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Invertebrate services within Cape to City and comparison of environmental DNA with conventional invertebrate community monitoring: Research Synthesis 2015/2016

Invertebrate services within Cape to City and comparison of environmental DNA with conventional invertebrate community monitoring: Research Synthesis 2015/2016

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Contents

Summary v

1 Introduction 1

2 Invertebrates and ecosystem services review 2

2.1 Ecosystem services and invertebrates 2

2.2 Invertebrates, mammalian predators and predation pressure 6

2.3 Long-term changes in ecosystem services 9

3 The invertebrate fauna at Mohi Bush Scenic Reserve, Hawke's Bay, assessed using pitfall and malaise traps 11

3.1 Introduction 11

3.2 Objectives 11

3.3 Methods 12

3.4 Results 16

3.5 Discussion 24

3.6 Recommendations 28

4 The invertebrate fauna at Mohi Bush Scenic Reserve, Hawke's Bay, assessed using environmental DNA 29

4.1 Introduction 29

4.2 Objectives 30

4.3 Methods 30

4.4 Results 33

4.5 Discussion 40

4.6 Recommendations 41

5 Overall recommendations 42

6 Acknowledgements 43

7 References 43

Appendix 1 – Abundance of invertebrates from different Orders collected in malaise traps at MBSR 53

Appendix 2 – Abundance of beetles collected in malaise traps at MBSR 54

Appendix 3 – Abundance of invertebrates from different Orders collected in pitfall traps at MBSR 56

Appendix 4 – Abundance of beetles collected in pitfall traps at MBSR 57

Appendix 5 – Invertebrate homogenisation procedure 59

Summary

Project and Client

- The goal of the Cape to City project is to restore indigenous biodiversity across 26 000 ha of productive landscape in the Hawke's Bay, through mammal control and habitat restoration. When restoring and managing native biodiversity within productive landscapes, it is particularly important to consider invertebrates, a diverse and functionally important component of biodiversity. Hawke's Bay Regional Council contracted Landcare Research to compare a novel technology, environmental DNA, with conventional invertebrate monitoring techniques within the Cape to City project, and to review the role of invertebrates and ecosystem services and the likely impact of mammals.

Objectives

- Briefly review the role of invertebrates in ecosystems services.
- Provide information on how invertebrates contribute to the diet of mammalian predators.
- Discuss how invertebrates may respond to release from mammal predation over the short and long term (50+ years).
- Characterise the invertebrate fauna, and in particular the beetle community, of Mohi Bush Scenic Reserve, using conventional invertebrate community monitoring techniques.
- Undertake an assessment of the invertebrate fauna of Mohi Bush Scenic Reserve using environmental DNA extracted from soil and conventional invertebrate community monitoring techniques, and compare and contrast these results.

Methods

- Google scholar, web of knowledge and expert knowledge was used to identify key literature on the role of invertebrates and ecosystem services in New Zealand.
- Twelve 20 x 20 m plots were located at Mohi Bush Scenic Reserve, six along the forest-pasture edge ('edge' plots) and six within the interior (>100 m from the forest-pasture edge) of the forest fragment ('interior'). Three groups of edge and interior plots were positioned along the northern and southern boundaries respectively of Mohi Bush Scenic Reserve.
- Within the 12 plots, flying insects and foliage-dwelling invertebrates were sampled using malaise traps, while ground-dwelling invertebrates were collected with pitfall traps.
- DNA was extracted from 24 soil cores taken from the twelve plots. In addition, DNA was extracted from identified invertebrates collected from malaise and pitfall traps.

Results

- Invertebrates play an essential role in ecosystem services in New Zealand; examples include their roles in pollination, nutrient cycling and soil formation. Invertebrates can also be a large and important component of the diets of mammalian predators, especially for hedgehogs and rats. It is unclear how invertebrates respond to predator control in New Zealand; however, it appears large-bodied species such as weta do show increases in population after mammalian predator control. Invertebrate monitoring for the Cape to City project can provide key information on how mammalian predator control can influence invertebrate communities. Incorporating measures for ecosystem services is essential if the Cape to City project wish to understand if or how ecosystem services change due to release from mammalian predators.
- In total, 7503 invertebrates from 22 Orders were collected from the malaise and pitfall traps. In the malaise traps, invertebrate abundance was highest at edge plots and was significantly lower at the interior plots at Mohi Bush Scenic Reserve. Native beetles dominated the samples from all plots, with only a few introduced species being found, and the majority of these were from the edge plots. Ordination analyses showed that beetle communities differed between the edge and interior plots, reflecting differences in vegetation structure and composition, including average canopy height, canopy density, % introduced plant cover <0.3 m and % ground cover litter.
- Environmental DNA sampling detected 749 OTUs, spanning 4 different taxonomic Phyla (Table 2), 22 Orders and 102 Families. Only a small fraction (6%) of the total OTUs matched the reference sequence to a sufficiently high enough level to be considered a potential species-level match (>97% match), with 11% matching at >95% (approximately genus level) 35% matching at >90% (approximately family level). Ordination of OTUs showed clear separation between the communities from the bulk invertebrate samples and the soil samples, indicating that sample media had a significant effect on the observed invertebrate community. Within each of these sample media there was a clear distinction between samples from inside the forest fragment and from the edge for some Orders and not for others. Community ordinations of the bulk invertebrate beetle community and the eDNA-based beetle community were statistically correlated and showed similar ecological separation of forest edge versus forest interior.

Recommendations

- Invertebrates provide important ecosystem services and need to be considered as part of Cape to City's goal of enabling indigenous taxa to co-exist with human habitation, food production and recreation at large scales in an agricultural landscape.
- We recommend monitoring large-bodied taxa, such as the Hawke's Bay tree weta, because of their known responsiveness to mammal control. As this iconic tree weta species is restricted to the Hawke's Bay, it could stimulate public participation and ownership. Landcare Research is monitoring Hawke's Bay tree weta with artificial retreats focussing on sites with rat control. A further recommendation is to survey for rare and threatened species within the Cape to City footprint and determine whether

host-specific threatened invertebrate species are habitat or predator limited. This could be achieved through trial restoration plantings, including the host plant taxa in areas with or without predator control.

- Environmental DNA can provide high detail data on entire invertebrate communities for similar cost to conventional monitoring, which typically targets well-known groups such as beetles or weta. A number of areas for methodological improvement have been identified (e.g. more reference data, optimised bioinformatics pipelines, further comparisons with conventional data). These methodological issues need to be addressed before eDNA can be rolled out as an established monitoring technique for invertebrates within Cape to City.

1 Introduction

The Cape to City project was launched in the Hawke's Bay in 2015 with a strategic objective of enabling indigenous taxa to co-exist with human habitation, food production, and recreation at large scales in an agricultural landscape. The 5-year project involves integrating possum control with large-scale control of feral cats, stoats, and ferrets across 26 000 ha, with rats targeted at specific sites, and is supplemented by additional habitat restoration (Norbury & McLennan 2015).

Invertebrates constitute a substantial proportion (58%) of New Zealand biodiversity (Gordon 2010) and are critical to ecosystem functions such as pollination and nutrient cycling. Therefore, when restoring and managing native biodiversity within productive landscapes, it is particularly important to consider invertebrates. However, their inclusion in biodiversity monitoring and conservation planning and management has lagged behind better-known, more widely appreciated taxa. One of the reasons for this is because collecting and sorting invertebrates using conventional monitoring techniques is often expensive, time-consuming, and restricted by taxonomic expertise. However, as novel technologies have advanced, including environmental DNA, it could be easier and more cost-effective to undertake invertebrate monitoring.

Landcare Research is monitoring invertebrate presence and abundance using artificial retreats, weta houses, and funnel traps set beneath tree canopies within the Cape to City project focussing on sites where rat control will take place. In addition to this monitoring, Hawke's Bay Regional Council contracted Landcare Research to compare a novel technology, environmental DNA, with conventional invertebrate monitoring techniques within the Cape to City project. We also review the role of invertebrates and ecosystem services and the likely impact of mammals. This report is therefore divided into three sections. We begin with a brief review (Section 2) of the role of invertebrates and ecosystem services and the likely impact of mammals. With this background information, the remainder of the report investigates the possibility of using environmental DNA as an invertebrate monitoring tool within Cape to City. Specifically, we characterise the invertebrate fauna of Mohi Bush Scenic Reserve using conventional invertebrate monitoring (Section 3), and compare these results with environmental DNA analysis (Section 4). We conclude the report with overall recommendations (Section 5) arising from this research.

2 Invertebrates and ecosystem services review

Productive landscapes make up a large proportion of New Zealand's land area, including exotic forest (7.5%), pasture (39.8%) and cropping/horticulture (1.8%; Statistics New Zealand 2015). Maintaining and incorporating biodiversity within productive landscapes is a realistic goal for regions around New Zealand. Cape to City is a wide-scale predator control and ecological restoration project over 26 000 ha of land between Hastings and Cape Kidnappers, and extends southwards to include Waimarama and forest remnants at Kahuranaki. The strategic objective of Cape to City is that native species thrive where people live, work, and play (C2C 2016). The Cape to City project identifies agricultural primary productive landscapes as having a key role in achieving this vision (C2C 2016). Within the Hawke's Bay, the Cape to City project will achieve this vision through transformational change in pest management, research, and community engagement in ecological restoration initiatives. Hawke's Bay Regional Council requested Landcare Research to provide a short review of the role of invertebrates covering 3 major topics:

- Provide a summary of the key contributions that invertebrates make to ecosystem services.
- Provide information on how invertebrates contribute to the diet of mammalian predators.
- Discuss how invertebrates may respond to release from mammal predation over the short and long term (50+ years).

This review ends with a brief discussion on robust methods for invertebrate monitoring.

Google scholar, web of knowledge and expert knowledge were used to identify key literature on the roles of invertebrates and ecosystem services in New Zealand. Information was then briefly summarised for this report.

2.1 Ecosystem services and invertebrates

Ecosystem services are the benefits people obtain from ecosystems and can be divided into four key groups based on the Millennium Ecosystem Assessment (2003);

- Provisioning – products obtained from ecosystems, e.g. food, freshwater, fuelwood, fibre, biochemical, genetic resources
- Regulating – benefits obtained from regulation of ecosystem processes, e.g. climate regulation, disease regulation, water regulation, water purification, pollination, biological control.
- Cultural – nonmaterial benefits from ecosystems, e.g. spiritual and religious, recreation and ecotourism, aesthetic, inspirational, educational, sense of place, cultural heritage.
- Supporting – services necessary for the production of all other ecosystem services, e.g. soil formation, nutrient cycling, and primary production

Throughout New Zealand, invertebrates contribute directly and indirectly to a multitude of ecosystem services. Here we summarise the major contribution invertebrates make to ecosystem services in New Zealand across productive landscapes.

2.1.1 Provisioning services

Food

Invertebrates contribute to provisioning services in New Zealand both directly, e.g. through food production or filtering of water, and indirectly, e.g. through nutrient cycling. One of the major provisioning services an invertebrate species directly provides in New Zealand is honey production by the introduced honey bee (*Apis mellifera*). Over 12 000 tonnes of honey are produced in New Zealand each year, with half of it exported overseas, resulting in export earnings of $140 million and growing (MPI 2016).

For Māori three valued invertebrate kai species are kōura (freshwater crayfish; *Paranephrops planifrons* and *Paranephrops zealandicus*), kākahi (freshwater mussel; *Echyridella menziesi*), and takaka (Huhu grub) (NIWA 2016). Currently, commercial kōura aquaculture is present at low levels in New Zealand, but can be incorporated into productive landscapes. One commercial venture has used plantation forest water storage ponds to farm Kōura (Rae 2015).

2.1.2 Regulating services

Biological control of pests

Invertebrates can control crop-feeding insects and disease vectors through multiple pathways: parasitism, direct predation, altering pest behaviour, and transmission of bacteria, viruses or toxins to the pest species. Invertebrates control invasive weeds by consuming biomass, reducing reproductive output or increasing plant susceptibility to stresses. Vegetation bordering crop fields can provide habitat for invertebrates that prey on pest species, and having a range of crop types within a landscape can enhance natural enemy populations, increase pest suppression and lower crop damage (Letourneau et al. 2011). Furthermore, creating more diverse vegetation types around crops can reduce pest abundance and enhance natural enemy abundance and parasitism rates within the fields (Morandin et al. 2014). The overall result is a reduction in pest abundances and subsequently lower needs for chemical pest control (Morandin et al. 2014).

Biological control can also involve the introduction of new species into the environment to control a pest species. In New Zealand there are many examples of introduced invertebrate biocontrol agents, including St John's wort beetle *Chrysolina* spp., which is used to control St John's wort. It is estimated that net present value of the introduced beetle is between $140 and $1490 million dollars (Hayes et al. 2013).

Pollination and seed dispersal

In New Zealand most agricultural products (e.g. apples, kiwifruit and avocados) and some pastoral species (e.g. clover) depend on animal-mediated pollination. Birds and invertebrates are New Zealand's main pollinators, with the introduced honey bee, *Apis mellifera*, the major pollinator in agricultural systems. *Apis mellifera* pollination services are estimated to contribute $5 billion to New Zealand's GDP (Newstrom-Lloyd 2013). New Zealand's native invertebrate pollinators tend to be solitary bees (Hymenoptera), flies (Diptera), moths and butterflies, (Lepidoptera) and beetles (Coleoptera). Native Diptera and hymenoptera are known to pollinate onion, brassica, radish, carrot and white clover crops in New Zealand (Newstrom-Lloyd 2013).

Dispersal services by invertebrates are not well studied in New Zealand; however, worldwide earthworms, ants, wētā, and grasshoppers are all known to disperse seeds. One experimental study of an agricultural weed found earthworms collected and buried 90% of seeds placed on the soil surface at a rate 8-fold faster than abiotic seed burial (Regnier et al. 2008).

2.1.3 Supporting services

Primary production

Primary production, i.e. biomass produced from photosynthesis, is influenced both positively and negatively by invertebrates. This influence can be direct, through consumption, pollination and seed dispersal (both covered in regulating services), or indirectly through trophic cascades, soil formation and nutrient cycling (see below). Herbivores and detritivores convert primary production into energy or other resources that benefit the food-web, i.e. higher trophic levels or lower subsystems. Herbivorous invertebrates can reduce primary productivity directly, especially during large population outbreaks by consuming plant material, resulting in reduced yields (Eubanks & Finke 2014). An example of an endemic pest invertebrate in productive landscapes is the widespread New Zealand native grass grub (*Costelytra zealandica)*. The larval stage of the grub feeds on the roots of plants, especially in grasslands and pastures, while the adult beetles consume foliage in horticultural systems, e.g. vineyards and orchards (AgResearch Limited 2016). At infestation levels of 200/m^2 in mixed clover grasslands, 11–44% of the grass component can be lost, up to 49% in pure ryegrass swards (East et al. 1979; Zydenbos et al. 2011). Invasive introduced pests can also cause severe losses in productive landscapes. The clover root weevil (*Siltona lepidus*) was first discovered in 1996 and now is found throughout the country. The young larvae feed on the roots of clover, reducing clover production by ca 35% in the North Island (AgResearch Limited 2016). MAF have estimated clover root weevil may cost New Zealand between $200m and $1b per annum (AgResearch 2016).

Nutrient cycling and soil formation

Invertebrates play an important role in nutrient cycling and soil formation in both terrestrial and aquatic systems. They influence soil formation through the mixing and redistribution of sediments (bioturbation), the erosion of mineral rock (bioerosion), the alteration of soil porosity, and the decomposition of organic material. In soil, annelids (earthworms) often represent the largest component of animal biomass and are commonly referred to as "ecosystem engineers" (Blouin et al. 2013). Depending on the species, earthworms compact and loosen soil, and can contribute to soil erosion. In a New Zealand pasture the presence of earthworms significantly increased the amount of sediment in run-off (due to surface casts) but reduced surface run-off two fold (Sharpley et al. 1979; Blouin et al. 2013).

Invertebrates can redistribute and alter nutrient availability within ecosystems through the consumption and egestion of plants and detritus, and by physically moving materials and disturbing sediments via bioturbation and bioerosion. Direct impacts by earthworms can be species specific; however, in general they accelerate organic matter degradation through reducing matter to smaller sized particles (Blouin et al. 2013). Nitrogen mineralization is enhanced directly through the release of their metabolic products (casts, urine, and mucus that contains NH_4+, urea, allantoin and uric acid), and dead tissue, or indirectly through changes in soil physical properties and fragmentation of organic material, and through interactions with other soil organisms (Blouin et al. 2013). Another key invertebrate in nutrient cycling is the nematode. Nematodes have been linked to increased leaching in nitrogen and dissolved organic carbon (de Vries et al. 2013). Bacterivorous and predatory nematodes are estimated to contribute to 8% and 19% of nitrogen mineralisation in conventional and integrated farming systems respectively (Neher 2001).

Within aquatic environments, one of the most important roles invertebrates play is to break down and recycle organic matter (Macadam & Stockan 2015). Invertebrates break down plant detritus, turning it into dissolved organic matter (DOM), fine particulate organic matter (FPOM), and living biomass. Filter-feeding invertebrates remove POM and redistribute nutrients in the water column (Macadam & Stockan 2015).

2.1.4 Cultural services

Sense of place and inspiration

A key aspect of the Cape to City Project is participation by the public, iwi, and landowners. Iconic native invertebrates can capture the imaginations of people, helping build a greater connection to the environment. Examples of iconic New Zealand invertebrates include wētā, peripatus (ngaokeoke), huhu beetle, puriri moth, freshwater crayfish (kōura), Powelliphanta snails and giant earthworms (DOC 2016a). Increased visibility (due to increased invertebrate populations) and understanding of native invertebrates will help inspire participation in conservation programmes. Invertebrates are good candidates for reintroductions or population supplementation for several reasons: 1) high rates of reproduction; 2) the ability of some, e.g. wētā, to thrive in captive breeding programmes; 3) easy adaptation of some invertebrates to highly modified habitats; and 4) they often require smaller areas to survive

than vertebrates, allowing them to survive in tiny fragments of original habitat often found across productive landscapes (DOC 2016a).

Education and Ecotourism

Invertebrate biology can be successfully incorporated into education programmes. This is being demonstrated already with the Cape to City project, which has a 5-year education programme. Robyn McCool and the "the Bug Man" Ruud Kleinpaste have been working with teachers and school children to educate them about invertebrates and their habitats (C2C 2016).

Tourism is New Zealand's largest non-primary sector export earner, with international tourism contributing 11.8 billion to the economy (Tourism New Zealand 2016). The main reason for tourist visits to New Zealand is "its spectacular landscapes and natural scenery"; its "environmentally friendly image" is rated in sixth place (Tourism New Zealand 2016). Seventy-two percent of visitors rate New Zealand's environmental management among the best or ahead of most countries (Tourism New Zealand 2016). The Cape to City project, including conservation land and sanctuaries within Hawke's Bay, can provide tourists with hands-on experience of New Zealand conservation techniques (restoration and predator control) and provide opportunities for interactions with iconic endemic taxa, including glowworms (*Arachnocampa luminosa*) and wētā.

2.2 Invertebrates, mammalian predators and predation pressure

Hedgehogs, rodents, cats, possums and mustelids are being controlled as part of the Cape to City project. Each of these species has different dietary preferences, with invertebrates contributing varying amounts to their diets. Across all mammal diets, the proportion of invertebrates will vary seasonally, annually, and by habitat, based on availability of prey (Innes et al. 2010).

2.2.1 Invertebrate component of mammalian diets

Hedgehogs

Hedgehogs (*Erinaceus europaeus*) have a predominately insect diet (Moss & Sanders 2001; Jones & Toft 2006), with Innes et al. (2010) suggesting hedgehogs consume 89% of the estimated 740 g of invertebrates consumed per hectare per night by introduced mammals in North Island podocarp-broadleaved forest. The few studies of hedgehog diets in New Zealand have shown substantial variation in the invertebrate species eaten, which is dependent on the surrounding invertebrate populations and landscape use. Beetles (Coleoptera), including agricultural pests such as *Costelytra zealandica*, are often a dominant component of hedgehog diets (Campbell 1973). Butterflies and moths (Lepidoptera), especially at the larval phase, are also common. At Boundary Stream Mainland Island in the Hawke's Bay, hedgehogs were also found to target millipedes (Berry 1999). Consumption of soft-bodied invertebrates such as earthworms or slugs is likely to be

underestimated due to difficulties in identifying remains (Jones & Toft 2006). Native invertebrates, such as wētā, juvenile snails, earthworms, and rare giant centipedes can be part of hedgehog diets (DOC 2016b).

Rodents

Norway rats (*Rattus norvegicus*) and ship rats (*Rattus rattus*) eat a variety of foods, including plant material (seeds, seedlings, bark, fruit, foliage), vertebrates, and invertebrates. Invertebrates frequently consumed by Norway rats include beetles, spiders, wētā, and flies (Innes 2001). Innes et al. (2010) suggested that ship rats probably consumed a mean of 39 g of invertebrates per hectare per night in North Island podocarp–broadleaved forest and frequently consume wētā, cockroaches, harvestmen, spiders, and beetle larvae (Innes 2001). A study in the Pureora Forest Park during the first year after control, found the invertebrate component of ship rat diets increased as the population increased; this stabilised in the second year as the population stabilised (Sweetapple & Nugent 2007). Mice (*Mus musculus*) have been implicated in the decline of invertebrate populations in New Zealand (Brignall-Theyer 1998), with litter-dwelling caterpillars, beetles, and ground weta at particular risk from predation (Ruscoe & Murphy 2005). Van Aarde et al. (2004) and Miller and Webb (2001), while acknowledging that mice consume large numbers of invertebrates, suggest mice were unlikely to regulate invertebrate populations. However, mice may limit invertebrates directly by predation or indirectly by competition for food such as seeds, fruits, and other invertebrates (Watts et al. 2014).

Cats

Invertebrates can be a significant proportion of feral cat diets, occurring in 36% of feral cat guts in the Mackenzie basin (Murphy et al. 2004). Murphy et al. (2004) found prey included the locally endemic and endangered robust grasshopper (*Brachaspsis robustus)*. Domestic cats in urban environments also hunt invertebrates, with 47% of cat prey items brought into Auckland owners' homes being invertebrates (Gillies & Clout 2003). Common prey taxa of cats are cicadas, praying mantis, crickets, wētā, lepidopterans (moths and butterflies) and other orthopterans (grasshoppers) (Gillies 2001; Gillies & Clout 2003). The majority of invertebrates were brought in by cats that were less than 6 months old, supporting the evidence that invertebrates are common prey for young cats (Gillies 2001; Gillies & Clout 2003).

Possums

Foliage followed by flowers and fruit comprise the bulk of possum diets in New Zealand. However, their diet also routinely includes invertebrates. For example, in the Orongorongo valley, 45% of possum faeces contained invertebrate remains (Sadleir in Montague et al. 2000). Possums consume a large range of invertebrates, including Phasmatodea (stick insects), Hemiptera (plant bugs, cicadas), Orthoptera (grasshoppers and wētā), Coleoptera (beetles), Acari (ticks, mites), and native snails (Sadleir in Montague et al. 2000). At Waihaha, the insect larvae component of possum diets increased from 1% to 7% after the

possum population was reduced by >90%, suggesting that insect larvae might be a highly valued food source (Nugent et al. in Montague et al. 2000).

Mustelids

Stoats, weasels, and ferrets are flexible and opportunistic in their diet preferences. In addition to birds, lizards and other vertebrates, invertebrate taxa are an important part of stoat diets in New Zealand (Murphy et al. 2004). From 44 stoat guts from lowland podocarp forest in South Westland, 81.8% had invertebrates present, which is 44.3% of the total volume. In non-forest areas of the seaward Kaikoura Mountains, the alpine Murchison Mountains, and McKenzie Basin, invertebrates occurred in 7.9%, 33–59%, and 25% of stoat guts respectively (King et al. 2001; Smith & Jamieson 2003; Murphy et al. 2004). Ferret diets are more rabbit focused, with invertebrate occurrences in ferret guts ranging between 8.1 and 20% (Smith et al. 2995). Although diet preference studies have often not identified specific invertebrate taxa in mustelid guts, wētā have been noted (DOC 2016c).

2.2.2 Expected impacts of predator control

Population density, body size, and meal size of mammal predators are key factors in determining the impact of mammalian predators on invertebrates. Innes et al. (2010) estimated the mean weight of invertebrates eaten per hectare per night in North Island broadleaved–podocarp forest by some mammals as 740 g, with hedgehogs consuming the majority (89%).

Impacts from mammalian predators can be direct through predation, or indirect through intermediary taxa, e.g. suppression of an invertebrate predator allowing invertebrate prey populations to increase. The interactions between mammalian pest reduction and invertebrate populations can be complex and hard to predict. For example, the removal of mammalian pests is likely to coincide with increases in insectivorous bird species, resulting in varied responses of invertebrate populations (Table 1). In addition to the complexity of foodweb dynamics, a lack of studies that examine the impacts of mammal eradication on invertebrate populations in New Zealand hampers predictive scenarios for many invertebrate taxa. Watts et al. (2014) suggested that major changes in invertebrate communities should not be expected after mammalian predator control, although populations of large-bodied invertebrates may increase. Apart from abundance, invertebrate behaviour may also change with a reduction in mammalian predators. In Fiordland, tree wētā, spiders, and cockroaches have been shown to have stronger escape responses on a rat-invaded island compared with those on a rat-free island (Bremner et al. 1989). Predicting the impact of reducing the densities of one or a few key insectivorous mammal species (e.g. rodents and/or hedgehogs) compared with a suite of mammalian predators that are less likely to feed on invertebrates (e.g. cats, mustelids and possums) remains unknown. As invertebrates are the dominant prey items in the diet of rodents and hedgehogs it is likely that controlling these taxa would make the most difference to invertebrate populations (Watts et al. 2014).

Table 1 Summary of papers looking at impacts of mammal control on invertebrates in New Zealand. Common names provide guidance of species in the taxonomic group

Invertebrate Taxa	Controlled Species	Result	Location	Reference
Wētā species: *Hemideina thoracica*, *Hemiandrus pallitarsis*, *Gymnoplectron* species	Ship rat, mouse, cat, stoat, weasel, ferret, hedgehog, rabbit, hare, possum, red deer, goat and pig	Increase in abundance for all wētā species	Maungatautari (eradication except mice)	Watts et al. (2011)
Beetles (282 species)	Ship rat, Norway rat, house mouse, feral cat, stoat, weasel, hedgehog, rabbit, hare, brushtail possum, fallow deer, feral goat, feral pig and feral cattle.	Overall abundance and species richness of beetles declined	Zealandia (eradication except mice) and Otari-Wilton's Bush, Wellington (sustained control a few pests only)	Watts et al. (2014)
Wētā (*Hemideina thoracica* and Rhaphidophoridae) , spiders, and cockroaches	Rats – species not defined (possums partially)	Only *Hemideina thoracica* abundance increased after control	Whirinaki Forest Park and Mokaihaha Ecological Area; (short-term control, various mammals)	Ruscoe et al. (2013)
Carabidae (ground beetles), Amphipoda (Crustaceans), Scarabaeidae (Beetles), Zoropsidae (Spiders), Hemiptera (true bugs), Diptera (True flies), Formicidae (Ants), Orthoptera (Wētā, crickets etc), Isopoda (Crustacean)	Cow, goat, pig, deer, cat, brushtail possum, Norway rat, Kiore (eradicated over a century)	Decrease in catch frequency and diversity in Carabidae and Amphipoda.	Kapiti Island (final eradication – kiore and Norway rats)	Sinclair et al. (2005)

2.3 Long-term changes in ecosystem services

Worldwide, little attention has been given to long-term temporal changes in ecosystem services across production landscapes (Birkhofer et al. 2015). Studies have often been short-term and based on experimental plots. Many factors will influence invertebrate communities and the ecosystem services they provide in the future, including climate change, new pest species (e.g. the Queensland fruit fly and the brown marmorated stink bug) and changes in land-management practices such as changes in insecticide use. In New Zealand, linking changes in invertebrate communities after predator control to changes in ecosystem services is an important direction for future research. Watts et al. (2014) made five key recommendations for conservation managers or researchers attempting to quantify the benefits of mammal removal or control on the insect communities (Fig. 1). Monitoring the quality of ecosystem services during invertebrate monitoring would allow analysis of the impacts of changes in invertebrate communities on ecosystem services. Cape to City should

consider monitoring ecosystem services before and after predator control in addition to their baseline monitoring of bird, mammal, and invertebrate species. The resulting data would provide invaluable information to help guide conservation managers on the true impacts of a "predator free" New Zealand.

Figure 1 Recommendations for long term monitoring of invertebrates after mammalian predator control (adapted from Watts et al. 2014).

3 The invertebrate fauna at Mohi Bush Scenic Reserve, Hawke's Bay, assessed using pitfall and malaise traps

3.1 Introduction

Although almost all the lowland and coastal forest in Hawke's Bay occurs in very small fragments (< 100 ha) surrounded by pasture, vineyards or exotic forestry, these fragments provide a major opportunity for conservation of indigenous biodiversity within a modified landscape. When restoring and managing native biodiversity within productive landscapes, it is particularly important to consider invertebrates, not only for conservation of overall biodiversity, but because invertebrates are critical to ecosystem function. However, collecting and sorting invertebrates using conventional invertebrate community monitoring techniques is often expensive and time-consuming, and is restricted by taxonomic expertise. Innovative technologies, including environmental DNA, have recently been developed that could potentially overcome these limitations and make it easier and more cost-effective for regional councils and community groups to undertake invertebrate monitoring, for example, to evaluate the performance of restoration projects within productive landscapes.

Because conventional invertebrate monitoring programmes are expensive, time-consuming, and rely on specialist knowledge, there is a paucity of documented information on indigenous invertebrate assemblages surviving in forest fragments within productive landscapes. Within the Hawke's Bay, the Department of Conservation considers Mohi Bush Scenic Reserve (MBSR) to be the best example of a lowland native forest fragment in the region. It is likely that taxonomists with an interest in certain invertebrate taxa will have surveyed within MBSR, but there are few published accounts of their findings. One exception is Seldon (2015), who described a unique species of ground beetle (Carabidae) only known from MBSR. To the authors knowledge there are no published accounts of entomological community surveys at MBSR.

3.2 Objectives

The objective of this survey was to characterise the invertebrate fauna, and in particular the beetle community, of MBSR within the Cape to City project. These data will allow comparison between conventional invertebrate community monitoring techniques and novel environmental DNA surveys within the same plots at MBSR (4.4.3). Invertebrates were sampled from within and on the edge of Mohi Bush Scenic Reserve to allow comparison between these locations.

3.3 Methods

3.3.1 Study area and design

MBSR (61 ha) is a remnant of partially logged podocarp/broadleaved forest on the Maraetotara Plateau, within the eastern Hawke's Bay Ecological District. The vegetation is dominated by tawa (*Beilschmiedia tawa*), pigeonwood (*Hedycarya arborea*) with scattered miro (*Prumnopitys ferruginea*), and matai (*Prumnopitys taxifolia*; John MacLennan, pers. comm.) and the fragment is approximately 1.5 km long by 325 m wide (Fig. 2). Possum control using bait stations occurs within MBSR and rodent control is planned to commence in summer 2015/2016.

Figure 2 Map of Mohi Bush Scenic Reserve in the Hawke's Bay showing locations of sampling plots.

A total of twelve 20 x 20 m plots were located at MBSR: six along the forest-pasture edge ('edge' plots) and six within the interior (>100 m from the forest-pasture edge) of the forest fragment ('interior' plots). Plot locations were determined by placing points 25 m apart along the northern and southern pasture-forest boundary of MBSR using an aerial photograph and a programme implemented through a purpose-built extension to ArcView 3.2 (ESRI 1999). In the field, edge plots were rejected if they 1) were less than 120 m apart, 2) contained forest trees that had fallen into the pasture interrupting the abrupt pasture-forest edge, or 3) contained large amounts of ongaonga (*Urtica ferox*). Three edge plots

were located along the northern ('N' plots) and 3 along the southern ('S' plots) boundaries of MBSR and were numbered 1–3 (Fig. 2). Edge plots were positioned so that half the plot was in pasture and the remaining half was in the forest. From each edge plot, the interior plots were situated by moving 100 m on a bearing into the forest. The bearing for the N plots was 192° and for the S plots was 9°. As each interior plot had to be at least 120 m apart, the bearings were different for the N and S plots. Due to two interior plots being positioned on and very near tracks, the bearing and distance into the forest were slightly adjusted (forest N3 bearing 203°, distance 130 m; forest S1 bearing 6°, distance 131 m; Fig. 2). The easting and northing of each plot was recorded using a Garmin 60CSx GPS.

3.3.2 Invertebrate sampling techniques

There are numerous techniques available for sampling invertebrate communities, including pitfall traps, malaise traps, visual searching, suction traps, insecticide fogging, sticky traps, light traps, and sweep-netting, which guarantee rapid acquisition of considerable collections and provide researchers with specimens. The method chosen for sampling often depends on the invertebrate group selected for study. In the present study, pitfall traps were used to sample the ground-dwelling invertebrate fauna and malaise traps were used to collect the flying insect fauna inhabiting foliage. Both types of traps are passive, easily transported and installed in the field, and can be left unattended for several weeks.

Numerous studies in New Zealand have shown that invertebrates are frequently caught in traps between November and February when there is a peak in invertebrate activity and abundance (Moeed & Meads 1985, 1987; Hutcheson 1990; Hutcheson & Jones 1999; Watts & Gibbs 2002). The survey at MBSR therefore focussed on December–January as an optimum sampling period.

Sampling the invertebrate fauna using malaise traps

Malaise traps, which resemble open-sided tents made of fine mesh cloth, were used to collect insects that fly or are blown into the trap (Townes 1972; Moeed & Meads 1987; Hutcheson 1990; Hutcheson & Jones 1999). The standard malaise trap design used in forest ecosystems was modified to endure the increased exposure to wind in New Zealand. This trap design has been extensively tested and is now used routinely to sample invertebrates within New Zealand wetlands (Watts et al. 2012; 2015). The design of the malaise trap (Fig. 3) remains the same, but the dimensions of the trap were halved. The two end poles were each secured to a flat wooden plate on the ground for increased stability. This mini-malaise trap was used to sample the invertebrate communities at MBSR.

At each plot (six edge and six interior plots, 12 malaise traps in total), one malaise trap was placed in the centre of the 20 x 20 m plot. At the edge plots the malaise trap was located in the forest (<3 m from the pasture-forest edge) so that it was not visible to the public from the pasture. On all malaise traps, the collecting jar containing 150 ml of 50% monopropylene glycol was orientated northward. Traps were set for 1 month from 10 December 2015 to 7 January 2016. Invertebrates were preserved in 70% ethanol.

Figure 3 A malaise trap used to collect flying insects, particularly flies, wasps and beetles.

Captured invertebrates were sorted and counted to Order level using a binocular microscope. Due to the lack of taxonomic knowledge of a number of invertebrate groups in New Zealand, we concentrated on identifying beetles to species and counting their abundance. Beetles are routinely selected for study in New Zealand as they (1) represent a large component of the invertebrate biodiversity, (2) account for approximately 65% of the known New Zealand insect fauna, (3) have representatives in all trophic groups, and (4) have a wide range of habitat preferences (Watts et al. 2008, 2015). Beetles were sorted on the basis of external morphology to recognised taxonomic units (hereafter referred to as species) and, where possible, given generic and species-level identifications by Stephen Thorpe (Research Associate, University of Auckland). Each species was classified as native, introduced or of unknown status.

Sampling the ground-dwelling invertebrate fauna using pitfall traps

Pitfall traps have been used extensively to sample ground-dwelling invertebrates in New Zealand (Moeed & Meads 1985; Kuschel 1990; Crisp et al. 1998; Reay & Norton 1999; Watts & Gibbs 2000, 2002; Watts et al. 2008); they rely on the invertebrate falling into the trap, which contains a chemical solution that kills and preserves the specimens. Ground-dwelling invertebrates were sampled using pitfall traps consisting of a 100-mm-deep plastic cup (105-mm diameter) containing 100 ml of 50% monopropylene glycol (Fig. 4). Four pitfall traps were placed 5 m away from the each corner of the malaise trap within the 20 x 20 m plot (total of 48 pitfall traps). Traps were set for 1 month from 10 December 2015 to 7 January 2016. Invertebrates were preserved in 70% ethanol. Captured invertebrates and beetles were sorted and counted as described above.

Figure 4 A pitfall trap used to collect ground-dwelling invertebrates. A plastic cup was sunk vertically into the ground so that the rim of the cup was flush with the ground. A cover (positioned beside the trap for the purpose of the photo) was held a few centimetres immediately above the trap to minimise the amount of debris and water entering the trap.

3.3.3 Vegetation sampling

Within each 20 x 20m plot, we estimated the % cover of each plant species in six height tiers (<0.3 m, 0.3–2 m, 2–5 m, 5–12 m, 12–25 m, and > 25 m) using the RECCE method outlined by Hurst & Allen (2007). From these data, the following variables were derived: maximum canopy height, average canopy height, canopy density, total number of plant species, total number of introduced plant species, total number of native plant species, and % introduced or native cover in each height tier. At the edge plots, individual pasture grass species were noted and then grouped into one category entitled 'pasture grasses' and estimated for each plot. The average % ground cover divided into vegetation, non-vascular (moss and lichen), litter and bare ground as well as the average ground cover height was estimated for each plot. In addition, the physiography (ridge, face, gully or terrace), slope (convex, concave or linear) and drainage (good, moderate, poor or very poor) were also determined.

3.3.4 Data analysis

Total invertebrate abundance

Means per plot ± 95% confidence intervals (CI) using GenStat 14 (VSN International 2013) were calculated for the total invertebrate abundance and the number of Orders collected using the malaise and pitfall traps so that differences (P=0.05) between the plots were apparent by inspection of graphical representation. Initially, plots were assessed by those located in 1) north versus south and 2) edge versus interior plots. However, there were no significant differences detected in the plots located in the north and south of MBSR so these data were combined and presented as edge versus interior plots.

Beetle community data

The average total beetle abundance and species richness were analysed as described above.

Variation in beetle species composition and abundance between plots were analysed using classification and ordination analyses in PATN multivariate analysis package (Belbin 1995). Classification differentiated the main beetle groups based on variation in beetle composition and abundance in each plot at the edge and interior plots. In addition, we used SSH scaling to compare the similarity of beetle species composition and their abundance between the plots. SSH ordination scores (indicating site similarity) were correlated with beetle species distributions using PCC analyses, in which taxa with the highest correlations have the most influence on the ordination patterns. A three-dimensional ordination with a stress value of 0.2015 was considered appropriate to summarise the beetle data from the malaise traps adequately (see Belbin (1995)). For the pitfall trap data, a stress value of 0.1868 (2-dimensional ordination) summarised the data satisfactorily, as solutions of other dimensions did not markedly change ecological interpretability (Belbin 1995). To examine the beetle species responses to the environmental variables recorded (distance from edge, maximum canopy height, average canopy height, canopy density, total number of plant species, total number of introduced plant species, total number of native plant species, and % introduced or native cover), we used a vector-fitting approach, also implemented within PCC; the length and angle of the vectors plotted on the ordination indicate the direction of best fit of each environmental variable and the strength of the correlation.

3.4 Results

3.4.1 Invertebrates collected by malaise traps

Total invertebrates

A total of 2981 invertebrates were captured representing 15 Orders (see Appendix 1). The most common Orders caught were Diptera (53.1%), Lepidoptera (21.9%), Hymenoptera (16.8%), and Coleoptera (11.5%). Overall, average invertebrate abundance was highest at edge plots (328±38.4 SE) and was significantly lower at the interior plots (160±4.8 SE; Fig.

5A). The number of Orders sampled did not significantly differ between the edge and interior plots sampled (Fig. 5B).

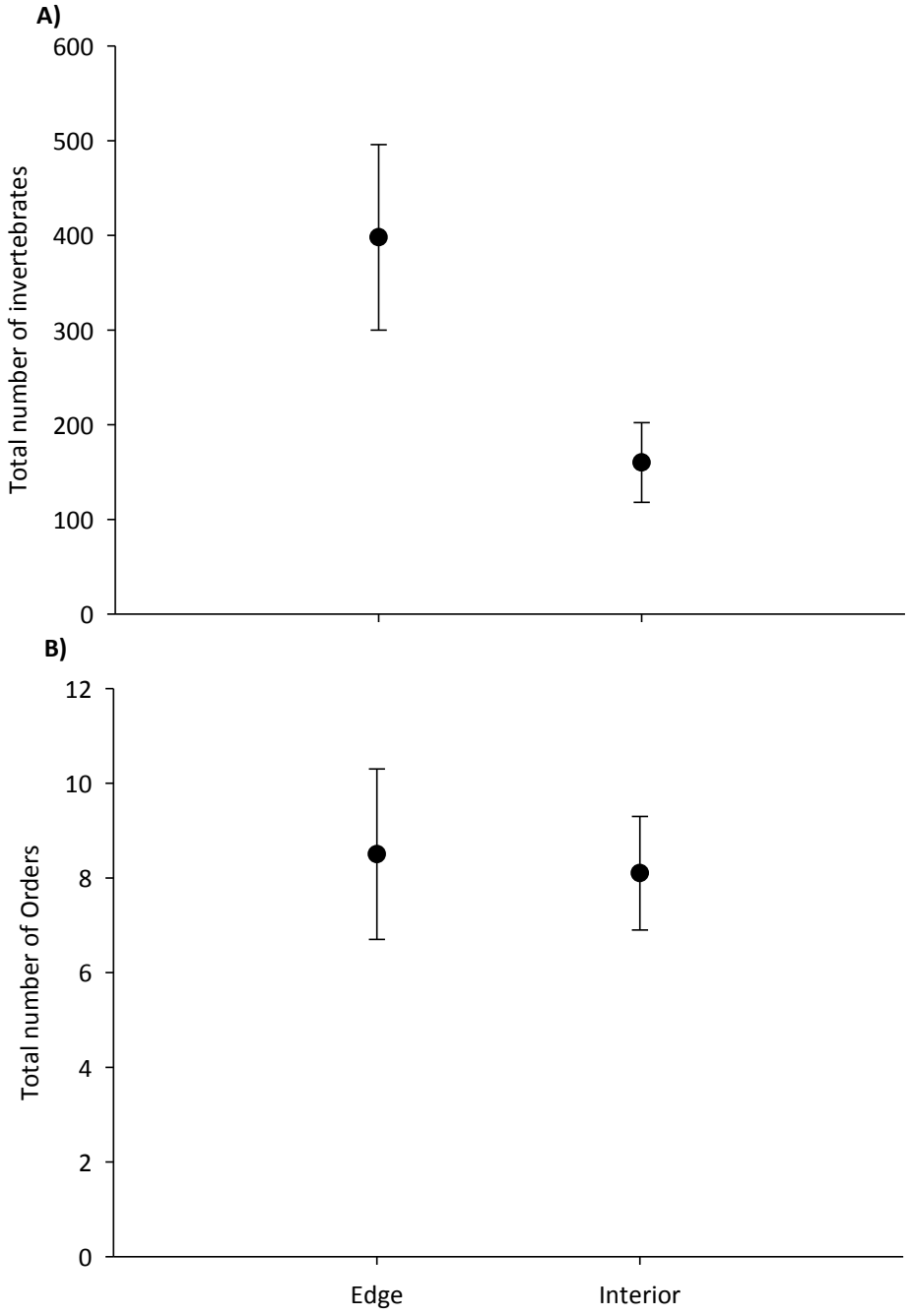

Figure 5 A) Average (±95% CI) total abundance and **B)** number of Orders of invertebrates collected from malaise traps (*n*=6) set at the edge and in the interior of Mohi Bush Scenic Reserve.

The majority of Diptera were sampled from the edge plots with the introduced striped dung fly (*Oxysarcodexia varia*) being dominant. Lepidoptera were also more abundant in the edge plots dominated by the introduced common blue butterfly (*Zizina labradus labradus*). The majority of Hymenoptera caught were adults of Ichneumonidae parasitic wasps. A frequently found species in the interior plots was *Xanthocryptus novozealandicus*.

Beetle communities

A total of 331 beetles, comprising 72 species, were collected (see Appendix 2). The most species-rich families in the samples were Curculionidae (weevils; 18 species), Cerambycidae (longhorn beetles; 6 species), and Anthribidae (fungus weevils; 6 species). The most abundant beetle caught was the native Chrysomelid *Eucolaspis* sp. 1 (12.5% of the total sample). Significantly higher beetle abundance (33.0±6.8 SE) was sampled in the edge plots than in the interior plots at MBSR (19.2±1.9 SE; Fig. 6A). There were no significant differences in the number of beetle species collected between the edge and interior plots sampled (Fig. 6B).

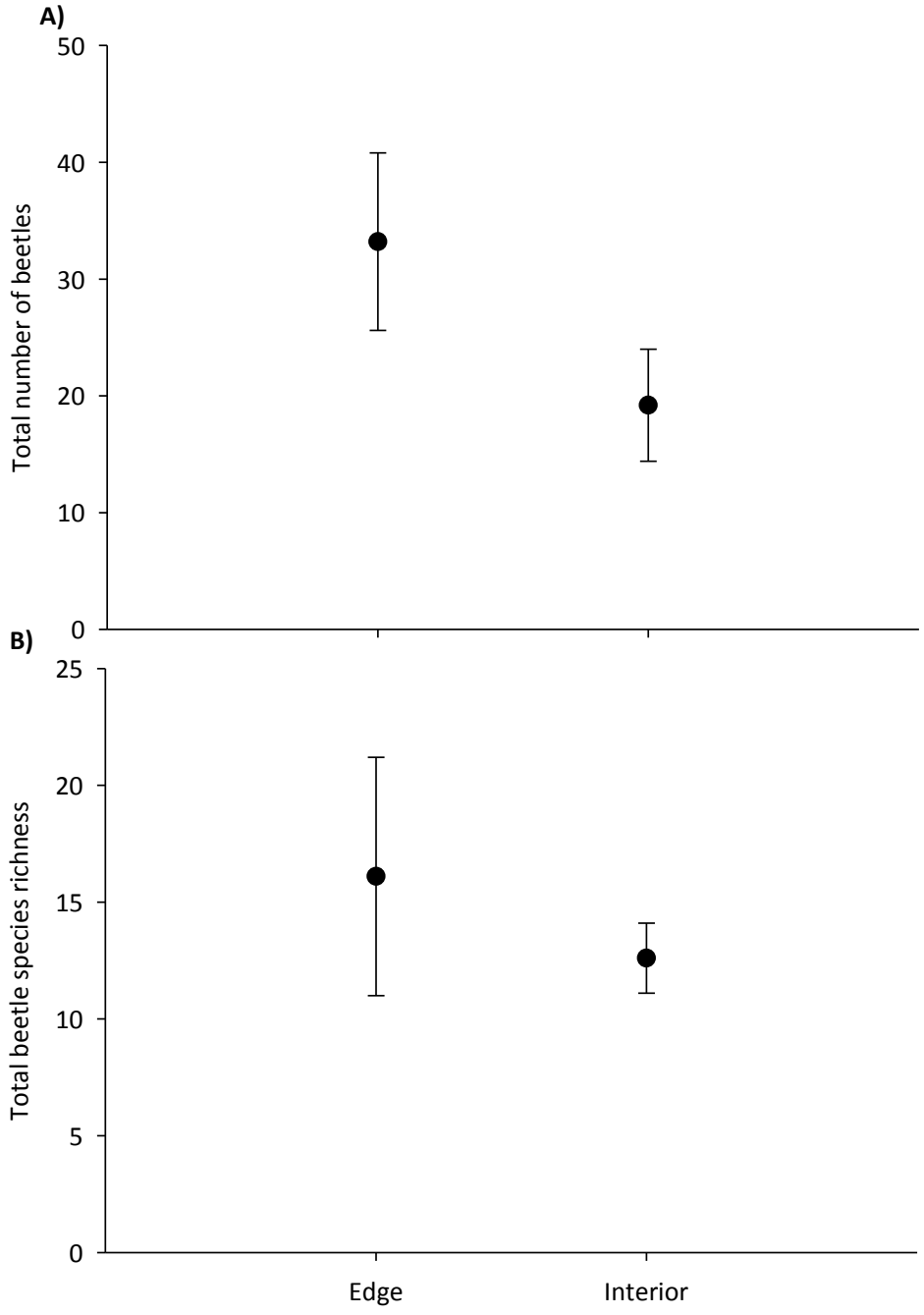

Figure 6 A) Average (±95% CI) total beetle abundance and **B)** number of beetle species collected from malaise traps (*n*=6) set at the edge and in the interior of Mohi Bush Scenic Reserve.

Of the total 72 beetle species caught, 63 were native species, 6 were introduced species, and the status of 3 were unknown (see Appendix 2). Native beetles dominated the samples from all plots, with 4% of species in the edge plots being introduced and only 1% of species in the interior plots being introduced.

The ordination based on species composition and abundance showed that beetle communities differed between the edge and interior plots (Fig. 7). Three groups with differing beetle species composition were identified by the FUSE clustering analysis, and these groupings were overlaid onto the SSH ordination to identify trends in the beetle community composition within the survey (Fig. 7). Beetles collected from the interior plots (Group I) had comparable beetle assemblages. The beetles collected from the edge plot S3 were Group II and were dominated by *Eucolaspis* sp. 1. The remaining edge plots formed a distinct group with similar beetle compositions (Group III; Fig. 7). There were distinct patterns in habitat variables in this ordination, reflecting differences in vegetation structure and composition, including average canopy height, canopy density, and % introduced plant cover <0.3 m (Fig. 7). The abundances of 28 (out of 72) beetle species were significantly correlated with plots along SSH axes in relation to the vegetation structure and composition. *Gerynassa* sp. 1 (Curculionidae) were associated with the interior plots (Group I), while *Hylobia sexnotata* (Melandryidae) were predominantly found within the edge plots (Fig. 7). *Eucolaspis* sp. 1 appears to be associated with the edge plot S3 (Group II), as 95% of the specimens were collected from that plot (Fig. 7).

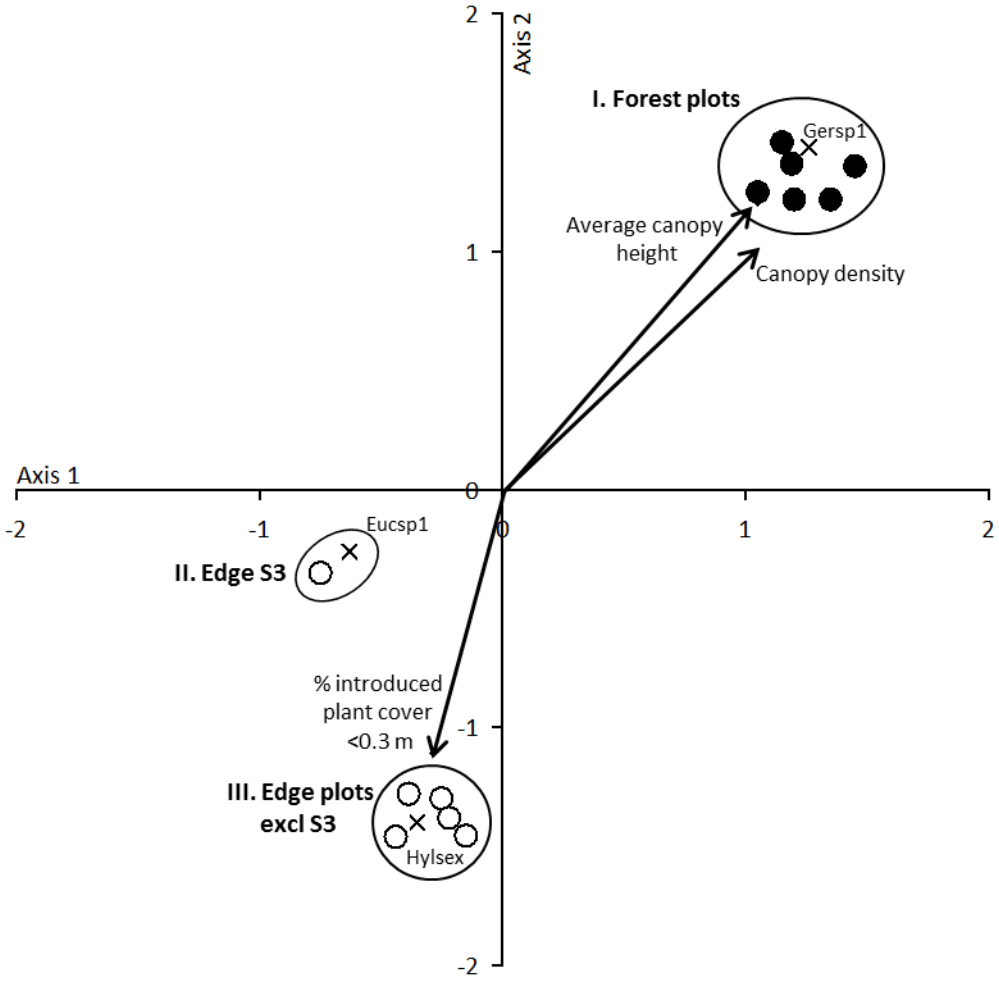

Figure 7 Distribution of beetle species (centroids = ×) caught in malaise traps (symbols) defined by the three-dimensional SSH ordination analyses from the edge and interior plots sampled at Mohi Bush Scenic Reserve. Plots which are closer together have similar beetle communities. Edge plots = open circles and interior plots = solid circles. Significant (*P*-value<0.01) environmental variables defined by the SSH ordination analyses. The length of the environmental arrow indicates the degree of corrections and the angle between the arrows shows the degree of intercorrelation in their effects on beetle community composition. Gersp1 = *Gerynassa* sp.1; Eucsp1 = *Eucolaspis* sp. 1; and Hylsex = *Hylobia sexnotata.*

3.4.2 Invertebrates collected by pitfall traps

Total invertebrates

In total, 4522 invertebrates from 20 Orders were collected (see Appendix 3). Collembola (28.0%), Coleoptera (13.5%), and Lepidoptera (13.4%) were the most abundant Orders caught. Overall, invertebrate abundance was similar in both interior and edge plots (Fig. 8A). The average number of Orders sampled at the plots ranged between 14 and 17, and did not significantly differ between the edge and interior plots (Fig. 8B).

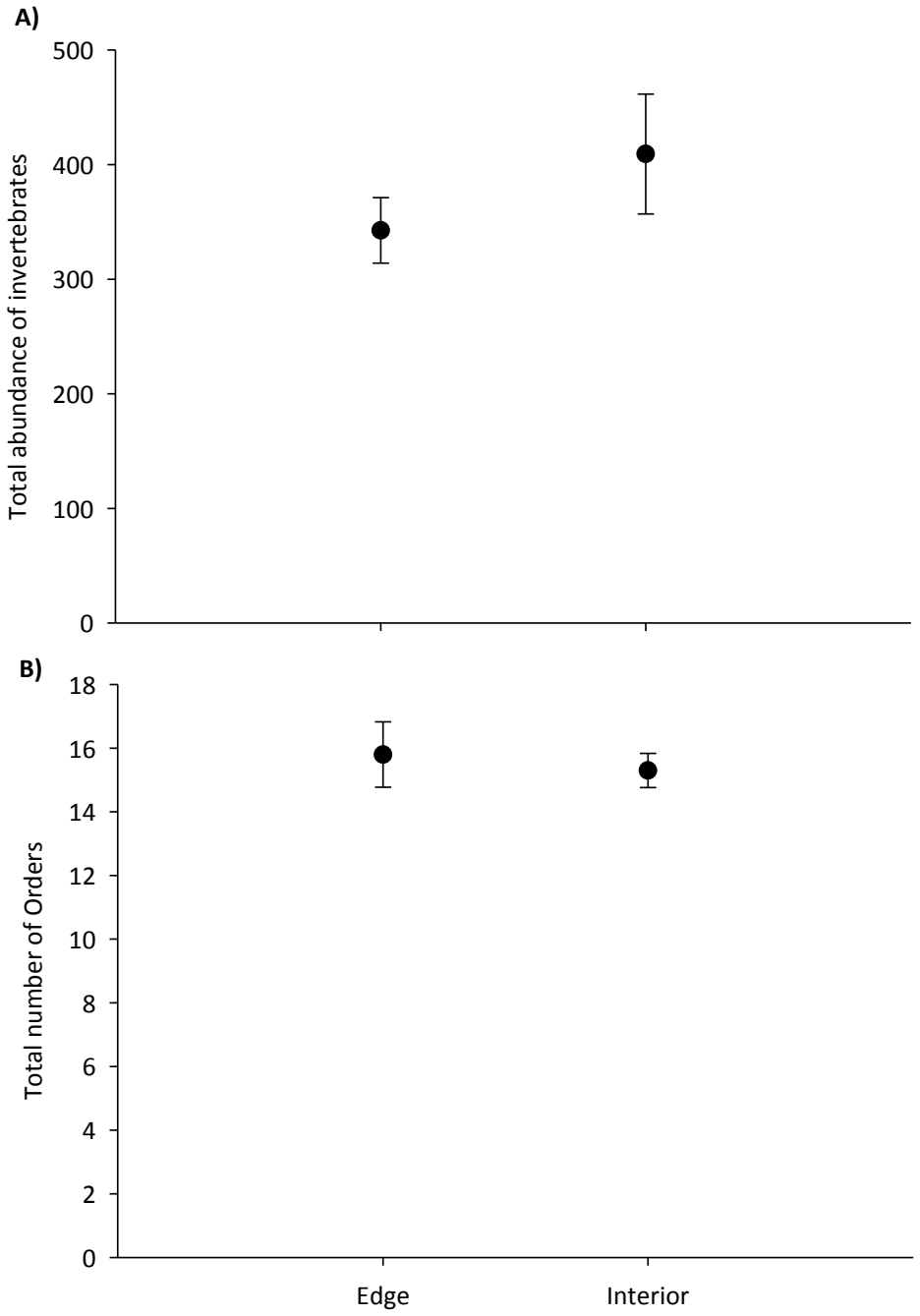

Figure 8 A) Average (±95% CI) total abundance and **B)** number of Orders of invertebrates collected from pitfall traps (*n*=6) set at the edge and in the interior of Mohi Bush Scenic Reserve.

Within the edge plots, large abundances of (271 individuals) of Orthoptera (crickets, weta, and grasshoppers) were found along with many Opiliones (harvestman). The Orthoptera collected from the edge plots were dominated (99%) by black field crickets (*Teleogryllus commondus*: Orthoptera). In contrast, no black field crickets were found in the interior plots where cave weta were common. Approximately, more than three times as many Collembola were collected from pitfall traps in the interior plots compared with the edge plots. In addition, Lepidoptera larvae were more abundant in the interior plots (546 specimens) compared to the edge plots (60 specimens). The larvae sampled from the interior plots were all individuals of litter-dwelling *Gymnobathra tholodella*.

Beetle communities

A total of 609 beetles, comprising 63 species, were collected (see Appendix 4). The most species-rich families in the samples were Carabidae (ground beetles; 12 species), Staphylinidae (rove beetles; 12 species), and Curculionidae (weevils; 10 species). The most common beetles sampled were *Ctenognathus* sp. 1 (Carabidae; 20.5% of the total sample) and *Mecodema oconnori* (Carabidae; 12.2% of the total sample). Overall, beetle abundance was similar in interior and edge plots (Fig. 9A). The average number of beetle species sampled at the plots ranged between 13 and 23 and did not significantly differ between the edge and interior plots (Fig. 9B).

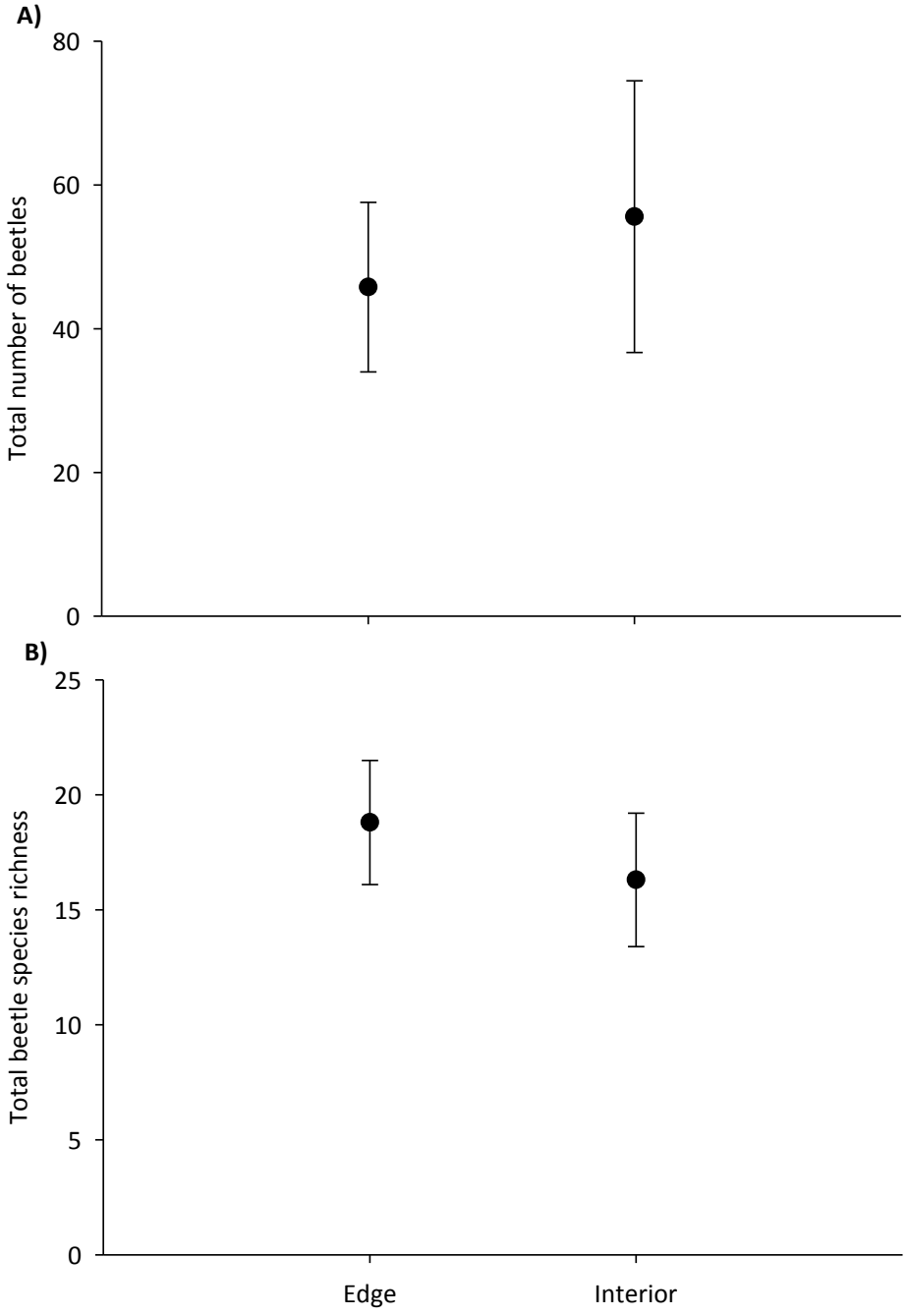

Figure 9 A) Average (±95% CI) total beetle abundance and **B)** number of beetle species collected from pitfall traps (*n*=6) set at the edge and in the interior of Mohi Bush Scenic Reserve.

Of the total 63 beetle species caught, 57 were native species, 4 were introduced species, and the status of 2 was unknown (see Appendix 2). Native beetles dominated the samples from all plots. Only 13 introduced beetles from 4 species (*Thyreocephalus chloropterus* (Staphylinidae); *Bruchidius villosus* (Chrysomelidae); *Harpalus affinis* (Carabidae) and *Anomotarus illawarrae* (Carabidae)) were captured, and 85% of these were collected from the edge plots.

The ordination analysis showed that beetle communities differed between the edge and interior plots (Fig. 10). Three groups with differing beetle species composition were identified by the FUSE clustering analysis, and these groupings were overlaid onto the SSH ordination to identify trends in the beetle community composition (Fig. 10). Beetles collected from the interior plots excluding plot N3 (Group I) had similar beetle assemblages. The beetle communities sampled at interior plot N3 were distinct and were dominated by *Ctenognathus* sp. 1. The edge plots formed a separate group with similar beetle compositions (Group III; Fig. 10). Variation in beetle species composition was explained by the environmental and vegetation variables – namely % introduced plant cover <0.3 m and % ground cover litter (Fig. 10). The abundances of 33 (out of 63) beetle species were significantly correlated with plots along SSH axes in relation to the vegetation structure and composition. The carabid *Mecodema oconnori* was associated with the interior plots (Group I), while another carabid *Ctenognathus* sp. 1 was predominantly found at the interior plot N3 (Group II; Fig. 10). The introduced rove beetle *T. chloropterus* and the native grass grub (*Costelytra zealandica*; Scarabaeidae) were clearly associated with the edge plots (Group III), as all specimens caught were from the edge plots (Fig. 10).

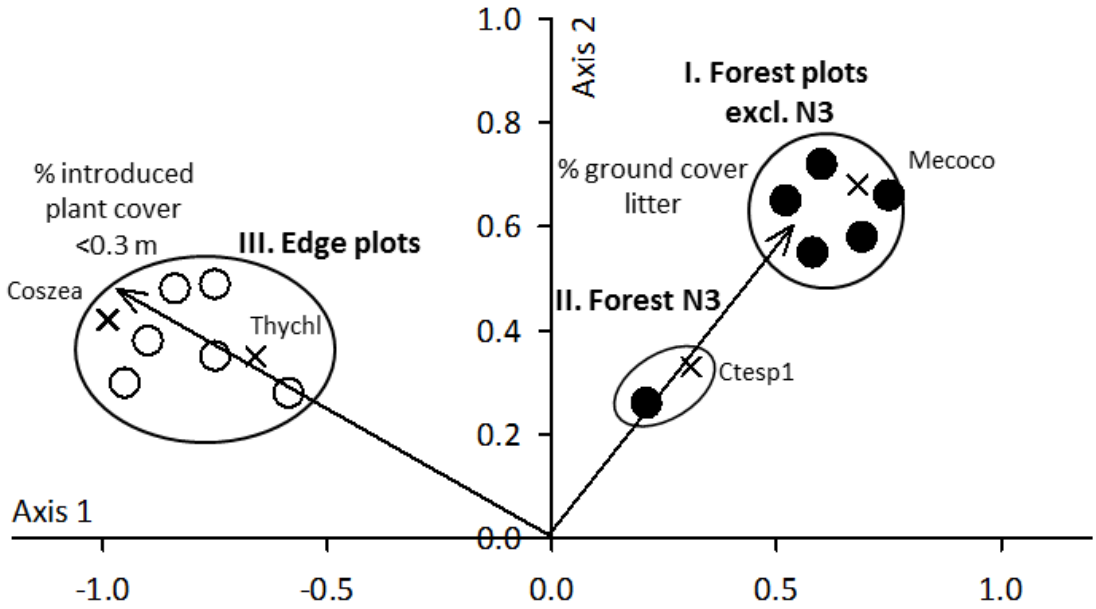

Figure 10 Distribution of beetle species (centroids = ×) caught in pitfall traps (symbols) defined by the two-dimensional SSH ordination analyses from the edge and interior plots sampled at Mohi Bush Scenic Reserve. Plots which are closer together have similar beetle communities. Edge plots = open circles and interior plots = solid circles. Significant (*P*-value<0.01) environmental variables defined by the SSH ordination analyses. The length of the environmental arrow indicates the degree of corrections and the angle between the arrows shows the degree of intercorrelation in their effects on beetle community composition. Mecoco = *Mecodema oconnori*; Ctesp1 = *Ctenognathus* sp. 1; Thychl = *Thyreocephalus chloropterus;* and Coszea = *Costelytra zealandica.*

3.5 Discussion

3.5.1 The invertebrate fauna at Mohi Bush Scenic Reserve

The samples from malaise traps were dominated by Diptera, Lepidoptera, Hymenoptera, and Coleoptera. In addition, some non-flying invertebrates were also collected, such as Araneae (spiders) and harvestman, which presumably climbed into the traps. Due to the surrounding intensively pastoral landscape, a number of introduced taxa were found in the edge plots. For example, a large number of introduced striped dung fly were caught. This species was accidently introduced from South America and is common in summer when it breeds in sheep and cow dung. In addition, Lepidoptera at the edge plots were dominated by the introduced common blue butterfly. This small butterfly is frequent in farmland where its caterpillars eat legumes such as white clover.

A frequently found native parasitic wasp in the interior plots was *X. novozealandicus,* which is widespread and common in New Zealand. It has been recorded parasitizing several species of longhorn beetles (Ward & Schnitzler 2013). The large abundances of *X. novozealandicus* found in MBSR could be a result of the surprisingly high diversity of longhorn beetles (6 species) found in the interior plots.

As expected, pitfall trap samples were dominated by ground-dwelling taxa, including Collembola, Coleoptera, and Lepidoptera. As with the invertebrate assemblage sampled with malaise traps, introduced taxa were more common in the edge plots. For example, large abundances of black field crickets were found. This insect is a serious pasture pest in northern New Zealand and can reach plague proportions over summer. In contrast, no black field crickets were found in the interior plots where native cave weta were common. Approximately, more than 3-times as many Collembola were collected from pitfall traps in the interior plots compared with the edge plots probably relating to a better quality and quantity of leaf litter present. In addition, Lepidoptera larvae were more abundant in the interior plots compared with the edge plots. The larvae sampled from the interior plots were all individuals of the case-bearing litter browser, *G. tholodella*, which feeds on freshly fallen green leaves and is a widespread endemic species that can be abundant in the litter of native forests over the summer months (Dugdale 1996).

Hawke's Bay tree weta (*Hemideina trewicki*) is restricted to southern and central Hawke's Bay, inhabiting urban gardens and bush remnants (Morgan-Richards 1995). It is locally sympatric with Auckland tree weta (*H. thoracica*) in the northern area of its range where individuals from both species may be found in different holes on the same tree. Hawke's Bay tree weta is known from MBSR but was not found in the present study.

3.5.2 The beetle community found at Mohi Bush Scenic Reserve

Larger abundances of beetles were caught in malaise traps in the edge plots than in the interior plots. In contrast, Harris and Burns (2000) found that the abundance and species richness of malaise trapped beetles was higher within kahikatea (*Dacrycarpus dacrydioides*) fragments in the Waikato than in the surrounding pasture. The result in the present study could be because the edge plots were a mixture of pasture and forest and had increased

availability of a diverse range of habitats in which different beetles could survive. For example, the presence of pasture allows the herbivorous grass-infesting Argentine stem weevil (*Listronotus bonariensis*) to persist within an edge plot along with a variety of fungus weevils which are associated with deadwood and litter in the forest (Holloway 1982).

The pitfall traps found a high diversity of carabids particularly within the interior plots at MBSR. *Mecodema oconnori* was common in interior plots at MBSR. Elsewhere in New Zealand it is frequently found in podocarp-broadleaf forests (Larochelle & Lariviere 2001). Another carabid *Ctenognathus* sp. 1 dominated the beetle fauna collected from the interior plot N3. This genus is mostly restricted to North Island where it inhabits wet native forests; it is commonly found on streambanks and in seepages with ferns present (Larochelle & Lariviere 2001). The vegetation at plot N3 was different from the other interior plots within MBSR and was dominated by *Dicksonia squarosa* and situated 5 m above a wet gully. The introduced rove beetle *T. chloropterus* and the native grass grub (*Costelytra zealandica*; Scarabaeidae) were clearly associated with pasture present in the edge plots, as all specimens caught were from the edge plots. The native grass grub is considered a pest species in productive landscapes as the larvae feed on the roots of plants, especially in pastures, while the adult beetles consume foliage, particularly in agricultural ecosystems. All introduced *Thyreocephalus* species in New Zealand came from Australia and help control insect pests in pasture (Eyles 1973).

For both malaise- and pitfall-trapped beetles, the communities differed between the edge and interior plots. Variation in beetle species composition was explained by vegetation variables such as % introduced plant cover <0.3 m, % ground cover litter, average canopy height, and canopy density. These results agree with other studies, which suggest that the composition of beetles present at a site is correlated with habitat characteristics and vegetative composition/physiognomy (Grimbacher & Catterall 2007; Watts et al. 2008).

It is encouraging that the beetle fauna from MBSR were dominated by native taxa, which indicates that relatively small areas of forest are useful for native invertebrate conservation. Considering that MBSR is a 'small' forest fragment within a pastoral landscape, it was surprising that few introduced beetle species were collected and that the majority of these were found in the edge plots suggesting that the beetle community is persistent. Watts et al. (2014) also found very few (1.1%) introduced beetle species in Zealandia despite its urban location.

The majority of species caught in the present study were small in size (<10 mm), which is distinctive of beetle communities in modified ecosystems. Habitat fragmentation and the presence of a suite of mammalian predators, especially rodents, strongly filter invertebrate communities and produce a fauna with few large representatives.

No threatened beetle species were found in the survey. *Mecodema chaiup* sp. nov. has been recently described and is known from only one specimen that was collected from MBSR. This large (31 mm in length) reddish/brown beetle was hand collected from under a log near the north-eastern edge of MBSR (Seldon 2015). It was hoped that the pitfall trap survey in this study would find the taxon but no specimens were found. Seldon (2015) notes that other smaller native forest fragments within the Maraetotara Plateau are likely to have *M. chaiup* sp. nov. present but have not been surveyed.

Using both malaise- and pitfall-trapped beetles, beetle abundance showed the same trends as general invertebrate abundance. Beetles have been considered to be representative of insects in general (Hutcheson 1990) largely because of their functional and taxonomic diversity (New 2010). Preliminary research in New Zealand wetlands indicates that beetles could potentially be used as biodiversity indicators as they reflected similar short-term (few months) patterns of change in abundance observed across total invertebrate abundance and some Orders (e.g. Lepidoptera, Diptera and Araneae) (Watts et al. 2015). Further research is required regarding the suitability of beetles as biodiversity indicators in a range of ecosystems.

The abundance and diversity of the beetle community found within MBSR were comparable those found in kahikatea fragments in the Waikato (Harris & Burns 2000). Comparison with assembages within larger tracts of forest that have not undergone such fragmentation would be needed to determine those beetle species that may be missing. However, in the Hawke's Bay such undistrubed coastal ecossytems do not occur. Hutcheson (1996) conducted Malaise trapped beetles sampling in a large tract of *B. tawa* forest in the central North Island and caught between 140 and 430 beetles and between 53 and 99 sepcies for a 4-week sampling period in December. The trap catches from MBSR were lower but this could be due to varaiation in micro-climate and habitat between the two sites and because the mini-Malaise traps were used in the present study were smaller in size.

3.5.3 Possible outcomes of predator control on the invertebrate fauna at Mohi Bush Scenic Reserve

Predation of New Zealand's native invertebrate fauna by introduced mammals has been widely recognised as a major conservation concern (Buckley et al. 2012; Leschen et al. 2012; Mahlfeld et al. 2012; Sirvid et al. 2012; Stringer et al. 2012; Trewick et al. 2012). Although invertebrates are frequently reported in the diet of invasive mammals, few papers have quantified the impact of introduced mammals on native invertebrate populations or communities. In New Zealand, the majority of evidence regarding how mammals may affect invertebrate populations is derived from invertebrate response to island rodent eradications (Green 2002; Rufaut & Gibbs 2003; Sinclair et al. 2005), and to mainland rat control (Spurr 1996; Hunt et al. 1998; King 2007; Ruscoe et al. 2013). Eradication of mammals (particularly rodents) has usually resulted in altered invertebrate abundance (Green 2002; Watts et al. 2011; Watts et al. 2014), species richness (Sinclair et al. 2005), and behaviour (Rufaut & Gibbs 2003; Watts et al. 2011). Some invertebrates, however, have shown no response to rodent control (Craddock 1997; Van Aarde et al. 2004; Sinclair et al. 2005; Rate 2009). These studies illustrate that the interactions between reducing mammal densities and invertebrate populations can be complicated and complex to predict. For example, the removal of mammal pests is likely to coincide with increases in insectivorous bird species, resulting in varied responses of invertebrate populations (Sinclair et al. 2005; Watts et al. 2011). In addition to the complexity of food-web dynamics, a lack of studies examining the impacts of mammal control or eradication on invertebrate populations in New Zealand hampers predictive scenarios for many invertebrate taxa. Watts et al. (2014) suggested that significant increases in the abundance of invertebrates should not be expected after mammalian predator control, although populations of large-bodied invertebrates may increase. Recently, analysis of ground-based control by TBFree New Zealand and

conservation agencies found that the only widespread species that has been shown to increase after mainland pest control is *H. thoracica*, probably because it is a favoured prey of ship rats (Byrom et al. 2016).

While small introduced mammals, particularly rodents and hedgehogs, have highly plastic and seasonably variable diets, they are considered the main predators of invertebrates in New Zealand ecosystems (King 2005). For example, weta were found in 39–76% of ship rat (*Rattus rattus*) stomachs and beetles, spiders, moths, sticks insects and cicadas were often present (numerous studies collated in Innes (2005)). Not only are these mammals likely to affect the abundance of their primary prey items, they are also predicted to prey selectively on specific types of invertebrates and in particular, those that are large because this makes them more rewarding food items (Pyke et al. 1977). Studies comparing invertebrate abundance across a range of size classes often show that larger-bodied taxa were more frequently affected by introduced mammals than smaller-bodied taxa (Bremner et al. 1984; Craddock 1997; St Clair 2011).

3.5.4 Scope for restoration

Emerging evidence from studies elsewhere in New Zealand indicate that major changes in invertebrate communities should not be expected after mammalian predator control, although some taxa – especially large-bodied invertebrates – will benefit (Watts et al. 2014). The insect community surviving at MBSR has survived in the presence of 700+ years of kiore (*Rattus exulans*) and over 150 years with a diverse mammal guild. Therefore, this insect community is likely to be resistant to mammal predation and taxa that are most affected by mammals would already be extinct. This is consistent with finding a fauna that was dominated by small (<10 mm) beetles. Mammal pest control for ground-dwelling invertebrate restoration should target insectivorous mammals, probably in order of importance hedgehogs, ship rats, mice, Norway rats, feral cats, possums and stoats, but German wasps *Vespula germanica* may perhaps also be important pests.

Monitoring large bodied taxa, such as the Hawke's Bay tree weta within the Cape to City project is important. Byrom et al. (2016) found that the only widespread taxa shown to increase after mainland pest control were the Auckland tree weta *H. thoracica*. In addition, monitoring iconic species such as the Hawke's Bay tree weta, which is restricted to the Hawke's Bay, could stimulate public participation and ownership.

An invertebrate restoration programme in the Cape to City project could focus on threatened invertebrate species. Four threatened moths are known from the Hawke's Bay. All these species are threatened by habitat destruction and modification. *Asaphodes stinaria* was formerly known from Taupo/Hawke's Bay to Invercargill but it is now found only in coastal Westland and Otago. The host plant is likely to be a native *Ranunculus* found in damp grassy clearings in native forests (McGuinness 2001). The Northern *Pimelea* cutworm moth, *Meterana pictula,* is a High Priority I threatened species whose larvae feed on *Pimelea* species. Another *Pimelea* feeding species is *Ericodema aerodana*, a leafroller inhabiting coastal areas in the region. Another threatened moth is an unnamed bright yellow *Pyroderces* species, the larvae of which feed in dead bark/twigs of *Sophora tetraptera*, and which is known from Esk State Forest and Lake Tutira but is probably widely distributed in

Hawke's Bay wherever its host occurs (Robert Hoare, pers. comm.). No threatened moth species are known from MHSR but this is probably due to the lack of survey by Lepidopterists (Robert Hoare, pers. comm.). Because these species are host-specific, the plight of these threatened moth taxa illustrates the importance of considering habitat restoration, which may be more important for some threatened invertebrates than pest control. In the case of Hawke's Bay, which has been little surveyed for invertebrates, we should add that primary survey targeting particular habitats for particular rare species is an important additional step.

If we are to learn anything about invertebrate responses to restoration we need to have specific objectives in mind. Watts et al. (2014) made five key recommendations for conservation managers or researchers attempting to quantify the benefits of mammal removal or control on the invertebrate communities: 1) use consistent protocols at both treatment and associated non-treatment sites; 2) plan in advance to allow for sufficient monitoring before mammal eradication starts; 3) simultaneously measure environmental variables that could contribute to changes in the invertebrate community; 4) monitor over a long time scale (10+ years) to account for lagged invertebrate responses and seasonal and climatic variation; and 5) reconstruct the fossil invertebrate community to sharpen restoration objectives. However, these recommendations could be applied to any restoration activity.

3.6 Recommendations

We recommend monitoring large-bodied taxa, such as the Hawke's Bay tree weta, because of their known responsiveness to mammal control. In addition, as this iconic tree weta species is restricted to the Hawke's Bay, it could stimulate public participation and ownership. Landcare Research is monitoring Hawke's Bay tree weta with artificial retreats focussing on sites with rat control.

A further recommendation is to survey for rare and threatened species within the Cape to City footprint and determine whether host-specific threatened invertebrate species are habitat or predator limited. This could be achieved through trial restoration plantings including the host plant taxa in areas with or without predator control.

4 The invertebrate fauna at Mohi Bush Scenic Reserve, Hawke's Bay, assessed using environmental DNA

4.1 Introduction

Currently, there is a paucity of documented information on invertebrate assemblages associated with forest fragments within productive landscapes. This limits our ability to assess the impacts of mammals, and of mammal control, on invertebrate communities and the ecosystem services they provide (see Section 2). Emerging environmental DNA (eDNA) techniques could potentially revolutionise biodiversity monitoring for cryptic groups such as invertebrates by providing the ability to characterise entire communities from a single easily-collected environmental sample (Taberlet et al. 2012; Bohmann et al. 2014; Thomsen & Willerslev 2015). Environmental DNA analyses work by extracting trace DNA from bulk environmental samples such as soil, water or leaves, and then sequencing specific gene regions that can act as genetic barcodes to identify species whose DNA was present in the sample.

Examples of eDNA studies to date include the use of trace amounts of mammal eDNA extracted from carrion flies has to monitor cryptic mammal species in dense tropical rainforest (Calvignac-Spencer et al. 2013). In New Zealand, multi-gene eDNA approaches have been used to assess biodiversity of many groups including invertebrates on Little Barrier Island (Drummond et al. 2015), and across a wide variety of land use types in Marlborough (Wood et al. in review). Furthermore, DNA-based identification of bulk invertebrate samples (such as malaise trap collections) offers great potential to circumvent the need for time consuming, costly, and taxonomically demanding microscope-based identification of bulk invertebrate samples (Morinière et al. 2016). Environmental DNA could therefore be an important monitoring tool for invertebrates within the Cape to City project.

The overall aim of this research was to explore the potential for environmental DNA to be used as an invertebrate monitoring tool within Cape to City. Specifically, we aimed to characterise the invertebrate fauna of Mohi Bush Scenic Reserve (MBSR) using environmental DNA analysis, and to compare these results with data from conventional invertebrate monitoring (Section 3). Two different eDNA sample media were used: 1) DNA extracted from bulk invertebrate samples collected using malaise and pitfall traps at the same location; and 2) eDNA extracted from soil. This design enabled comparison of soil and terrestrial (pitfall & malaise trap) communities, as well as comparisons between conventional monitoring data and eDNA data.

4.2 Objectives

The objectives of this research were to:

1. Undertake an assessment of the invertebrate fauna of MBSR using environmental DNA extracted from soil

2. Assess the utility of eDNA to as a species identification tool for invertebrate samples collected using malaise or pitfall traps

3. Compare and contrast the results of the eDNA analysis with the results obtained from conventional invertebrate monitoring (Section 3).

4.3 Methods

4.3.1 Study area and design

MBSR (61 ha) is a remnant of partially logged podocarp/broadleaf forest on the Maraetotara Plateau, within the eastern Hawke's Bay Ecological District. A total of twelve 20 x 20 m plots were located at MBSR: six along the forest-pasture edge ('edge' plots) and six within the interior (>100 m from the forest-pasture edge) of the forest fragment ('interior' plots). These plots were the same as those used in the conventional assessment (Section 3). A full description of the study area, sampling design, and the location of the sampling plots is provided in Section 3, and only brief details are given here.

4.3.2 Field sample collection

Soil is a well-known repository for environmental DNA from both soil-dwelling invertebrates (mites, nematodes, earthworms, larvae) and terrestrial invertebrates (dead carcases, exoskeletons, frass) (Drummond et al. 2015). As such, soil samples could provide a single easy to collect sample media for eDNA invertebrate analysis. Since extra-cellular DNA does not persist in the soil for extended periods, DNA extracted directly from soil will be dominated by DNA from organisms currently present in some form or another (e.g. larvae of beetles, worms, etc.). Soil was collected from the same 14 20 x 20 m plots as used in the conventional invertebrate monitoring (Section 3). A total of 24 soil cores (organic and mineral horizons) were collected from each plot with samples randomly located following standard sampling protocols (Fig. 11) using sterilised trowels. Approximately 4–6 kg of soil were collected per plot. This soil was stored at 4 degrees and transferred to the lab within 5 days of collection for DNA extraction.

DNA was also extracted from a single bulk invertebrate sample per plot, consisting of the invertebrates collected from both the malaise traps and the pitfall traps located at each plot (see section 3.3.2). This was the same sample that underwent conventional identification, and this allowed direct comparison of the results with those presented in Section 3.

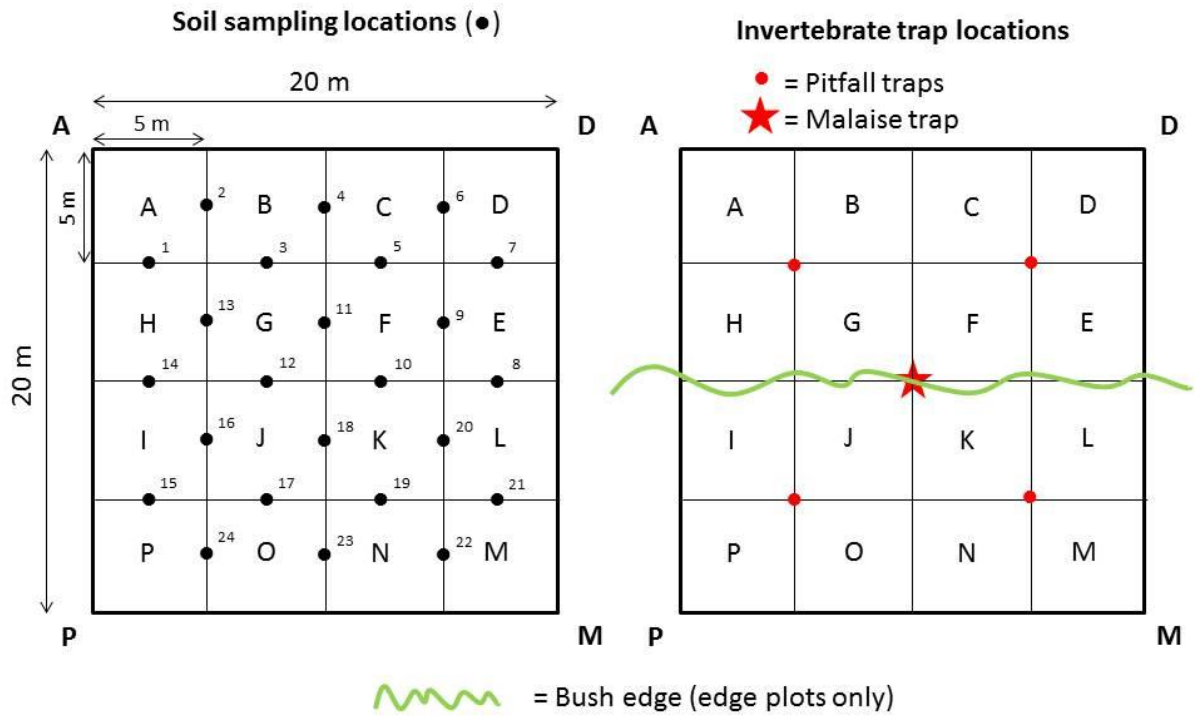

Figure 11 Layout of sampling points within the 20 x 20 m plots.

4.3.3 Molecular methods

Soil samples were homogenised and a 10-g sample was taken for DNA extraction. DNA extraction was done using PowerMax soil DNA extraction kits, following the manufacturer's standard protocol. Bulk invertebrate samples were ground into a paste and homogenised following the protocol described in Appendix 5. Approx. 300 mg of the homogenised sample was used for DNA extraction, using the Machery-Nagel NucleoSpin 96 Tissue extraction kit. The initial lysis used 400 ul of the Stable Digestion Buffer, left to incubate at 56^{o} with shaking overnight. The remainder of the protocol followed the manufacturer's instructions, but with 500 ul MN Buffer BQ1 and 500ul Abs EtOH added to the sample. The automated parts of the protocol were carried out on the Janus Automated Liquid Handling System (PerkinElmer).

Invertebrate DNA from both the soil and bulk invertebrate samples were isolated and amplified using PCR using single Invertebrate-specific primers (mlCOIintF and HCO2198; Leray et al. 2013) and plot and sample-media specific identification tags. The initial template amplification stage was carried out following the Touchdown protocol (Leray et al. 2013). This was then followed by the second stage of the MoTasp protocol (Clark et al. 2014) to add the barcodes and sequencing adaptors. Samples were sequenced by New Zealand Genomics Limited using a single run on Illumina MiSeq platform.

4.3.4 Bioinformatics and statistical analyses

A database of all available CO1 genes from animals was downloaded from NCBI using the search string "Metazoa"[Organism] AND (COI[All Fields] OR "CO1"[All Fields] OR "cytochrome oxidase"[All Fields] OR "cytochrome c oxidase"[All Fields] OR "COX1"[All Fields]) AND ("100"[SLEN] : "5000"[SLEN]) NOT ("UNVERIFIED:"[All Fields] OR "Uncultured":[All Fields])". This resulted in 1 801 017 sequences. This database was then filtered to retain only non-duplicate sequences matching the invertebrate forward primer and containing >300 base pairs. This resulted in a clean reference database containing sequences representing 100 134 species.

Sequences were analysed with a modified UPARSE (Edgar 2013) pipeline with elements from Usearch and Vsearch. This represents an interim solution as we work to move from a proprietary pipeline with poorly documented algorithms (Usearch) to an open-source but fundamentally similar pipeline (Vsearch). Paired-end reads were merged using Usearch -fastq_mergepairs, filtered using Vsearch -fastq_filter at a maximum allowable expected error of 1, dereplicated using vsearch -derep_fulllength with a minimum sequence length of 200 and excluding singletons, and then clustered into OTUs using usearch -usearch_global at 97% identity. All filtered sequences were then matched against these clusters using vsearch --usearch_global at 97%. Otus were identified by matching against the above reference database at using vsearch --usearch_global at 80% identity, with any sequence not matching the database at 80% or higher being discarded. Application of this pipeline to our raw sequence data resulted in 8 524 742 retained sequences representing a total of 5,602 OTUs.

On inspection of the phylum level results, additional quality filters were imposed on the resulting dataset based each sequences percent match to an OTU, with only those sequences matching at >84% retained. Matches below this threshold were more likely to be incorrectly assigned. This threshold removed all OTUs matching non-invertebrate phyla (e.g. Chordata) and phyla solely from marine environments (e.g. Cnidaria), and reduced the total number sequences to 2 573 272 and the total number of OTUs from 5602 to 749.

Data were transformed into a community-level matrix (plot by OTU). OTU abundance was standardised within each plot by standardising actual reads into percent reads based on total reads per plot. Data were further transformed using both sqrt and Wisconsin transformations. Community ordinations were conducted on the standardised and transformed data using the metaMDS function of the vegan package in R (Oksanen et al. 2015) using Bray distances. The use of a quantitative distance metric assumes that sequence abundance is related in some way to species abundance in samples, which although supported by some empirical evidence can also be biased by experimental factors (such as amplicon length and primers used) (Amend et al. 2010; Engelbrektson et al. 2010; Egge et al. 2013). Nonetheless, treating next-generation sequencing data as simply presence-absence would over-inflate the importance of rare sequences, which are more prone to include errors (Dickie 2010; Lindahl et al. 2013). Correlations of composition were tested using procrustes rotations and the function '*protest*' in the vegan package of R (Oksanen et al. 2015).

4.4 Results

4.4.1 Taxonomic coverage and composition of detected OTUs

Our sampling detected 749 OTUs, spanning 4 different taxonomic Phyla (Table 2). OTUs assigned an 'unknown' Phyla were matched to unidentified environmental invertebrate sequences. Arthropoda was by far the most common Phylum detected, which potentially reflects the specificity of the primer used, rather than an overall lack of diversity in the other invertebrate Phyla.

Table 2 Total number of OTUs detected by Phylum for soil and bulk invertebrate samples

Phylum	Soil sample OTUs	Bulk invertebrate OTUs	Total unique OTUs
Annelida	8	1	9
Arthropoda	152	590	655
Mollusca	1	2	2
Rotifera	35	0	35
Unknown	48	2	48
Totals	244	595	749

A total of 22 orders and 102 Families were detected, with the same number of Orders (19) detected in both the bulk invertebrate samples the soil samples (Fig. 12). In the bulk invertebrate sample, Diptera, Lepidoptera, Orthoptera, and Coleoptera were the four most abundant orders (in terms of total number of sequences) across the entire dataset. For soil samples, Hemiptera was the most abundant order, followed by Diptera, Hymenoptera, and Haplotaxida. There was an order of magnitude fewer quality sequence reads from soil samples (16,080 +/- 95%CI 12,872 per plot), compared with bulk invertebrate samples (198,258 +/- 95%CI 17,893).

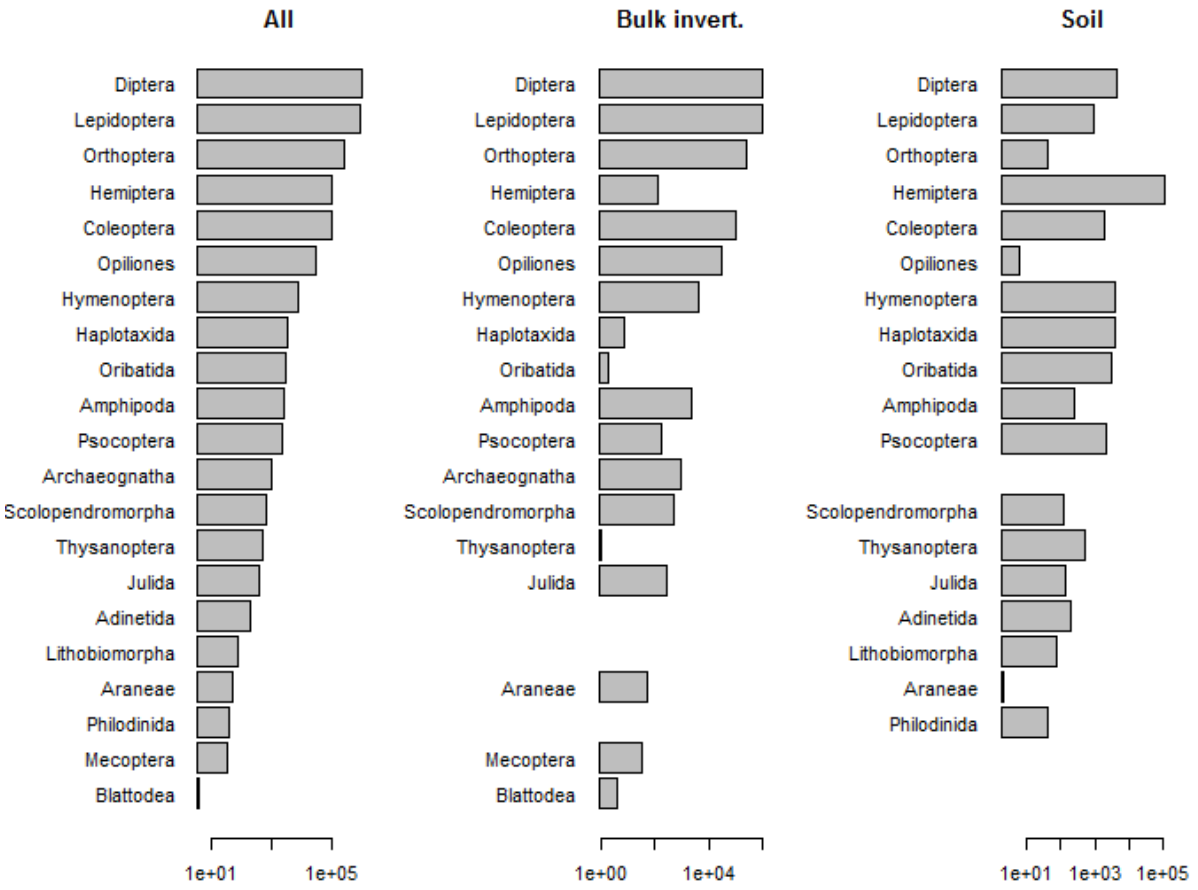

Figure 12 Total sequence reads for each order detected across the entire eDNA dataset (All), and divided into eDNA from bulk invertebrate samples and eDNA from soil samples. Note log x axis.

Only a small fraction (6%%) of the total OTUs matched reference sequence to high enough level to be considered a potential species-level match (>97% match), with 11% matching at >95% (approximately genus level) 35% matching at >90% (approximately family level). This indicates relatively poor reference data coverage and limits our confidence in (and ability to make) species-level assignments. Despite this, examination of OTUs with good levels of matching (>95%) and >5 sequences revealed a number of interesting genus/species level matches, illustrating the ability of eDNA to pick up a range of different invertebrates given adequate reference data (Table 3). The dominance of large invertebrates (e.g. cicadas, ants, bumblebees) in this list potentially reflects a bias in the reference data coverage towards these groups, or a bias in the DNA sample towards large-bodied organisms.

Table 3 Examples of species detected using eDNA

Order: Family	Species	Common name	Notes
Hemiptera: Cicadidae	*Amphipsalta zelandica*	Common cicada	Throughout NZ, common in forest areas
Coleoptera: Curculionidae	*Sitona lepidus*	Clover root weevil	Pest on white clover throughout NZ; arrived in NZ in 1996
Hymenoptera: Apidae	*Bombus terrestris*	Large earth bumblebee	Common throughout NZ; has the shortest tongue of 4 species in NZ so less effective at pollination
Lepidoptera: Oecophoridae	*Tingena armigerella*		Common in North Island forests; larvae feed in leaf-litter; bright yellow moth
Lepidoptera: Noctuidae	*Agrotis ipsilon*	Greasy cutworm	Common in pasture throughout NZ; larvae feed on range of plants including pasture spp; self-introduced

4.4.2 Comparison of invertebrate communities from forest edge and forest interior

Overall ordination of all group OTUs (across all taxa) showed clear separation between the communities from the bulk invertebrate samples and the soil samples (Fig. 13), indicating that sample media had a significant effect on the observed invertebrate community. Within each of these sample media there was no clear distinction between samples from inside the forest fragment and the edge (Figs 14 and 15). Looking within the relatively abundant taxonomic orders revealed subtle substrate and taxon-specific effects of forest edge vs interior on community composition (Fig. 16).

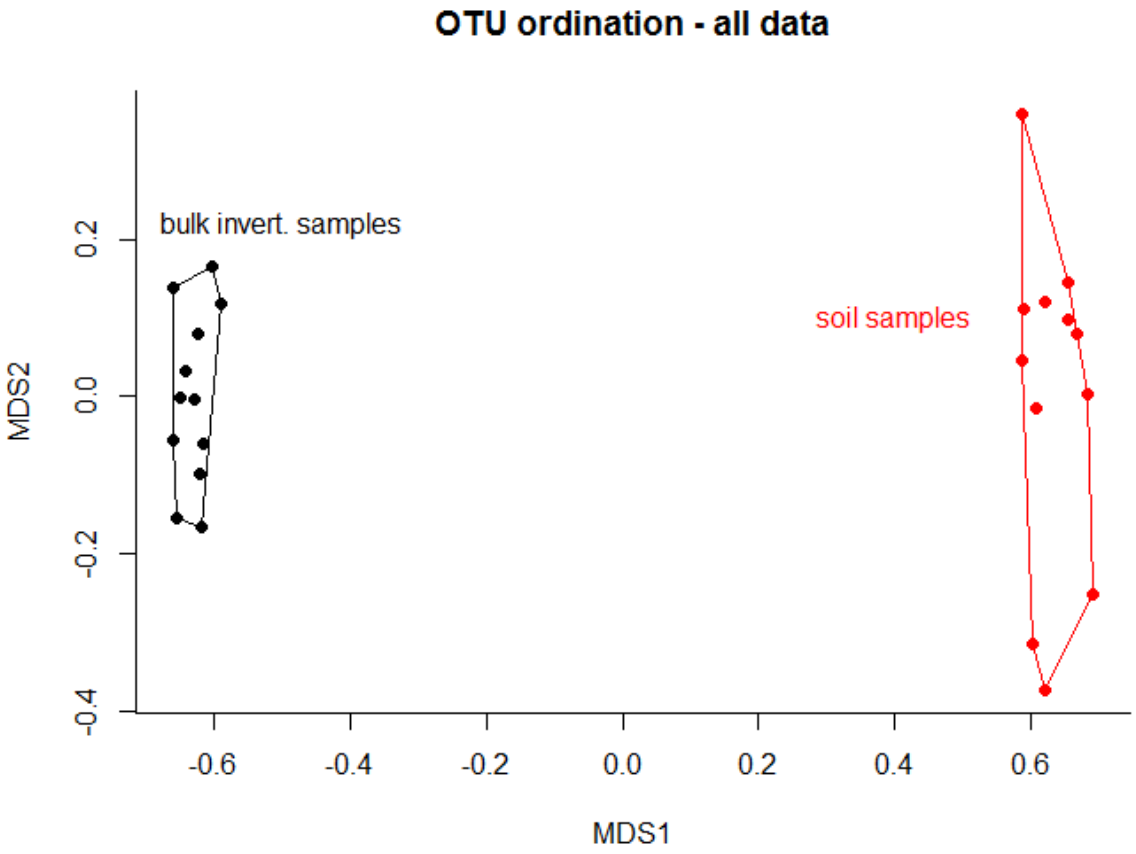

Figure 13 Ordination results based on OTU standardised abundance data. Polygons are shown for soil samples (black) and for bulk invertebrate samples (red). Highly distinct polygons indicate soil and bulk invertebrate samples had very different community composition at the OTU level.

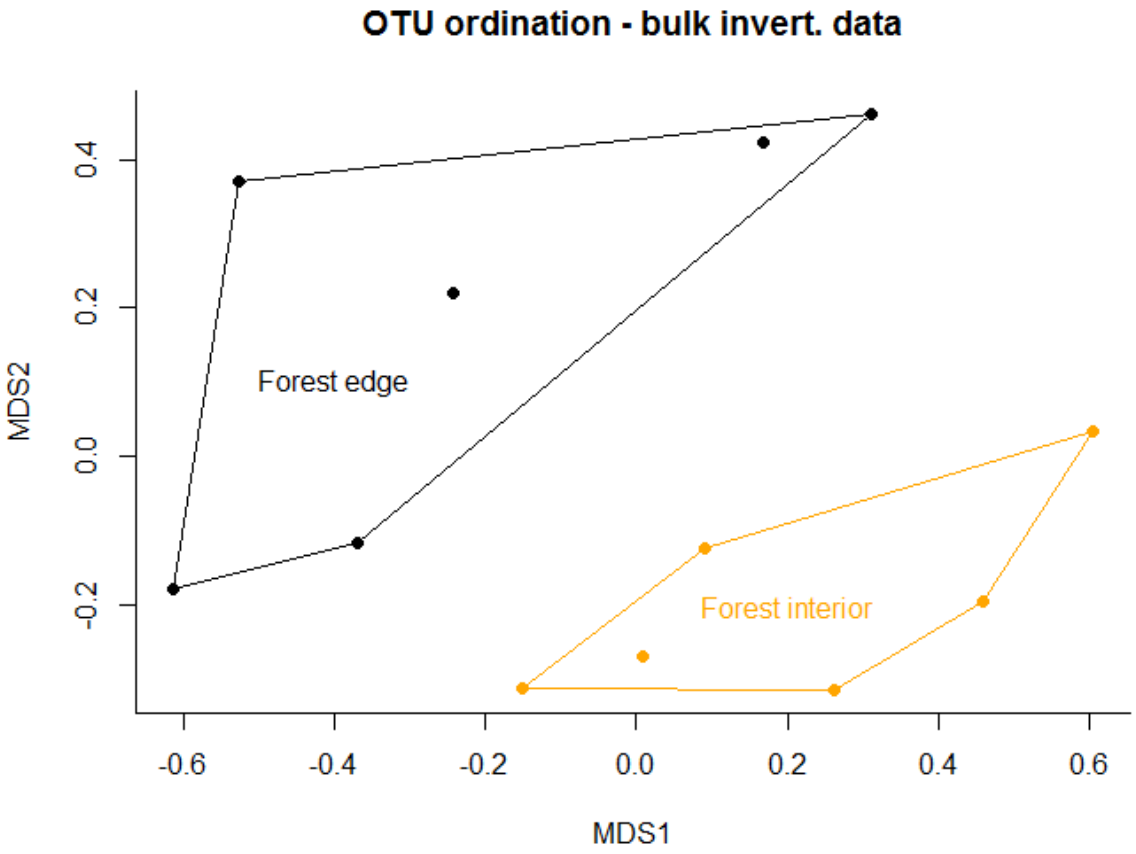

Figure 14 Ordination results based on group OTU abundance data for bulk invertebrate samples only. Polygons are shown for plots located on the forest edge (black) and plots located in the forest interior (orange). Forest edge communities were different to those in the forest interior (indicated by non-overlapping polygons) at the OTU level.

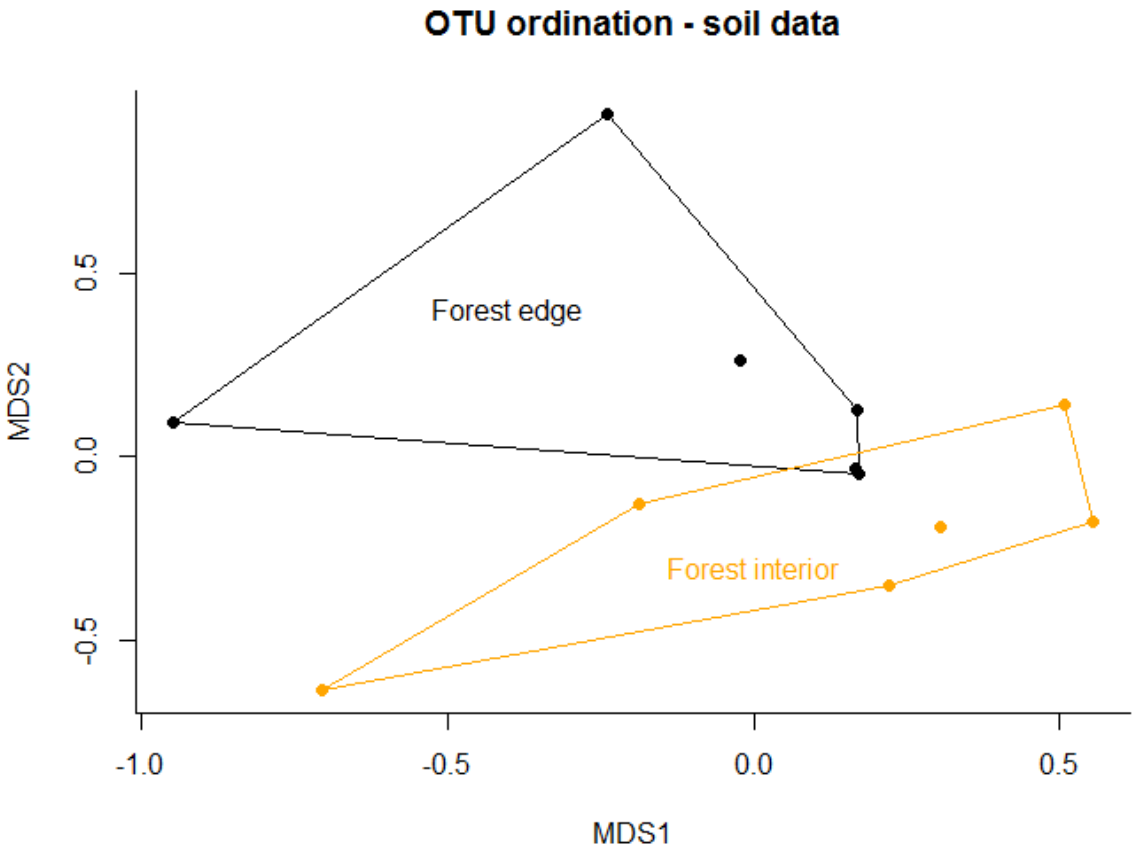

Figure 15 Ordination results based on standardised OTU abundance data for soil samples only. Polygons are shown for plots located on the forest edge (black) and plots located in the forest interior (orange). Forest edge communities were generally different to those in the forest interior (indicated by almost non-overlapping polygons) at the OTU level.

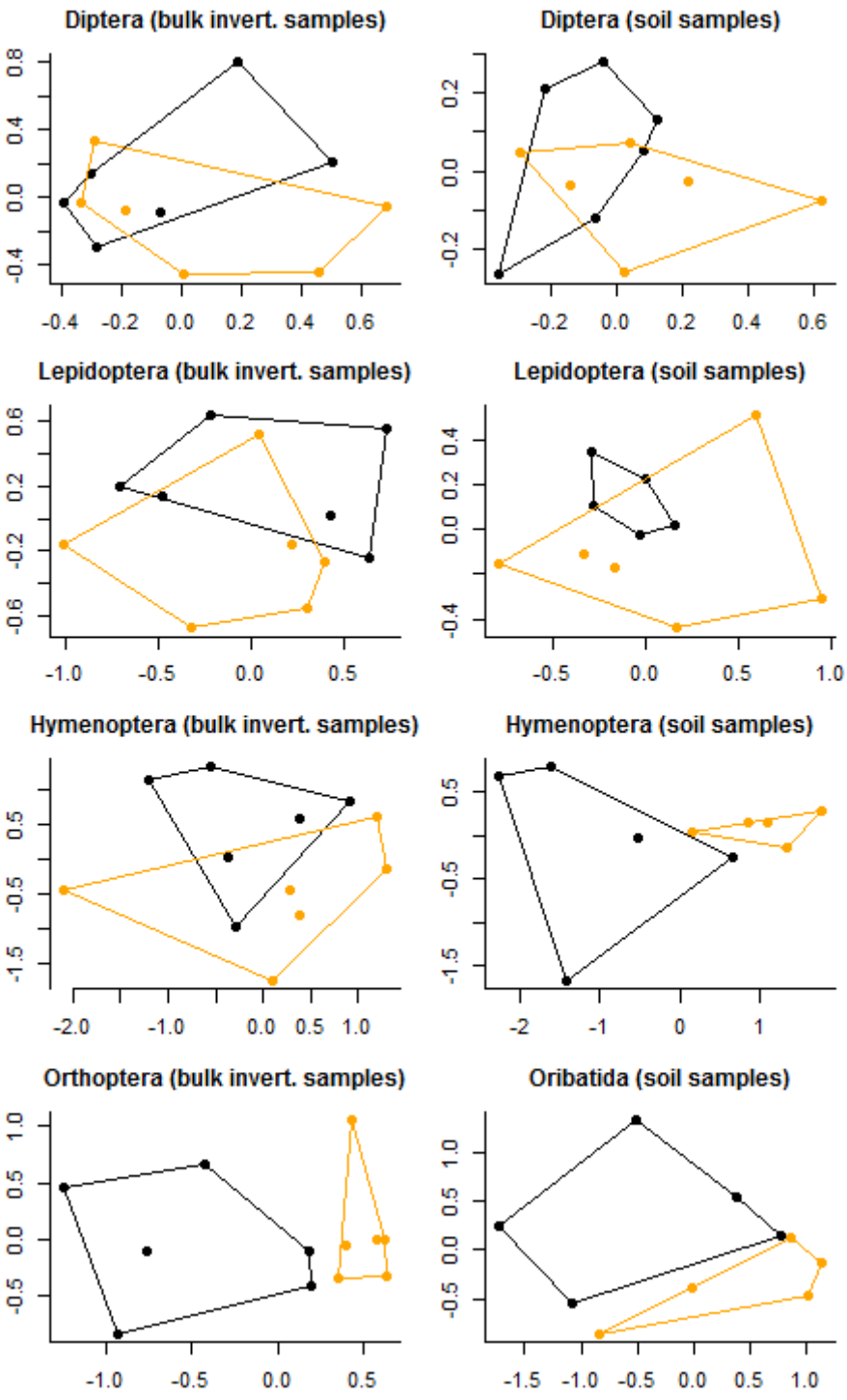

Figure 16 Ordination results based on group OTU abundance data for specific taxonomic orders, split into soil samples and bulk invertebrate samples. Diptera = flies, Lepidoptera = moths and butterflies, Hymenoptera = wasps, bees and ants, Orthoptera = grasshoppers crickets and weta, Oribatida = beetle mites. Polygons are shown for plots located on the forest edge (black) and plots located in the forest interior (orange). The more separated the polygons are the more distinct the communities are.

4.4.3 Comparison with conventional data

A direct comparison on conventional versus eDNA data was done for beetle communities. Observed beetle communities were compared using ordinations of the beetle community based on observed genus/species identities (conventional data) and observed OTUs matched to Order Coleoptera (eDNA data). Beetle communities based on conventional data showed clear separation of forest edge and forest interior, and a similar pattern was observed in the eDNA beetle communities (Fig. 17). The two beetle ordinations were statistically correlated (Procrustes rotation test, *P*=0.029), indicating that the resulting community patterns were similar for the conventional and eDNA beetle datasets.

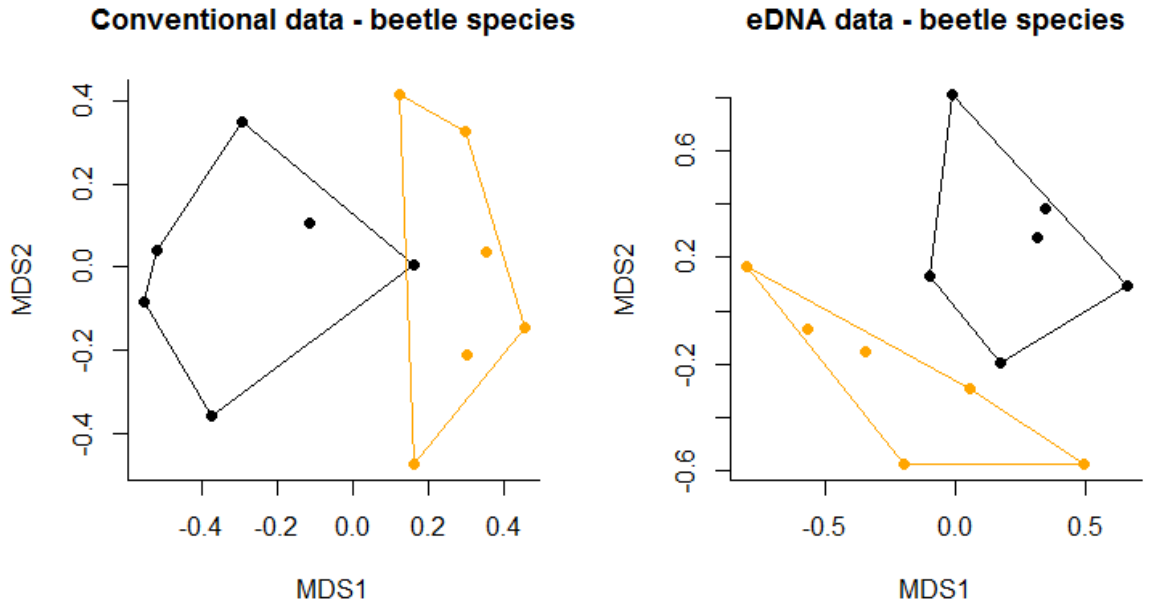

Figure 17 Direct comparisons of beetle community ordination results on beetle abundance data obtained from the same samples assessed using conventional taxonomic identification (to genus/species level) and eDNA OTUs. Polygons are shown for plots located on the forest edge (black) and plots located in the forest interior (orange). Community patterns in the two ordinations are significantly correlated (Procrustes rotation test, *P*=0.029).

4.5 Discussion

Our results support the idea that eDNA can be used a species identification tool to characterise whole invertebrate communities from either bulk invertebrate samples collected using malaise or pitfall traps, or from soil samples (Morinière et al. 2016), but that the sampling method has very strong effect on the resulting community. The eDNA data provided information on diverse array of invertebrate taxa – many more than conventional analysis for the same level of cost. The use of eDNA as a species identification tool for bulk invertebrate samples (e.g. those collected using malaise or pitfall traps) therefore has considerable potential (Drummond et al. 2015; Morinière et al. 2016).

However, the accuracy and resolution of this approach is currently limited by a lack of DNA reference data for New Zealand invertebrates. Building reference libraries is a relatively easy undertaking and can be done on either fresh samples or existing collections (e.g. Herbert et al. 2013) and should be prioritised for future work.

The eDNA extracted from soil represented different invertebrate community to those observed in the pooled malaise and pitfall trap bulk invertebrate samples (Fig. 13), with some orders being more abundant in the soil samples (e.g. Orabatida) and others in the bulk invertebrate samples (e.g. Orthoptera). Many of the observed ecological patterns (i.e. separation of communities between forest edge and forest interior; Ewers & Didham 2008) were observed in both soil and bulk invertebrate samples. The eDNA data allowed for investigation of these edge effects for a diverse range of invertebrate taxa, not just beetles (Fig. 16).

Soil has the advantage of being easy to sample in the field (collected during a one-off visit), whereas over malaise or pitfall traps require specialised sampling technology and repeat visits to the sample location to establish and empty the traps. However, the quality and abundance of invertebrate DNA obtained from soil samples in our study was low, with significant amounts of junk DNA being sequenced. Further analyses are needed (with updated reference data) to explore more comprehensively the effect of sample media on the detected patterns in the invertebrate community.

A number of methodological issues remain to be resolved. The most pressing is the need for better reference libraries, as discussed above. Another key area for improvement is the bioinformatics method (i.e. the method of going from DNA sequences to OTU's and then assigning species names to those OTUs). Further uncertainties exist about the presence of primer biases, and the relationship of the resulting sequence abundance data to actual abundance data. The effects of these uncertainties need to be explored more fully in order for eDNA to become an accepted tool for invertebrate monitoring.

Overall, this study represents one of the first attempts to apply eDNA methods to monitor invertebrate communities in New Zealand. Comparison with traditional monitoring data allows for verification of the eDNA methods and identification of key areas for improvement. Despite the limitations, the amount of information that eDNA can provide on invertebrate communities is immense and these benefits warrant further investment in this approach.

4.6 Recommendations

To improve the utility of eDNA for monitoring invertebrates, we recommend that:

1. Future work should first focus on collecting DNA reference data for taxonomically identified specimens collected from the study area. This is urgently needed to improve the taxonomic coverage of the DNA reference libraries. Doing so will greatly improve confidence in taxonomic assignments, increasing the resolution, quality and interpretability of the eDNA data.

2. The above analysis should then be re-run using the updated DNA reference libraries. This is easily done based on the existing sequence data without the need to re-sample or do further molecular lab work.

3. To handle invertebrate DNA, alternative bioinformatics pipelines should be explored and optimised, and methods developed for improved ecological interpretation of the resulting data.

4. Once these methodological improvements have been made, future work should focus on the ability of eDNA to detect changes in invertebrate communities over time in response to management such as restoration and rodent control. This work should be carried undertaken alongside conventional invertebrate monitoring as a means of verifying the resulting eDNA datasets.

5 Overall recommendations

Overall recommendations arising from this work are:

- Invertebrates provide important ecosystem services and need to be considered as part of Cape to City's goal of enabling indigenous taxa to co-exist with human habitation, food production and recreation at large scales in an agricultural landscape.
- We recommend monitoring large-bodied taxa, such as the Hawke's Bay tree weta, because of their known responsiveness to mammal control. As this iconic tree weta species is restricted to the Hawke's Bay, it could stimulate public participation and ownership. Landcare Research is monitoring Hawke's Bay tree weta with artificial retreats focussing on sites with rat control. A further recommendation is to survey for rare and threatened species within the Cape to City footprint and determine whether host-specific threatened invertebrate species are habitat or predator limited. This could be achieved through trial restoration plantings, including the host plant taxa in areas with or without predator control.
- Environmental DNA can provide an unprecedented level of detail on entire invertebrate communities for similar cost to conventional monitoring, which typically targets well known groups such as beetles or weta. A number of areas for methodological improvement have been identified (e.g. more reference data, optimised bioinformatics pipelines, further comparisons with conventional data). These methodological issues need to be addressed before eDNA can be rolled out as an established monitoring technique for invertebrates within Cape to City.

6 Acknowledgements

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Appendix 1 – Abundance of invertebrates from different Orders collected in malaise traps at MBSR

	Order	Common name	Edge (n=6 plots)	Interior (n=6 plots)
Arachnida	Acari	ticks, mites	2	3
	Araneae	spiders	37	31
	Opiliones	harvestman	3	1
Insecta	Blattodea	cockroaches	2	0
	Coleoptera	beetles	198	115
	Diptera	two-winged flies	882	569
	Hemiptera	bugs	18	10
	Hymenoptera	wasps, bees, ants	341	117
	Lepidoptera	moths, butterflies	506	92
	Mantodea	mantids	1	0
	Neuroptera	lacewings	9	8
	Orthoptera	crickets, weta, grasshoppers	11	14
	Phasmatodea	stick insects	2	4
	Plecoptera	stoneflies	0	1
Turbellaria	Annelida	flatworm	0	4

Appendix 2 – Abundance of beetles collected in malaise traps at MBSR

Family	Genus/Species	Biostatus	Edge (n=6 plots)	Interior (n=6 plots)
Anthribidae	*Cacephatus* sp. 1	Native	1	0
	Dysnocryptus pallidus	Native	3	1
	Lawsonia variabilis	Native	3	0
	Lophus rudis	Native	1	0
	Phymatus sp. 1	Native	1	0
	Pleosporius bullatus	Native	1	0
Cantharidae	*Asilis* sp. 1	Native	1	2
Carabidae	*Amarotypus edwardsi*	Native	3	3
	Euthenarus bicolor	Introduced	1	0
	Pentagonica vittipennis	Native	1	0
Cerambycidae	*Calliprason sinclairi*	Native	0	2
	Hybolasius sp. 1	Native	2	0
	Psilocnaeia sp. 1	Native	2	1
	Somatidia sp. 1	Native	0	2
	Spilotrogia sp. 1	Native	2	2
	Xylotoles sp. 1	Native	1	1
Chrysomelidae	*Adoxia* sp. 1	Native	23	10
	Eucolaspis sp. 1	Native	36	3
	Peniticus sp. 1	Native	1	0
	Trachytetra rugulosa	Native	0	1
Clambidae	spp.	Native	0	1
Cleridae	*Lemidia aptera*	Native	4	0
	Phymatophaea sp. 1	Native	2	0
Coccinellidae	*Adoxellus* sp. 1	Native	2	1
	Coccinella undecimpunctata	Introduced	0	1
	Rhyzobius sp. 1	Native	5	1
Corylophidae	*Holopsis* sp. 1	Native	0	1
Cryptophagidae	*Micrambina* sp. 1	Native	0	1
Curculionidae	*Brachyolus punctatus*	Native	14	12
	Brachyolus sp. 1	Native	3	0
	Catoptes sp. 1	Native	5	3
	Cossininae sp. 1	Native	3	1
	Cryptorhynchinae spp.	Native	4	12
	Gerynassa sp. 1	Unknown	2	6
	Hoplocneme sp. 1	Native	1	0
	Listronotus bonariensis	Introduced	1	0
	Omoeacalles crisioides	Native	2	0

Family	Genus/Species	Biostatus	Edge (n=6 plots)	Interior (n=6 plots)
Curculionidae, con't	*Oreda notata*	Native	1	0
	Paedaretus hispidus	Native	1	0
	Rhinocyllus conicus	Introduced	1	0
	Rhopalomerus sp. 1	Native	2	0
	Sitona oboletus (=*S. lepidus*)	Introduced	0	2
	Stephanorhynchus lawsoni	Native	4	0
	Storeini spp.	Native	1	0
	Tychanopais sp. 1	Native	1	0
	Tysius bicornis	Native	1	0
Elateridae	*Panspoeus guttatus*	Native	0	1
	Protelater spp.	Native	4	0
	Sphaenelater collaris	Native	1	0
	spp.	Native	13	10
Eucinetidae	*Eucinetus stewarti*	Native	1	0
	spp.	Native	1	0
Latridiidae	*Cartodere* (*Aridius*) sp.	Unknown	1	6
	Corticariinae spp.	Native	3	2
Leiodidae	Cholevinae spp.	Native	1	1
Melandryidae	*Hylobia sexnotata*	Native	12	1
Mycetophagidae	*Nototriphyllus* sp. 1	Native	0	1
Nitidulidae	*Hisparonia hystrix*	Native	0	1
	Soronia asperella	Native	1	0
Oedemeridae	spp.	Native	2	3
Pyrochroidae	spp.	Native	4	0
Scarabaeidae	*Costelytra zealandica*	Native	6	2
Scirtidae	spp.	Native	1	9
Scraptiidae	*Nothotelus* sp.	Native	2	1
Staphylinidae	Aleocharinae spp.	Native	0	1
	Anotylus sp. 1	Unknown	1	0
	Quedius sp. 1	Native	0	1
	Sepedophilus sp. 1	Native	0	1
	Tachyporus nitidus	Introduced	0	1
Tenebrionidae	*Zolodinus zelandicus*	Native	1	0
Zopheridae	*Pristoderus asper*	Native	0	1
	Tarphiomimus indentatus	Native	1	1

Appendix 3 – Abundance of invertebrates from different Orders collected in pitfall traps at MBSR

	Order	Common name	Edge (n=6 plots)	Interior (n=6 plots)
Arachnida	Acari	ticks, mites	26	45
	Araneae	spiders	151	117
	Opiliones	harvestman	121	22
	Pseudoscorpiones	false scorpions	1	1
Crustacea	Amphipoda	hoppers	186	207
	Isopoda	slaters	40	42
Insecta	Archaeognatha	bristletails	3	3
	Blattodea	cockroaches	0	3
	Coleoptera adult	beetle adults	275	334
	Coleoptera larvae	beetle larvae	8	5
	Collembola	springtails	363	901
	Diptera	two-winged flies	64	28
	Hemiptera	bugs	25	18
	Hymenoptera	wasps, bees, ants	375	53
	Lepidoptera	moths, butterflies	60	546
	Mantodea	mantids	0	0
	Orthoptera	crickets, weta, grasshoppers	271	86
Mollusca	Gastropoda	slugs, snails	4	1
Myriapoda		millpedes, centipedes, symphylans	72	45
Turbellaria	Annelida	earthworm, flatworm	12	8

Appendix 4 – Abundance of beetles collected in pitfall traps at MBSR

Family	Genus/Species	Biostatus	Edge (n=6 plots)	Interior (n=6 plots)
Anthicidae	*Cotes* sp. 1	Native	1	0
Carabidae	*Anomotarus illawarrae*	Introduced	1	0
	Aulacopodus sp. 1	Native	0	2
	Ctenognathus sp. 1	Native	13	112
	Dicrochile sp. 1	Native	3	8
	Harpalus affinis	Introduced	0	1
	Holcaspis sp. 1	Native	21	8
	Mecodema oconnori	Native	41	33
	Megadromus capito	Native	30	27
	Megadromus vigil	Native	10	17
	Plocamostethus planiusculus	Native	0	1
	Scopodes sp. 1	Native	5	0
	Zolus sp. 1	Native	1	3
Cerambycidae	*Nodulosoma angustum*	Native	1	3
	Ptinosoma sp. 1	Native	1	1
Chrysomelidae	*Adoxia* sp. 1	Native	3	0
	Bruchidius villosus	Introduced	1	0
	Peniticus sp. 1	Native	1	0
Corylophidae	*Sericoderus* sp. 1	Unknown	2	0
Cryptophagidae	*Micrambina* sp. 1	Native	1	1
Curculionidae	*Brachyolus punctatus*	Native	4	0
	Catoptes sp. 1	Native	2	0
	Cossininae sp. 1	Native	1	0
	Cryptorhynchinae spp.	Native	5	7
	Hiiracalles sp. 1	Native	0	1
	Phrynixus sp. 1	Native	4	0
	Scelodolichus sp. 1	Native	1	2
	Styphlotelus fascicularis	Native	6	1
	Tropiphorini spp.	Native	0	3
	Tychanopais sp. 1	Native	1	1
Elateridae	spp.	Native	8	7
Erotylidae	*Cryptodacne* sp. 1	Native	1	0
Euxestidae	*Hypodacnella rubripes*	Native	4	0
Histeridae	*Parepierus* sp. 1	Native	1	0

Family	Genus/Species	Biostatus	Edge (n=6 plots)	Interior (n=6 plots)
Hydrophilidae	*Cyloma lawsonus*	Native	0	10
	Cyloma stewarti	Native	0	6
Latridiidae	*Aridius* sp. 1	Unknown	0	6
	Corticariinae spp.	Native	8	4
Leiodidae	Cholevinae spp.	Native	4	0
	Inocatops sp. 1	Native	5	0
Lucanidae	*Dendroblax earlii*	Native	1	0
	Paralissotes reticulatus	Native	1	2
Melandryidae	*Hylobia* sp. 1	Native	1	0
Oedemeridae	spp.	Native	0	2
Scarabaeidae	Costelytra zealandica	Native	11	0
	Odontria magnum	Native	0	2
Staphylinidae	Aleocharinae spp.	Native	14	26
	Anabaxis sp. 1	Native	1	0
	Eupines sp. 1	Native	1	0
	Euplectitae spp.	Native	0	1
	Falagria sp. 1	Native	1	0
	Hyperomma sp. 1	Native	1	0
	Maorothius sp. 1	Native	2	1
	Quedius spp.	Native	2	12
	Scydmaeninae spp.	Native	1	0
	Sepedophilus sp. 1	Native	2	7
	Thyreocephalus chloropterus	Introduced	10	0
	Tramiathaea cornigera	Native	12	1
Tenebrionidae	*Mimopeus* sp. 1	Native	3	0
Ulodidae	*Brouniphylax* sp. 1	Native	2	0
	Syrphetodes marginatus	Native	0	1
Zopheridae	*Pristoderus bakewellii*	Native	18	13
	Pycnomerus sp. 1	Native	1	0

Appendix 5 – Invertebrate homogenisation procedure

1. **EtOH evaporated off all sub-samples from the same plot. Process can be sped up by removal of EtOH by Pasteur pipette, if no tiny insects (at risk of being removed) are present.** During this time, the following cleaning procedures can be carried out.
2. **Spray bench with bleach solution (10%).** Wipe clean.
3. **Spray bench with ethanol (70%).** Wipe clean.
4. **Spray TriGene (green) over 'clean' pestle and mortar.** Wipe around with a tissue to ensure all surfaces are covered, leave for a minute or two, and then wipe out remaining liquid.
5. **Spray with bleach solution (10%).** Wipe around with a tissue to ensure all surfaces are covered, leave for a minute or two, and then wipe out remaining liquid.
6. **Spray with ethanol (70%)** and allow to evaporate dry (complete drying at this stage is not essential).
7. **Place a couple of metal spatulas into tube of cleaning bleach.** Swirl around for a minute or so.
8. **Spray with ethanol (70%)** and allow to evaporate dry (complete drying at this stage is not essential).
9. **Place all sterilised equipment in the UV steriliser machine, on a clean paper towel.** Push the button to start, takes around 5 min. Once complete, keep everything on paper towels on clean bench.
10. **Fill a red travel mug with liquid nitrogen.** Pour a small amount into the mortar and allow both pestle and mortar to cool.
11. **Tip all insect subsamples from the same plot into the mortar.** Any remaining EtOH will hiss and pop a bit, so be aware. Pour on more liquid nitrogen as required to freeze all the insect material.
12. **Bang and grind the material until it is a fine powder.** Add more liquid nitrogen as required. "Fine powder" is more than likely "gloop at a consistent size". Try to get all the big bits.
13. **Transfer the homogenized sample into a 50ml falcon tube.** Use the sterilised metal spatulas to get out as much of the sample as possible.
14. **Flash freeze the sample tube in liquid nitrogen.** Once frozen, transfer into the bag in the -80 freezer.
15. **Wipe out as much remaining sample from the mortar and pestle as possible with a tissue,** followed by a rinse with water and a splash of detergent.
16. **Repeat the process for all remaining samples.**