared dietary taxa. This is unsurprising given that skinks are generalist omnivores, consuming many species of plants and animals, whilst centipedes are exclusively carnivorous predators. Even so, the shared animal prey taxa are also dissimilar, presumably because skinks are more likely to prey on diurnal invertebrates on the ground, foliage, and arboreally, such as *Harmonia yedoensis* and Rhyparochromidae. Centipedes instead prey primarily on nocturnal groups on the ground, such as cockroaches and pyralid moths, as well as diurnal groups that they find whilst foraging under rocks\debris at night.

Interestingly, *S. abnormis* are consuming Bojer's skinks, *Gongylomorphus bojerii*, at a relatively high frequency. Roughly a quarter of centipedes in our study consumed Bojer's skinks. Predation of vertebrates by large *Scolopendra* centipedes has been anecdotally recorded wherever large scolopendrids occur alongside vertebrates, though formal articles also exist (McCormick and Polis 1982; Halpin et al. 2021). Telfair's skinks have been observed consuming Bojer's skinks, though the lack of any detections in this study suggests it is uncommon. For centipedes, Bojer's skinks appear to make up a significant part of the diet. Centipedes probably consume juvenile and sub-adult skinks hiding beneath rocks during the night that they can more easily overpower.

Considering the high relative biomass of Bojer's skinks compared to the other dietary taxa consumed, and to the centipedes themselves, it is possible *S. abnormis* centipedes specialise on consuming young Bojer's skink individuals as one of their major sources of nutrition. Bojer's skinks are reproductively active between July and February and eggs hatch between August and April, though low levels of reproductive activity occur throughout the year (Cole and Payne 2022). Centipedes consume Bojer's skinks more frequently during periods where Bojer's skinks are reproductively active (frequency of consumption: Bojer's skink breeding season = 40%, low breeding activity = 17.4%), corroborating this hypothesis further. These periods also coincide with the centipede's own breeding season, possibly allowing female centipedes to build up the protein stores required to produce eggs and the fat reserves needed to nurse them before they hatch. Previous observations of *S. abnormis* foraging on Serpent Island suggest seabirds may be a major component of its diet (Lewis et al. 2010), but seabirds were not detected in centipede diet in this study. The density and diversity of nesting seabirds on Round Island is far lower than on Serpent Island (Cheke and Hume 2008), possibly reducing the opportunities centipedes have to consume seabird tissue.

Solely from these dietary data, it is difficult to definitively conclude whether the non-native ant community is having a negative impact on *S. abnormis* centipedes or Telfair's skink. This is ultimately because there are no comparable surveys of Round Island before non-native ants invaded. However, on Ile Marianne, a small coralline islet measuring 2.135 ha and located approximately 4.5 km south-east of Mauritius, large scolopendrid centipedes were common in the early 2000s. Between 2003 and 2006, *P. megacephala* were introduced and the unidentified centipede species has now not been seen for at least four years (Nik Cole, pers. obvs., 2022). On Round Island, the hyper-abundant non-native ants are widely consuming many taxa, including native species, and this may have effects at multiple trophic levels directly and indirectly. Ants are known to substantially alter invertebrate communities in a range of different systems (Parr et al. 2016; Parker and Kronauer 2021), and this is especially true of non-native ants, which can cause dramatic compositional changes (Lessard et al. 2009; Parr et al. 2016) and diversity declines (Chapter 2) of native invertebrates after invasion. This may have knock-on effects to the diet of the centipedes and skinks, as well as other consumers, whereby the available food now consists of the species which are able to coexist with non-native ants. The current food resources available may have thus passed through the "biotic filter" of the non-native ant community (Parker and Kronauer 2021). It is impossible to test this hypothesis from metabarcoding data alone, though similar effects of non-native ants have been observed elsewhere (Hoffmann et al. 1999; Lessard et al. 2009). The broad dietary range and high abundance of non-native ants revealed here and in Chapter 4 suggests their role within the Round Island ecosystem is central to the ecological community, corroborated by our centrality analysis showing them to be comparable to the centrality of skinks (normalised degree centrality: ants = 53.1 %, skinks = 58.6 %). It is therefore likely that the non-native ants are exerting a strong top-down effect on the rest of the invertebrate community and indirectly to the consumers that rely on them.

5.5.2 Limitations

The limitations of the data generated for Chapters 3 and 4 that are used here are detailed in Sections 3.5.4 and 4.5.5, respectively. The limitations of dietary metabarcoding more broadly have been reviewed elsewhere (Symondson 2002; Nielsen et al. 2018; Alberdi et al. 2019; Lamb et al. 2019; Tercel et al. 2021; Cuff et al. 2022). However, I also identified some study-specific limitations.

The main limitation arises because of differences in how effectively the PCR primers amplify our different consumers. The universal animal PCR primers I used amplify invertebrates more effectively than vertebrates (Cuff et al. 2021a). Thus, ant and centipede dietary data have more host reads than skinks as a proportion of total read number. Moreover, the sample types varied. Ant digestive systems were not dissected from the gaster due to brittleness arising from storage in 100% ethanol, making dissection challenging and too time consuming for such a large sample size. Instead, whole gasters were pulverised to release dietary DNA. This resulted in a large proportion of total reads coming from host tissue. This was also true of centipedes, though guts were dissected and thus the host to prey tissue ratio was far lower. On the other hand, faecal samples were used to identify the diet of skinks. Faecal samples typically have the lowest host to prey tissue ratio of these three sample types and thus fewer host reads were found (Cuff et al. 2022). This is in addition to the PCR amplification bias described above, further reducing the number of host reads (and increasing the number of prey reads) seen in the skinks. To some extent, the dietary diversity detected may therefore be a result of how efficiently the primer pair amplifies the host rather than a true signal of how diverse the diet is (Cuff et al. 2022) given that there are a limited number of reads per sample. In practice, this suggests that ants and centipedes may have more diverse diets than our data suggests. Both ants and centipedes were screened with AntEx primers, but these data were removed to allow comparisons with skinks. When data from AntEx primers are included, ants and centipedes have far higher dietary diversity than when only Beren-Luthien are used (Chapter 5 Appendix 5.1, Figure S5.1). This supports the idea that many prey sequences are lost because of the efficient amplification of host reads by Beren-Luthien, and thus an underestimated dietary diversity. Some of these issues may be mitigated (or perhaps further confused) by the fact that skink samples were sequenced with a lower sequencing depth than those of ants and centipedes, possibly redressing the unequal efficacy to detect dietary diversity between consumers.

Another key limitation is that the effects of non-native ants on Round Island are difficult to conclusively quantify based on these data. Historical data from before ants arrived, or experimental data involving ant suppression plots, are needed to provide definitive conclusions. Despite this, I are able to make inferences through a combination of our results and the known effects of non-native ants more broadly.

5.5.3 Conclusions

This dietary study suggests it is likely that non-native ants are significantly affecting the Round Island ecosystem primarily through top-down pressures as hyper-abundant generalist consumers. This may also extend to sub-lethal processes that drive species into areas of lower ant activity, as seen in centipedes and their tendency to nest in areas with low numbers of ants. Non-native ants are central to the Round Island ecosystem and may be comparable to Telfair's skinks, the dominant native omnivore.

Whilst it is not possible to verify, the invertebrate community on Round Island may be effectively filtered by the abundant non-native ants and, in doing so, altering the availability of food resources for other consumers. Consumers that share a significant proportion of their dietary taxa with non-native ants may be at a greater risk of extirpation/extinction from Round Island, and I see that *S. abnormis* centipedes share 100% of their dietary taxa with non-native ants. Moreover, centipedes appear to be the most specialised of the three consumers I examined, relying primarily on native species such as the Bojer's skink. Thus, non-native ants could be affecting the endemic native centipedes in four ways: through direct predation, through sub-lethal effects, through competition for food resources, and by altering the wider community composition. The potential impact of non-native ants on Telfair's skinks appears relatively minimal and only the latter point is applicable.

Based on these findings, non-native ants may be a major threat to native invertebrate consumers on Round Island. Serpent Island centipedes appear to be threatened on multiple levels by non-native ants and this may be true of other endemic predators. A greater understanding of the role of non-native ants on Round Island is urgently required to determine to what degree and by which mechanisms non-native ants are affecting the Round Island community. There are several actionable conservation interventions that could help to reveal the role of ants more fully and to protect invertebrate consumers that are at a higher risk of being strongly adversely affected by non-native ants. For example, setting up ant suppression plots on Round Island would help to experimentally test their impact on invertebrate community composition. In addition, beginning captive breeding programs for *S. abnormis* centipedes and other threatened invertebrate consumers would safeguard their future and genetic diversity in the event of further non-native ant invasions and population growth.

The view from the helicopter that was used to evacuate Round Island a few days before the COVID19 pandemic. The original reason for the evacuation wasn’t because of COVID19, but because of a cyclone about to hit the island.

Chapter 6: General Discussion

Pheidole sp., Sapa, Vietnam.

6.1 Discussion

6.1.1 General discussion

One of the major caveats of the current study system is that there is only one Round Island. This makes experimental design difficult, given that non-native ants are found abundantly over the entire island. Therefore, definitively providing a quantitative estimate of how non-native ants affect native biodiversity on Round Island is currently impossible, because I do not have data describing the ecological community of Round Island before non-native ants colonised. Furthermore, using neighbouring islands (e.g., Gunner's Quoin, Flat Island) as proxies to quantify the effects of non-native is also not feasible for two reasons: 1) these islands are also heavily invaded by non-native ants and thus fall foul of the same experimental problem, and 2) the ecological communities and habitats on neighbouring islands differ from Round Island's, though they do share some specific native species. Suppression of non-native ants in experimental plots over Round Island may help to reveal some of the functional responses of the native community, and to quantify the scale of the current effect of non-native ants, though measuring the influence they have already had appears impossible. Nevertheless, over the preceding Chapters, I have presented a bank of evidence suggesting that non-native ants may be a significant threat to native taxa on Round Island through their hyper-abundance and direct and indirect interactions.

The first piece of evidence comes from our meta-analysis, presented in Chapter 2. This shows that non-native ants, on average, reduce local animal community abundance and richness by approximately 50 % in relatively undisturbed areas around the world. These diversity losses are ecologically significant: such dramatic declines in the number of individuals and species in an ecosystem suggest concomitant impacts on how that ecosystem functions. Though I were unable to conduct similar studies on Round Island, the very strong negative community responses to non-native ants in disparate habitats and locations around the world suggest they may have already reduced native species diversity on Round Island. Such reductions may have taken effect decades ago, given that non-native ants have been on Round Island since at least the 1970s (collected by D. Bullock) and may have been present for far longer. Revealing these effects, however, is challenging because scant entomological survey data is available for Round Island, especially before the 1970s, to provide baseline abundance and diversity data to compare to present-day data. Nevertheless, recent surveys (Dunlop et al. 2016) and the survey data presented here show that non-native ants now dominate the invertebrate community. The hyper-abundance of non-native ants on Round Island necessitates a large number of ecological interactions to support the nutritional requirements of the population (Hölldobler and Wilson 1977). Identifying which taxa non-native ants are interacting with can pinpoint how they may be directly influencing the community.

Chapters 3 and 4 present the next pieces of the puzzle. Firstly, Telfair's skinks are consuming ants, particularly *P. megacephala*, very frequently (Chapter 3). It is unlikely skinks are deliberately eating ants, given that, in 20 years of behavioural observation, only a few skinks have ever been seen preying upon ants. Instead, Telfair's skinks are probably consuming ants accidentally when ingesting other food. However, as of November 2022, at the very end of the dry season, several Telfair's skinks were seen consuming *P. megacephala* workers along a foraging trail on Gunner's Quoin, perhaps given the lack of other food resources. Nevertheless, the ability of ants to find food resources, and their high density over Round Island, suggests that ants may be swarming over food items that are subsequently consumed by skinks. This may not be ecologically meaningful for the skinks (in terms of nutrition) or for the ant colony from which the ant individuals originate (in terms of colony fitness) (Tercel et al. 2021; Tercel et al. 2022). The sheer abundance of non-native ants on Round Island, and their ability to find food rapidly, supports the hypothesis that they are an ecologically dominant group. Our DNA metabarcoding dietary analysis of the most populous twelve (of 18) species of ants on Round Island again corroborates this (Chapter 4). I recorded almost 2,000 dietary detections across 156 taxa being consumed by non-native ants. The diet primarily consisted of insect prey and secondarily of plant matter and all ants showed 'generalist' diet profiles, consuming taxa broadly across the animal and plant branches of the tree of life. Of these detections, 911 are of ants consuming other ants, suggesting that intra-guild predation is an extremely important dietary element for these species (and perhaps non-native ants more generally). Thus, our data go some way to support the long-held hypothesis that ant-ant interactions are particularly important in shaping the dynamics of ant community structure (Hölldobler and Wilson 1990; Lach et al. 2010). Moreover, our results describe a complex diet niche space of the non-native ant community in which non-native ants that have no shared evolutionary history can coexist, in part through dietary niche separation. It may be that the ant fauna of Round Island exists in its current form because the diets (and other traits) of each species are sufficiently different to avoid excessive competition. Alternatively, the diet of each species may be totally flexible, and each species can modify their highly generalist diet to avoid competition. Perhaps more likely is somewhere in-between these two hypotheses, whereby each ant species has an intrinsic generalist diet that it can modify based on the ecological context, including the other ant species around it. This analysis is the first to use dietary metabarcoding to reveal the species-level trophic interactions of individual ants at the community-scale, the first to show a large community of non-native generalist consumers showing dietary niche separation, and the first to show that non-native ants consume each other frequently. Finally, I also observed the non-native ant community consuming many native species, some of which are listed as threatened on the IUCN Red List.

Chapter 5 presents a comparative dietary analysis between the non-native ant community and two native consumers that are presumed to be important to the wider Round Island ecosystem: Telfair's skink and the Serpent Island centipede. The analysis shows that neither ants nor centipedes consume Telfair's skinks, whilst both native consumers are consuming ants. Ants are also consuming centipedes, albeit at relatively low frequency. If these detections are scaled-up to the tens of millions of ants present on Round Island, it may indicate an important predation pressure on the native centipedes. Furthermore, our ant activity surveys, taken in a 5 m radius around centipede sampling points, revealed that only four of 43 centipedes were near an ant colony or foraging trail. This contrasts with the 69 randomly generated 4 m^2 quadrats used to sample ants, where 100 % of quadrats were occupied by at least one species of ant. Thus, centipedes appear to be found in areas of low ant activity. This may represent different habitat preferences, or ants may exert non-lethal effects on centipedes by attacking them in their nests and whilst centipedes are foraging, causing them to flee. These non-lethal effects could elicit significant behavioural changes to centipede behaviour and, ultimately, their ecological function on Round Island, akin to the 'landscape of fear' in some vertebrate predator-prey systems (Gaynor et al. 2019). Moreover, ants consume 100 % of the dietary taxa that centipedes consume. Though the relative frequencies of prey taxa varies between ant and centipede diets, non-native ants may be competing with centipedes for certain food resources. Our data suggest that non-native ants could affect skinks and centipedes by altering the availability of food resources (especially invertebrates) through top-down pressures as hyper-abundant generalist consumers. It is feasible the processes described above may affect other native terrestrial invertebrate species in similar ways.

6.1.2 Conservation implications

In summary, five separate lines of evidence suggest non-native ants are having a significant effect on the Round Island ecosystem: 1) non-native ants reduce local animal community diversity by approximately 50 % on average in multiple locations and habitats around the world, and have thus plausibly had a comparable effect on Round Island; 2) due to their abundance, non-native ants are able to find and swarm over many food items consumed by a dominant native vertebrate omnivore, Telfair's skink; they may also be important sources of nutrition for native and non-native consumers; 3) the very high density of non-native ants on Round Island and their diet breadth suggest the non-native ant fauna may be affecting the wider community (especially invertebrates) through top-down pressures, which would have significant indirect implications for other consumers on Round Island; 4) non-native ants may be competing for food resources with the Serpent Island centipede, *Scolopendra abnormis*, and other native invertebrate predators; and 5) non-native ants may be exerting sub-lethal effects on a range of native animal species, as seen in Telfair's and Bojer's skinks, whereby ants bite the toes of skinks and eventually cause necrosis, and *S. abnormis*, whereby centipedes avoid areas of high ant activity. Whilst each of these inferences are inductive rather than deductive, the weight of evidence suggests non-native ants are continuing to drive significant changes to the Round Island ecosystem. The invertebrate community appears most susceptible to these changes. In light of these findings, the high number of endemic species, and very small area of occupancy, the native invertebrate community of Round Island might be considered one of the most threatened on Earth.

Unfortunately for conservation stakeholders, ant eradication is extraordinarily costly to employ and more than half of attempts fail (Hoffmann et al. 2016). However, trailing several long-term ant eradication plots (perhaps 30 x 30 m) across Round Island would begin to describe how the community might respond if ant populations are suppressed. Such an initiative could underpin the key taxonomic and functional responses of the invertebrate community on Round Island, and thus how the ants are currently affecting community composition. For a more long-term solution, continuing the island's extensive habitat regeneration program may eventually lower ant abundances passively. In denser stands of native forest, non-native ants are found in lower abundance than in more open and structurally homogenous areas. Non-native ants are often thought to be 'disturbance specialists' (Sanders et al. 2007; Vonshak and Gordon 2015) and increasing the amount of closed-canopy native forest cover on Round Island will disproportionately benefit native invertebrates and decrease non-native ant abundance. Finally, maintaining the stringent biological decontamination processes all people, food, and building materials go through before going to Round Island is vital to prevent further ant invasions. Several ant species present on mainland Mauritius, such as *Solenopsis geminata* and *Anoplolepis gracilipes*, may cause severe responses from the island's unique vertebrate community if they colonise Round Island. It appears unlikely that these results will directly influence conservation management in the near future. Whilst conservation managers typically want to solve problems caused by invasive ants (Hoffmann 2010; Hoffmann et al. 2016; Nik Cole, pers. comm.), there are numerous legislative, financial, and logistical barriers to, for example, establishing several long-term ant suppression plots on Round Island. This should be the first step taken in solving the potential problems arising from invasive ants on Round Island and elsewhere in the Mauritian archipelago. These plots would identify the exact ecological consequences of ant invasion in terms of biodiversity change, whether there are non-target effects of formicide use (e.g., effects on the lizards), and it could ultimately legitimise larger-scale invasive ant suppression.

More broadly, these results are confirmatory regarding the nature of invasive ants in terms of their ecological impact (Chapter 2) and their trophic interactions (Chapters 4 and 5). As stated previously, the highly generalist diets of non-native ants facilitates their global spread, allowing them to colonise many different habitats (Hölldobler and Wilson 1977; Holway 1998; Holway 1999; Holway et al. 2002; Krushelnycky et al. 2009). These results identify 156 dietary taxa – a huge diet breadth that presumably allows invasive ants to extract nutrition from a large number of disparately related different species. The use of metabarcoding to identify the diet of these ants finally corroborates, for the first time, the long-held assumption that invasive ants typically have very broad diets. This increases the likelihood that they will be able to successfully colonise new island and continental locations and is probably a factor governing the cosmopolitan distribution of many invasive ant species.

6.1.3 Questions raised and future research

This thesis presents one of the most comprehensive assessments of the diet of a community of generalists across fourteen focal species. However, there are several outstanding questions raised by our study.

How are certain dietary items being consumed? One of the shortfalls of dietary metabarcoding is the inability to identify *how* something was eaten. For some of our results, direct auxiliary behavioural observations would help tease apart the way in which some species are consumed, and this could improve the clarity of our ecological conclusions. For example, how ants are consumed by skinks, or whether different ant species consume other ants primarily through nest raiding or opportunistic predation etc. Determining how a consumer eats another species can reveal the dynamics of an ecological community further than the presence / absence data produced by dietary metabarcoding. To expand upon this using the above nest raiding example, if an ant species conducts nest raids on some species but consumes others through scavenging there may be more complex behavioural and nutritional implications of those interactions that cannot be gleaned solely from metabarcoding data. Successful nest raids typically result in a very high proportion of the raided nest's brood being consumed, which can severely limit the chances of colony survival, and these larvae may be more nutritionally rewarding per unit mass than adult workers. Moreover, many workers may be lost on both sides even during successful nest raids, suggesting colonies may need to balance the frequency and timing of nest raids to maintain a certain colony size and demography of workers. Thus, ants that raid the nests of other species may be able to exert a substantial local effect on the immediate community of other ants. Examining the mode by which consumers eat other species helps to illuminate the mechanistic underpinnings of ecological interactions and may therefore show a more detailed image of the ecology of a given system.

How does 'dominance' affect ant diet, if at all? As described in Chapter 1 (section 1.3.2), dominance can be defined in several ways, though the use of "ecological dominance", i.e., the foraging success of a species relative to its abundance (Parr and Gibb 2009), is desirable here because it provides a definition that incorporates numerical and behavioural definitions of dominance. Ecological dominance is believed to be an important trait governing the structure of ant communities (Hölldobler and Wilson 1990; Lach et al. 2010) and our results relating to *P. megacephala* suggest it could also affect the diet of ant communities. It might be possible to build a relative dominance hierarchy of an ant community which can then be used to explore whether diet and dominance are correlated, as well as the "dominance-release" hypothesis. I outlined this hypothesis in Chapter 4 (section 4.5.1). Ant species may modulate their diets in the context of changes to a dominance hierarchy but predicting these are challenging because dietary diversity may feasibly increase or decrease with a change to an existing hierarchy. This may also significantly alter the composition of a species' diet. Unfortunately, none of these aspects can be explored empirically in the current study. However, such data could be generated by conducting extensive baiting trials alongside dietary metabarcoding to correlate diet with dominance and spatial co-occurrence of different ant species. This should be done by experimentally removing dominant ants and comparing dietary composition of sub-dominant ants in areas with and without dominant ants. Furthermore, this experiment could continue further down the dominance hierarchy, where decreasingly dominant ants are excluded from communities to see whether ants that would normally hold a lower dominance rank eventually behave and consume food as if they were dominant. Answering how dominance may influence species-level trophic interactions of ant species and dietary dynamics of ant communities will add a new aspect to the decades of research attempting to reveal the role dominance plays in structuring communities of ants.

And finally, how do non-native ants influence the invertebrate community composition and abundance on Round Island? Though our results suggest non-native ants are widely affecting the ecological community on Round Island, it is not clear which taxonomic groups are responding most strongly. Setting up and maintaining long-term ant suppression plots on Round Island and measuring invertebrate diversity in paired suppression and control plots will go some way to quantify the current effect of non-native ants on the wider Round Island community.

The view from the south-west ridge at sunset, Round Island.

Thanks for reading. Have a nice day!

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Appendices

Chapter 2 Appendix

S2.1 Article search

Boolean search string used in www.webofknowledge.com "topic search":

> *TS=(("Pheidole megacephala" OR "African big-headed ant*" OR "Anoplolepis gracilipes" OR "Yellow crazy ant*" OR "Solenopsis invicta" OR "red imported fire ant*" OR "Myrmica rubra" OR "European fire ant*" OR "Linepithema humile" OR "Argentine ant*" OR "Wasmannia auropunctata" OR "little fire ant*" OR "electric ant*" OR "Paratrechina longicornis" OR "longhorn crazy ant*" OR "black crazy ant*" OR "Technomyrmex albipes" OR "white footed ant*" OR "white-footed ant*" OR "Brachymyrmex cordemoyi" OR "Nylanderia bourbonica" OR "Trichnomyrmex destructor" OR "Solenopsis geminata" OR "tropical fire ant*" OR "Tapinoma melanocephalum" OR "ghost ant*" OR "invasive ant" OR "invasive ants" OR "exotic ant" OR "exotic ants" OR "introduced ant" OR "introduced ants" OR "alien ant" OR "alien ants" OR "invading ants" OR "pest ants" OR "tramp ants" OR "tramp ant")*
>
> *AND*
>
> *(abundance OR divers* OR biodiversity OR richness OR community OR communities)*
>
> *NEAR/5*
>
> *(impact* OR effect* OR influence OR influences OR affect* OR damag* OR consequence* OR decline* OR increas* OR decreas* OR replace* OR destruction OR introduc* OR invasi* OR respon*))*

This returned 740 articles on 9th August, 2021. I chose to include only articles in English because I were unable to include collaborators sufficiently fluent in other languages. I also emailed Dr. Ben Hoffmann, an expert in invasive ant ecology, asking if they knew of any sources of grey literature or unpublished studies that might adhere to our selection criteria but they were not aware of any sources of additional literature. I paired this with extensive internet searches in non-academic search engines (e.g. Google), but revealed no additional sources of primary data.

Figure S2.1. PRISMA flow diagram of article screening, eligibility scrutiny, and inclusion in meta-analysis.

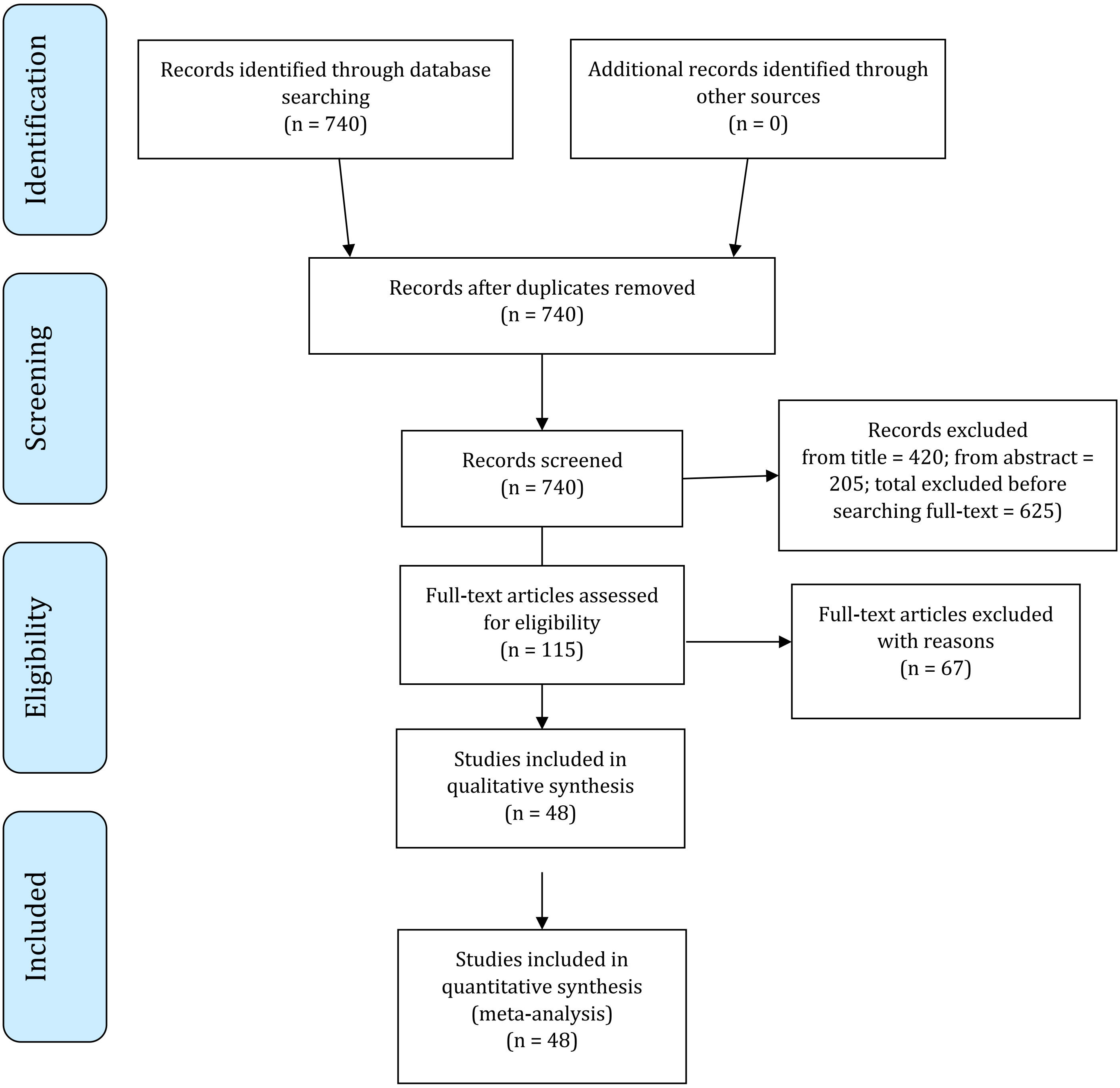


Table S2.1. Data extraction sheet used to aid data collection. Acc# denotes the accession number generated for an article. Authors, Date, Title, denote journal information; Invasive_ant denotes the focal species of introduced ant in a given study; Native_taxon denotes the native taxon responding to the introduced ant; Coordinates and Location relate to the geography of the study sites; Habitat denotes the primary habitat type of a site; Duration denotes the total sampling duration across months or years; Sampling_method denotes the type of sampling used to sample the given native taxon; Formicides denotes whether toxic formicides are used and the active ingredient if so; Page_of_acc is the page of the article data were extracted from; Parameter is either abundance or richness; Data_source is either Table(number), Figure(number), or text and denotes where values were extracted from in an article; X_uninv, SD_uninv, and N_uninv denotes the mean, standard deviation, and sample size of the native taxon parameter measurement in uninvaded sites; X_invade, SD_invade, and N_invade denotes the native taxon parameter measurement in invaded sites. Samples_per_site denotes the number of samples used per site; Comments provides the meta-analyst with additional space to make any further comments.

1. Acc#	2. Authors	3. Date	4. Title	5. Invasive_ant
6. Native_taxon	7. Coordinates	8. Location	9. Habitat	10. Duration
11. Sampling_method	12. Formicides	13. Page_of_acc	14. Parameter	15. Data_source
16. X_uninv	17. SD_uninv	18. N_uninv	19. X_invade	20. SD_invade
21. N_invade	22. Samples_per_site	23. Comments		

S2.2 Second meta-analyst

To ensure robust and valid data extraction, a second meta-analyst undertook full-text article screening and data extraction on a random subset of one-quarter of the articles. The second meta-analyst did not screen the full selection of articles because of time-constraints. This was conducted "blind", i.e., without knowledge of the results or articles selected by the first meta-analyst. Wilcoxon rank sum tests, non-parametric equivalents to unpaired t-tests, revealed no significant differences between calculated effect sizes or extracted data from the articles between the two meta-analysts (Table S3). Subsequent to these tests, any disagreements in article inclusion or notable differences in extracted data values were discussed between the analysts. These discussions then informed which studies in the subset would be included or disqualified. Two additional articles were included, whilst three were excluded during this step and both analysts agreed on the final list of included articles. Given that these tests ratify our robust and valid data extraction process, data extracted by the first analyst were used in all meta-analyses after these checks.

Table S2.2. Results of Wilcoxon rank sum tests to determine whether data or effect sizes were significantly different between the two meta-analysts.

Variable	Invasion status	Data type	*Z*	*p*
Abundance	Uninvaded	Mean	540.5	0.52
Abundance	Uninvaded	Standard deviation	418.5	0.31
Abundance	Uninvaded	Sample size	379.5	0.1
Abundance	Invaded	Mean	530.5	0.61
Abundance	Invaded	Standard deviation	466.5	0.72
Abundance	Invaded	Sample size	364	0.061
Richness	Uninvaded	Mean	33	0.96
Richness	Uninvaded	Standard deviation	28	0.71
Richness	Uninvaded	Sample size	29	0.7
Richness	Invaded	Mean	30	0.87
Richness	Invaded	Standard deviation	28	0.71
Richness	Invaded	Sample size	29	0.7
Abundance	-	Hedges' *g*	489	0.96
Richness	-	Hedges' *g*	40	0.44

Table S2.3. Possible effect modifiers coded for during data extraction and example codes for each.

Possible effect modifier	Example codes
Invasive ant taxon	Linepithema humile, Anoplolepis gracilipes, Brachyponera chinensis, Pheidole megacephala, Tapinoma sessile, Wasmannia auropunctata Solenopsis invicta, Solenopsis papuana, Myrmica rubra
Native taxon	Acari, Amphibians, Amphipoda, Ants, Arachnida, Araneae, Birds, Blattodea, Chilopoda, Coleoptera, Collembola, Decapoda, Dermaptera, Diplopoda, Diptera, Embioptera, Gastropoda, Hemiptera, Hymenoptera, Invertebrates, Isopoda, Lepidoptera, Orthoptera, Pscoptera, Pseudoscorpiones, Reptiles, Thysanoptera, Vertebrates
Location	Australia, USA, Spain, New Zealand, France (New Caledonia), Malaysia, USA (Santa Cruz Island), Samoa, New Zealand (Tokelau), South Africa, USA (Hawai'i), Australia (Christmas Island), Gabon, Kenya
Habitat type	Coniferous forest, deciduous forest, scrubland, tropical forest, grassland, shrub, coastal scrub, littoral forest, mixed forest, mixed habitats
Sampling method	Litter extraction, pitfalls, foliage beats, visual surveys, foliage removal, bait transect, hand sampling, pan traps, light trap, cover board, drift fence
Use of formicides	No, yes (“active ingredient”)

Table S2.4. Moderator analysis model statistics and permutation tests.

Model	Moderator variable	Q_M(df)	*p*	Sig.	R^2
Abundance by native taxon	Article	70.5 (38)	0.0011	*	51.51%
Permutation test	Article	70.5 (38)	0.004	*	-
Richness by native taxon	Invasive ant species	18.27 (7)	0.0108	*	39.67%
Permutation test	Invasive ant species	18.27 (7)	0.005	*	-
Richness by native taxon	Article	37.89 (27)	0.0796	.	43.62%
Permutation test	Article	37.89 (27)	0.09	.	-
Richness by article	Invasive ant species (excl. *M. rubra* studies)	9.04 (6)	0.17		8.39%
Permutation test	Invasive ant species (excl. *M. rubra* studies)	9.04 (6)	0.11		-

Table S2.5. Quality criteria checklist derived from Koricheva and Gurevitch, 2014

Quality criteria	Current study
1. Has formal meta-analysis been conducted (i.e. combination of effect sizes using standard meta-analytical methodology) or is it simply a vote count?	Yes, formal meta-analysis is conducted here, using a standardised mean difference approach to measure effect size. Vote counting was not used.
2. Are details of bibliographic search (electronic data bases used, keyword combinations, years) reported in sufficient detail to allow replication?	Yes, all extracted data and the reference list are provided, including a full list of screened articles. A PRISMA flow diagram is also provided (Figure S2.1).
3. Are criteria for study inclusion/exclusion explicitly listed?	Yes, all criteria are listed in the Methods section. Also, a full protocol for this meta-analysis is given.
4. Have standard metrics of effect size been used or, if nonstandard metrics have been employed, is the distribution of these parameters known and have the authors explained how they calculated variances for such metrics?	Yes, a standard metric of effect size is employed here – standardised mean difference (Hedges' *g* in this case).
5. If more than one estimate of effect size per study was included in the analysis, has potential non-independence of these estimates been taken into account?	I tested for non-independence of effect sizes and found that there was a strong significant non-independence between effect sizes estimated from the same articles. I attributed these differences to the highly variable and unique ecological communities examined in each article that responded to introduced ants; i.e. ecological communities are themselves interdependent and this was evidenced in our additional analyses of article-level effect sizes.
6. Have effect sizes been weighted by study precision or has the rational for using unweighted approach been provided?	Yes, effect sizes were weighted by variance.
7. Have statistical model for meta-analysis and the software used been described?	Yes, a random-effects model (due to significant heterogeneity) were run using the "metafor" package (11) in R version 3.6.1 (12).
8. Has heterogeneity of effect sizes between studies been quantified?	Yes, heterogeneity statistics are reported (p-values, I^2, H^2, tau^2, for each model), and confidence intervals are given for total effect size and for each study.
9. Have the causes of existent heterogeneity in effect sizes been explored by meta-regression?	Yes, changes to effect size due to different variables were investigated. See Table 2.1 in main Chapter 2 text.
10. If effects of multiple moderators have been tested, have potential non-independence of and interactions between moderators been taken into account?	Effect sizes of studies originating from the same article were combined for an overall article effect size to test for non-independence. I also tested for interactions between moderators, but none were significant.
11. If meta-analysis combined studies conducted on different species, has phylogenetic relatedness of species been taken into account?	Yes, this is a secondary question this systematic review aims to answer. Relatedness of species was taken into account and analysed from the results of the meta-analysis.
12. Have tests for publication bias been conducted?	Yes, funnel plots were generated and point distribution asymmetry tests (and subsequent corrections) were conducted (see Figures S2.2-2.5 below).
13. If meta-analysis combines studies published over considerable time span, have possible temporal changes in effect size been tested?	Yes, meta-regression was used to test whether effect size of studies varied with publication date.
14. Have sensitivity analysis been performed to test the robustness of results?	Yes, a leave-one-out analysis was run to test the robustness of the overall results.
15. Have full bibliographic details of primary studies included in a meta-analysis been provided?	Yes, a full list of all screened and used articles are included as a supplementary file. Excluded articles are given with a reason for exclusion.
16. Has the data set used for meta-analysis, including effect sizes and variances/sample sizes from individual primary studies and moderator variables, been provided as electronic appendix?	Yes, all data have been uploaded as a supplementary file.

Figure S2.2. Native species abundance response effect size and standard error per study (n = 158), used to test for publication bias. Zero additional studies are estimated to be required on the left side (SE = 7.18).

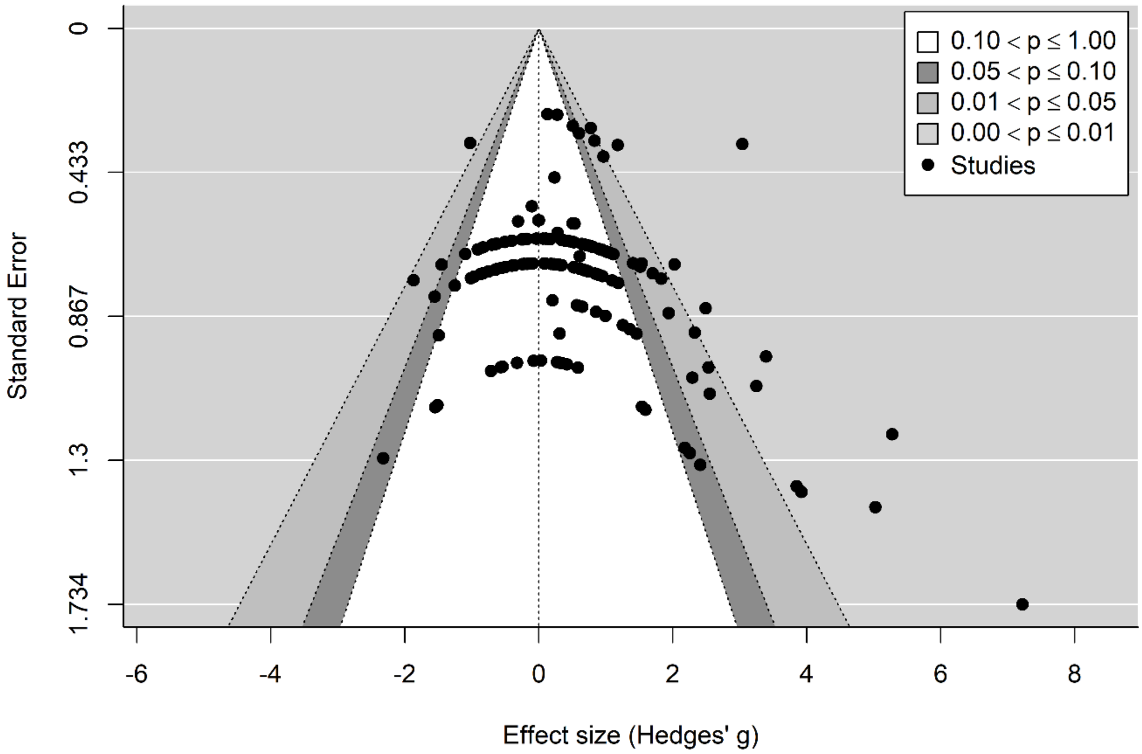


Figure S2.3. Native species richness response effect size and standard error per study (n = 54), used to test for publication bias. Zero additional studies are estimated to be required on the left side (SE = 4.17).

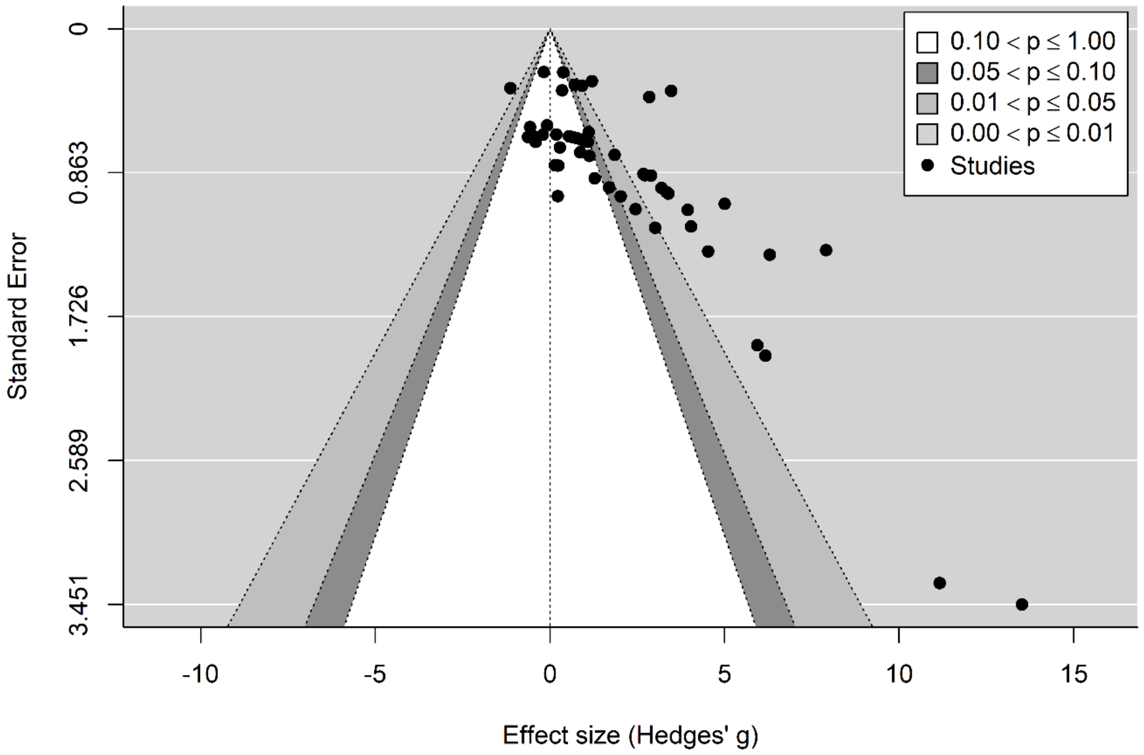


Figure S2.4. Community abundance response effect size and standard error per study (n = 41), used to test for publication bias. Zero additional studies are estimated to be required on the left side (SE = 3.1).

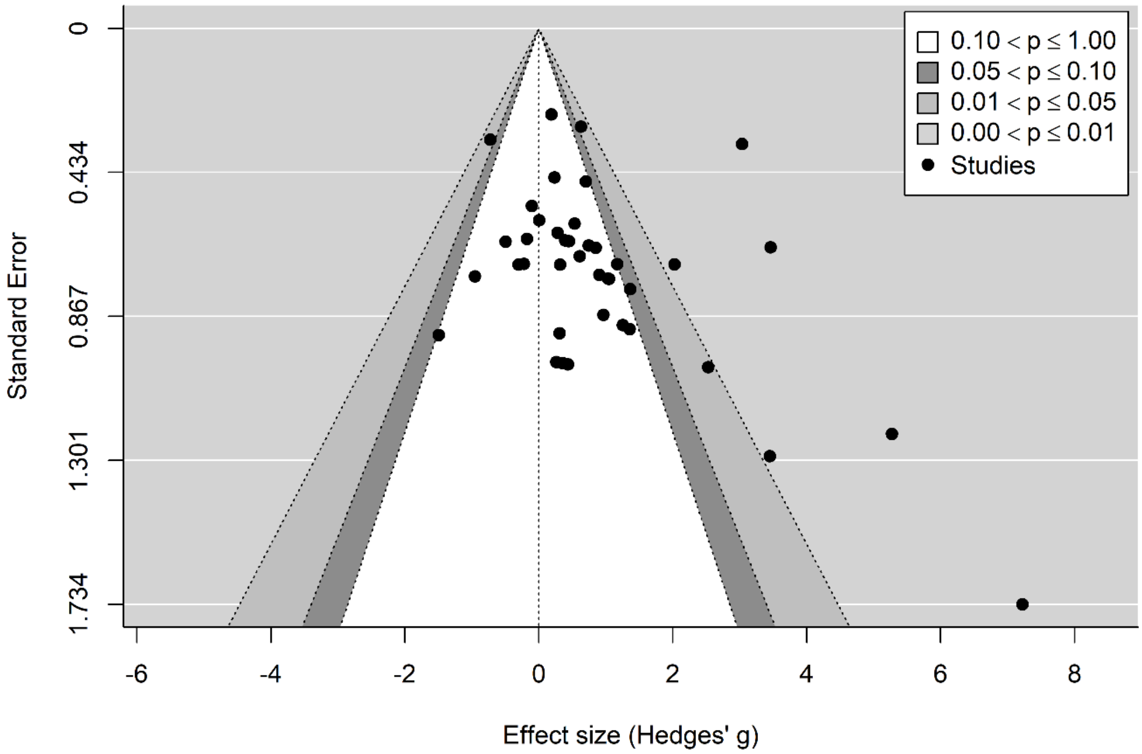
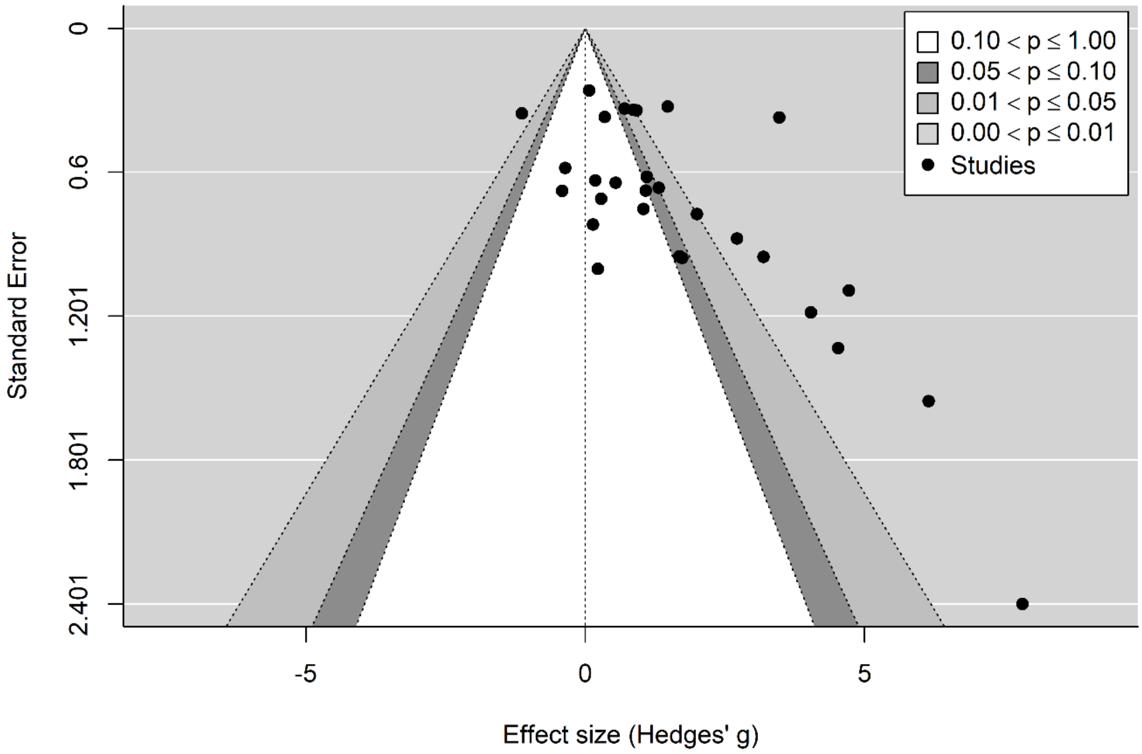


Figure S2.5. Community richness response effect size and standard error per study (n = 29), used to test for publication bias. Zero additional studies are estimated to be required on the left side (SE = 3.13).



S2.3 Limitations of the meta-analysis

Though I took many steps to ensure this analysis was as robust and reliable as possible, several limitations remain (see the main text discussion for tests of bias). One key issue is that it was extremely difficult to measure the external validity of screened articles. This is primarily because sampling strategies varied so widely that sample and site numbers were not representative of a consistent "value". For example, though one study may have used 120 total pitfall traps spread across 5 paired sites (12 traps per site), and another study may have used 150 traps across 5 paired sites (15 traps per site), the trap volume, aperture diameter, period of time left active, whether they were baited, trap spacing or layout, and the total site area may all be different. These values were not typically reported either. Nevertheless, I attempted to quantify article validity by proxy using "traps per site". I also separated each trapping type (pitfalls, pan traps, visual surveys etc.) and analysed these separately and together. These were all non-significant effect size moderators across all analyses.

Another key limitation is the lack of non-English articles, which may geographically bias results to Anglophone countries, or countries where English is typically learned/used in scientific publishing. It may be that there are biogeographical differences between regions I were unable to identify because of this patchy global coverage.

As previously acknowledged, many of the studies I included in our analysis investigating local invertebrate responses to invasive ants may not have been able to accurately distinguish whether all invertebrates captured were native. Some invertebrates in these communities may have been non-native, which could confound results. Our inference that primarily native communities are responding to invasive ants is greatly strengthened because of the stringent eligibility criteria I applied that disqualified studies where sites were considered 'degraded' (e.g. within or adjacent to anthropogenically modified habitats, presence of non-native plants or animals as noted by the authors of each study); 24% of the 246 articles screened at the abstract or full-text stage were disqualified because they violated these conditions explicitly or implicitly. However, even in otherwise 'intact' habitats, non-native species are often present, though typically in low numbers. Ultimately, this is a variable I cannot completely control for in our meta-analysis given that many included studies did not discuss this issue. Therefore, the invertebrate communities in our analysis should be viewed as predominantly native, with low abundances of non-native species.

Our sensitivity analyses did not detect any biases, but some may nevertheless remain. For example, responses to invasive ants may have been artificially inflated in studies which were purposefully carried out in areas where there was a prior knowledge of extreme declines in native species abundance or richness because of invasive ants. This is unfortunately impossible to reliably identify and could theoretically be detected from the sensitivity analyses conducted. As such, our results may present worst-case-scenarios. In contrast, there were several regions completely invaded by non-native ants and thus no comparisons could be made; some of these may be communities that have responded most strongly to invasion. Another potential source of bias could arise from where the local taxa studied follow the taxonomic expertise of the researchers. Response measurements may not include typically overlooked groups even if they are present and responding. This may conceal how certain components of the local communities react to ant invasion and biases observed community responses towards those of “charismatic” taxa.

For future syntheses, ideally a second (or third!) meta-analyst will conduct screening, data extraction and analyses on the full set of returned articles instead of only one-quarter. This will provide even more robust results.

S2.4 Bibliography of included articles

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Allen, C.R., Lutz, R.S., Lockley, T., Phillips, S.A. and Demarais, S. (2001). The non-indigenous ant, Solenopsis invicta, reduces loggerhead shrike and native insect abundance. Journal of Agricultural and Urban Entomology 18(4):249–259.

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Chapter 3 Appendix

S3.1. Bioinformatics

Bioinformatics followed Drake *et al.* (2022) for COI sequencing data (see main text). For ITS2 sequencing data, I followed Moorhouse-Gann *et al.* (2022):

Paired-end Illumina sequences were filtered for quality using Trimmomatic v0.3212 with a minimum quality score of 20 over a sliding window of 4 bp, retaining sequences with a minimum length of 135 bp. These were aligned using FLASH and demultiplexed into faecal sample-specific files using the MID tag sequence with the "trim_seqs" command in Mothur, which also removes the MID and primer sequences from the reads. I then used the "derep_fulllength" and "uchime2_denovo" commands in the USEARCH software v9.2.6415 to remove any sequences with fewer than 10 copies within a faecal sample and any potential chimeric sequences. For the skink dataset only, ITS2 sequences were extracted from all reads using ITSx16 and USEARCH was used once again to extract all unique ITS2 sequences. Analysis of species discrimination at the ITS2 region (this manuscript) suggests this region to be unsuitable for an approach of clustering similar sequences into molecular operational taxonomic units (MOTUs) due to the loss of ability to distinguish between species prior to the grouping of multiple polymorphisms within some plant species.

I took a sequence read-number approach to deal with any background contamination. The highest number of reads for any of these sequences was 139, so I re-ran our initial dereplication step (using "derep_fulllength" in USEARCH) with this new sequence read threshold. I then assigned the resulting sequences to taxonomic unit using the BLAST algorithm to search GenBank, combined with new sequences from our barcode library (GenBank accession numbers KT948614–KT948638). If a sequence had the smallest e-value matching only one species on GenBank, with >99 % sequence identity, I assigned the sequence to that species. If the sequence matched more than one species from the same genus, tribe or family (with a % match between 90 and 99), I assigned the sequence to the lowest common taxonomic unit up to the family level. Sequences with <90 % match to the closest matching species on GenBank, or for which BLAST returned no significant match were discarded, as was any sequence for which the closest match included a bacterium or fungus. Next, I examined each unique sequence found in a negative sample, including unused MID combinations, PCR negatives (n = 7), and extraction negatives (n = 9) to deal with any specific contaminants within our samples. For each sequence, I identified the highest read number within a negative sample and removed this sequence from any sample where the read number was below this threshold. For the Telfair's skink dataset only, the Blastn algorithm was used in Blast+ for taxonomic assignment, comparing all sequences to a comprehensive DNA barcode library of Round Island plants. Sequences were assigned to taxa based on BIT score: if the highest BIT score was reserved to a match with a single species, then species-level identification was achieved, and the same rule was applied to genus-level matches. If a sequence failed to match a plant in the barcode library, the blastn algorithm was used, as above, to search for matches on NCBI GenBank. Finally, for both datasets, sequences within each taxonomic unit were combined.

Table S3.1. Taxonomic information, status relative to Round Island (non-native, cryptogenic, native, endemic), number of detections, and frequency of occurrence (%) of all dietary items found in Telfair's skink faecal samples after data clean-up (n = 73).

Kingdom	Phylum	Class	Order	Family	Dietary taxon	Status	F_o (%)
Animal	Arthropoda	Arachnida	Araneae	Theridiidae	*Coleosoma floridanum*	non-native	1.37
				Theridiidae	Theridiidae sp.	cryptogenic	1.37
			Araneae	Thomisidae	*Ozyptila claveata*	non-native	5.48
			Mesostigmata	Laelapidae	Laelapidae sp.	cryptogenic	1.37
			Opiliones	Phalangodidae	*Bishopella laciniosa*	non-native	1.37
			Sarcoptiformes	Scheloribatidae	Scheloribatidae sp.	cryptogenic	1.37
		Collembola	Entomobryomorpha	Entomobryidae	Entomobryidae sp.	cryptogenic	1.37
		Crustacea	Decapoda	Grapsidae	*Geograpsus grayi*	native	1.37
			Isopoda	Porcellionidae	Porcellionidae sp.	cryptogenic	34.25
		Insecta	Blattodea	Blaberidae	Blaberidae sp.	cryptogenic	2.74
			Coleoptera	Coccinellidae	*Harmonia yedoensis*	non-native	20.55
			Diptera	Drosophilidae	*Drosophila* sp.	cryptogenic	2.74
				Drosophilidae	*Zaprionus africanus*	cryptogenic	1.37
				Drosophilidae	*Zaprionus indianus*	non-native	5.48
				Tachinidae	*Chetogena* sp.	cryptogenic	2.74
				Tephritidae	*Ceratitis capitata*	non-native	1.37
			Embioptera	Oligotomidae	*Oligotoma saundersii*	non-native	2.74
			Hemiptera	Aleyrodidae	*Dialeurodes hongkongensis*	non-native	2.74
				Cydnidae	Cydnidae sp.	native	1.37
				Diaspididae	*Hemiberlesia lataniae*	native	1.37
				Pseudococcidae	*Planococcus minor*	non-native	1.37
				Rhyparochromidae	Rhyparochromidae sp.	cryptogenic	8.22
			Hymenoptera	Apidae	*Inquilina* sp.	native	2.74
				Braconidae	*Heterospilus* sp.	cryptogenic	39.73
				Chalcididae	*Brachymeria* sp.	cryptogenic	1.37
				Formicidae	*Brachymyrmex cordemoyi*	non-native	19.18
				Formicidae	Formicidae sp.	cryptogenic	2.74
				Formicidae	*Monomorium floricola*	non-native	2.74
				Formicidae	*Pheidole megacephala*	non-native	39.73
				Formicidae	*Tapinoma* sp.	non-native	1.37
				Formicidae	*Tetramorium simillimum*	non-native	1.37
				Hymenoptera	Hymenoptera sp.	cryptogenic	1.37
				Platygastridae	Platygastridae sp.	cryptogenic	2.74
			Lepidoptera	Crambidae	Crambidae sp.	native	1.37
				Geometridae	Geometridae sp.	native	1.37
			Orthoptera	Gryllidae	Gryllidae sp.	native	1.37
			Thysanoptera	Thripidae	*Thrips parvispinus*	non-native	1.37
	Chordata	Aves	Procellariiformes	Procellariidae	*Ardenna pacifica*	native	1.37
	Mollusca	Gastropoda	Stylommatophora	Succineidae	*Succinea manuana*	non-native	1.37
Plant	Angiosperms	Eudicots	Asterales	Asteraceae	*Bidens pilosa*	non-native	1.37

Table S3.1. Taxonomic information, status relative to Round Island (non-native, cryptogenic, native, endemic), number of detections, and frequency of occurrence (%) of all dietary items found in Telfair's skink faecal samples after data clean-up (n = 73).

Kingdom	Phylum	Class	Order	Family	Dietary taxon	Status	F_o (%)
					Conyza canadensis	non-native	5.48
					Psiadia arguta	endemic	1.37
					Tridax procumbens	non-native	10.96
				Goodeniaceae	*Scaevola taccada*	native	10.96
			Caryophyllales	Amaranthaceae	*Achyranthes aspera*	non-native	19.18
					Amaranthus viridis	non-native	2.74
				Nyctaginaceae	*Boerhavia* sp.	native	19.18
				Portulacaceae	*Portulaca oleracea*	non-native	1.37
			Celastrales	Celastraceae	*Cassine orientalis*	endemic	1.37
					Maytenus pyria	endemic	2.74
			Fabales	Fabaceae	*Desmanthus virgatus*	non-native	1.37
					Desmodium incanum	non-native	6.85
					Gagnebina pterocarpa	native	10.96
			Gentianales	Apocynaceae	*Tylophora coriacea*	native	12.33
				Rubiaceae	*Morinda citrifolia*	non-native	1.37
			Lamiales	Lamiaceae	*Clerodendrum heterophyllum*	endemic	1.37
					Premna serratifolia	native	5.48
			Malpighiales	Euphorbiaceae	*Euphorbia thymifolia*	cryptogenic	4.11
				Passifloraceae	*Passiflora suberosa*	non-native	15.07
					Turnera angustifolia	non-native	1.37
				Phyllanthaceae	*Margaritaria anomala*	endemic	4.11
			Malvales	Malvaceae	*Abutilon indicum*	non-native	35.62
					Hibiscus tiliaceus	native	5.48
					Hilsenbergia petiolaris	native	4.11
					Thespesia populnea	native	1.37
			Myrtales	Myrtaceae	*Eugenia lucida*	endemic	4.11
			Sapindales	Meliaceae	*Turraea thouarsiana*	endemic	1.37
				Sapindaceae	*Dodonaea viscosa*	native	1.37
			Solanales	Convolvulaceae	*Ipomoea pes-caprae*	native	21.92
				Solanaceae	*Solanum lycopersicum*	non-native	4.11
					Solanum nigrum	non-native	17.81
		Monocots	Arecales	Arecaceae	*Hyophorbe lagenicaulis*	endemic	1.37
					Latania loddigesii	endemic	32.88
			Poales	Poaceae	*Cenchrus echinatus*	non-native	13.70
					Chloris barbata	non-native	2.74
					Dactyloctenium ctenoides	native	1.37
					Digitaria horizontalis	non-native	1.37

S3.2. Positive control species composition

The two positive controls used consisted of a standardised DNA concentration (4 ng / µl) comprised of equal proportions of the following species: *Anthocoris nemorum, Euproctis similis, Melieria crassipennis, Metopolophium dirhodum*, *Pardosa palustris*, *Philodromus aureolus*, *Promethes sulcator*, *Sminthurus viridis*, *Tenuiphantes tenuis*, *Tvetenia calvescens,* and *Utomaphora* sp..

Table S3.2. Dietary taxon richness, total dietary taxon detections, and mean (± SE) dietary taxon detections of Telfair's skinks by dietary taxon status relative to Round Island (cryptogenic, endemic, non-native, or native) and taxonomic kingdom. Mean dietary taxon detections per taxon within each category were calculated by dividing total detections by the number of dietary taxa detected.

Status	Kingdom	Dietary taxon richness	Total detections	Mean detections (± SE)
Non-native	Animals	16	80	5 (± 1.95)
	Plants	17	112	6.59 (± 1.64)
	Total	**33**	**192**	**5.82 (± 1.24)**
Cryptogenic	Animals	15	77	5.13 (± 2.32)
	Plants	1	3	3
	Total	**16**	**80**	**5 (± 2.11)**
Native	Animals	8	9	1.13 (± 0.13)
	Plants	11	71	6.45 (± 1.52)
	Total	**19**	**80**	**4.21 (± 1.04)**
Endemic	Animals	0	0	0
	Plants	10	37	4.11 (± 2.37)
	Total	**9**	**37**	**4.11 (± 2.36)**
Total		**77**	**389**	**5.05 (± 0.79)**

S3.3. Co-occurrence analysis

To discern whether accidental consumption or secondary predation explained any of the dietary detections, presence-absence data of dietary taxa were used to measure the co-occurrences in each individual Telfair's skink with package "cooccur" (Griffith *et al.* 2016) to determine positive, random, and negative co-occurrence relationships between all identified dietary taxa. I used the default threshold of the package to remove taxon pairs that did not co-occur in any skink faecal sample. All taxon pairs that co-occurred in at least one skink faecal sample are reported.

Co-occurrence analysis, using presence-absence data, produced taxon pairs that "positively" or "negatively" co-occurred (occurred together significantly more or less, respectively, than expected by chance, p = <0.05), as well as randomly co-occurred (did not co-occur together significantly more or less than expected by chance, p = ≥0.05). Of the 2,926 taxon pairs measured, 2,715 pairs (92.79 %) were removed from the analysis because of insufficient co-occurrences, i.e. those co-occurring fewer times than required for analysis. After these removals, 211 pairs were analysed, resulting in 19 positive, six negative, and 186 random taxon co-occurrences (Figure S3.1).

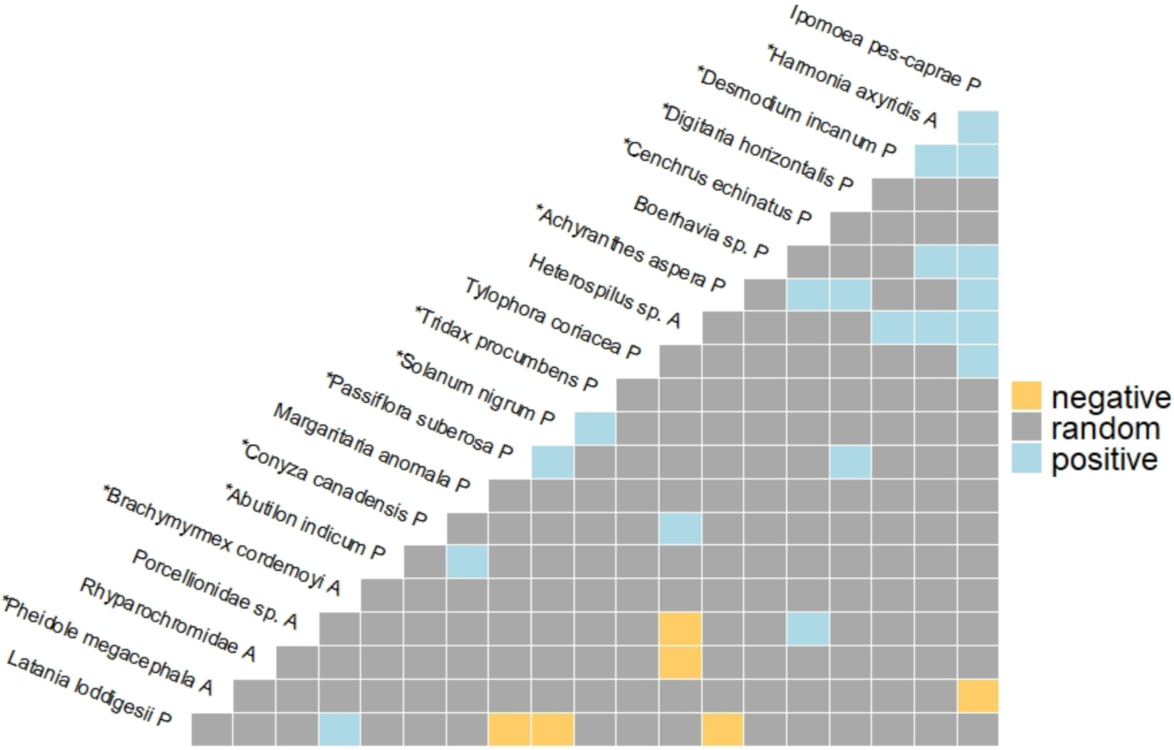

Figure S3.1. Co-occurrence matrix for species pairs found in Telfair's skink faecal samples. Yellow squares show species pairs that co-occur significantly less than expected by chance, grey squares show randomly co-occurring species pairs, and light-blue squares show species pairs that co-occur more than expected by chance. * = non-native species; A = animal; P = plant.

Whilst I did find several non-random co-occurrences, only one pair might have explained accidental consumption: a positive co-occurrence relationship between the ant *Brachymyrmex cordemoyi* and the palm *Latania loddigesii*. Because of the way ants forage, I thought it is possible that *B. cordemoyi* swarms over *L. loddigesii* fruits, and this has been observed, but many *B. cordemoyi* detections did not coincide with *L. loddigesii* detections. Furthermore, no other ant species showed this relationship. I also found negative co-occurrence relationships between plants solely or primarily consumed in different seasons. More generally, although co-occurrence analyses can be useful to disentangle results, they are not able to provide strong evidence of ecological interaction in this context (Blanchet *et al.* 2020), and may not facilitate interpretation of results (Tercel *et al.* 2021). This is because there could be a range of other reasons two taxa are co-occurring, which are discussed in detail elsewhere (Blanchet *et al.* 2020).

Chapter 4 Appendix

S4.1 AntEx primer design

I used two universal animal primers to reveal the carnivorous aspect of ant diet. The first was Beren-Luthien, a universal animal primer designed to amplify a very broad range of invertebrates (Cuff et al. 2021b). I aimed for the second primer pair to exclude ant DNA, thus overcoming the problems associated with excessive host reads flooding the dietary data (Cuff et al. 2022). No such primer pair existed, so I designed a new primer pair within the COI barcoding region (Folmer et al. 1994) to amplify as many invertebrates and vertebrates as possible whilst excluding ant DNA. Termed AntEx, our *in silico* tests using PrimerMiner (Elbrecht and Leese 2017) showed a complete exclusion of ant DNA whilst still amplifying a broad range of different invertebrates and vertebrates (Figure S4.1). I thus took this forward for *in vitro* testing and ultimately sequencing.

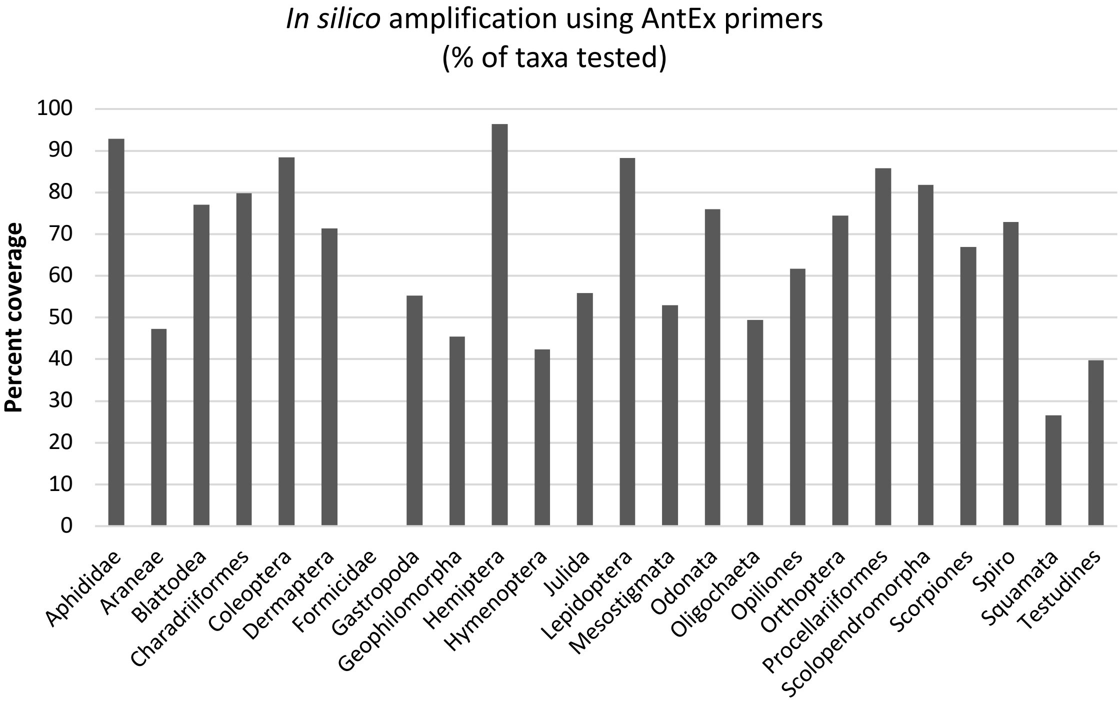

Figure S4.1. Results of *in silico* amplification testing using PrimerMiner to determine the amplification efficiency of AntEx for a range of different taxa present on Round Island.

S4.2 Mock community composition

Plants: *Corylus avellana, Digitalis purpurea, Hedera helix,* and *Taxus baccata.*

Animals: *Anthocoris nemorum, Cancer pagurus, Chernes cimicoides*, Epicriidae sp., *Folsomia candida*, *Geophilus truncorum, Lasius brunneus, Lutra lutra, Metopolophium dirhodum, Nossidium pilosellum,* Nudibranchia sp., *Trichoniscus pusillus,* and *Xysticus cristatus*.

S4.3 Invertebrate community between seasons

To test whether invertebrate communities were significantly different between dry and wet seasons, I conducted a generalised linear model in R package 'mvabund' (Wang et al. 2012) using that 'manyglm' function. The model showed that invertebrate community composition is significantly different between wet and dry seasons (LRT = 85.9, p = <0.001). I visualised the differences using non-metric multidimensional scaling (NMDS) in the 'vegan' R package (Oksanen et al. 2019) using the 'metaMDS' function (Figure S4.2).

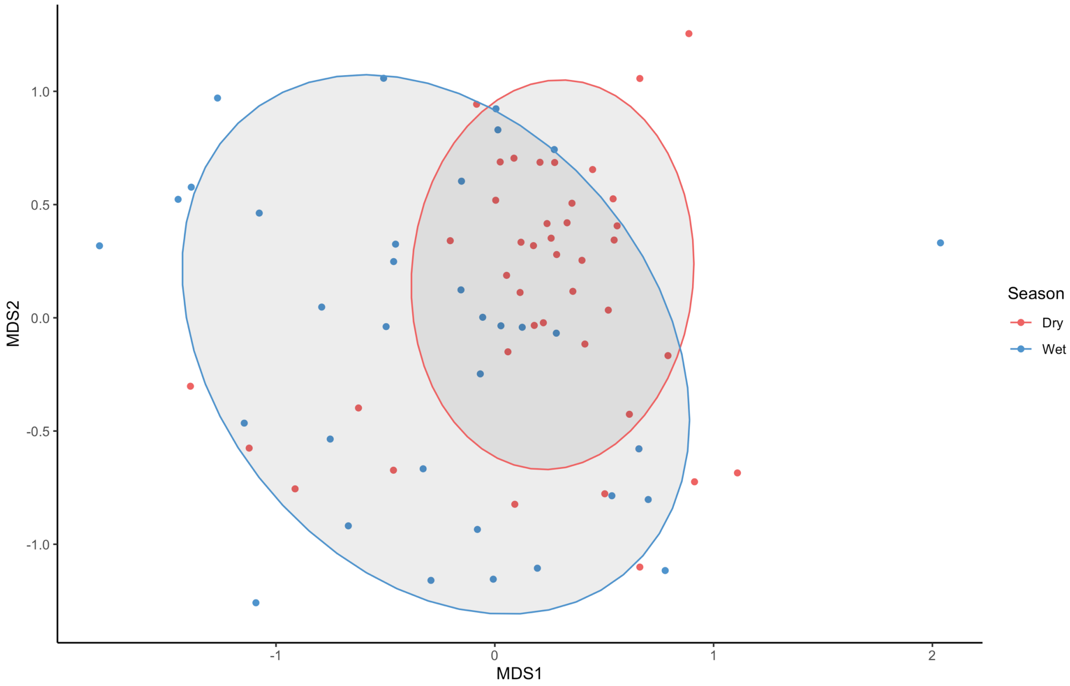

Figure S4.2. Invertebrate community composition between seasons, as visualised using non-metric multidimensional scaling analysis (NMDS). Red dots and ellipses show samples collected in the dry season; blue dots and ellipses show samples collected in the wet season. Ellipses are 80% data circles.

Chapter 5 Appendix

S5.1 Ant and centipede dietary diversity including AntEx primer pair data

Ants and centipedes were initially screened with three primer pairs: AntEx, Beren-Luthien, and UniPlant following the same methods as outlined in Chapter 4. However, skinks were screened only with Beren-Luthien and UniPlant. To allow for comparisons between all three consumer types, I removed data arising from AntEx for ants and centipedes. When dietary data were generated with both AntEx and Beren-Luthien for ants and centipedes, dietary diversity was higher for both groups than when Beren-Luthien is used alone (Figure S5.1).

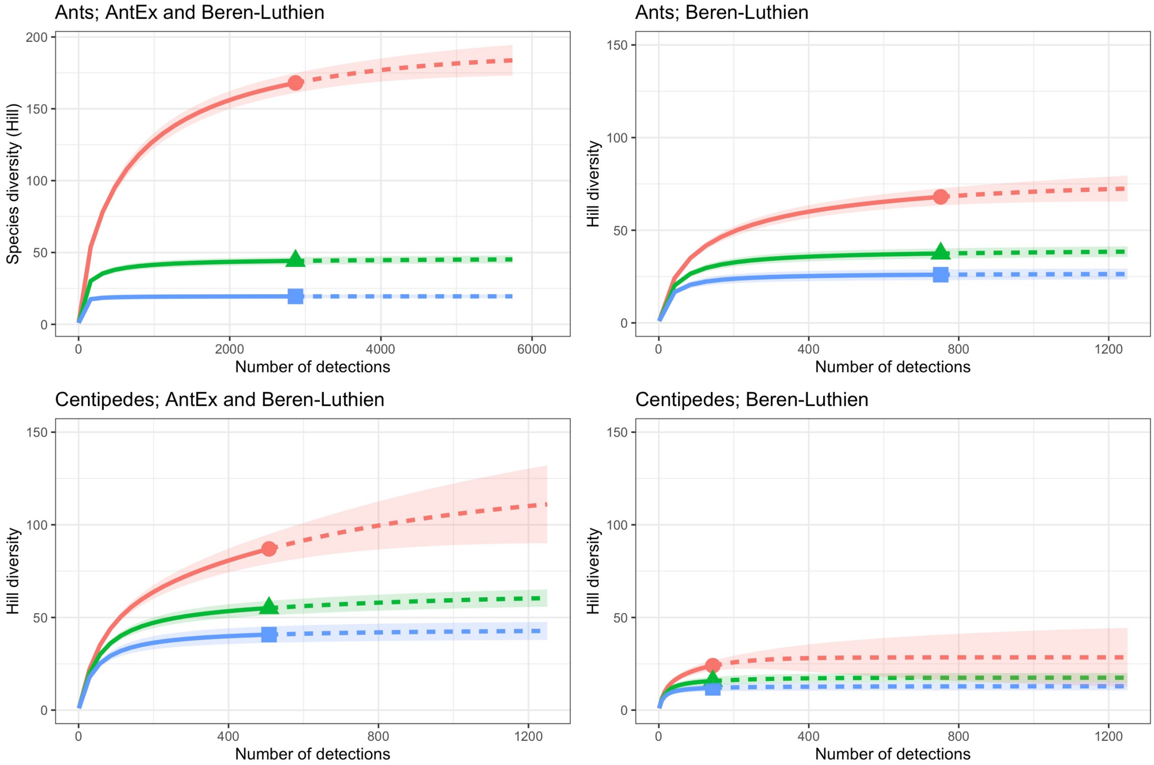

Figure S5.1. Hill diversity curves showing the observed and extrapolated dietary diversity of ants and centipedes using AntEx and Beren-Luthien primers (left plots) compared to only Beren-Luthien primers (right plots). Line colours denote values of the exponent ι that determines the rarity scale of different diversity estimates: Hill-richness, $\iota = 1$, red line with terminal circle; Hill-Shannon, $\iota = 0$, green line with terminal triangle; Hill-Simpson, $\iota = -1$, blue line with terminal square. Solid lines = observed, dashed lines = extrapolated. Confidence intervals (95%) are denoted by shading around the line. Note the different axes limits for the top left plot because of the far higher number of detections and dietary diversity.