Molecular insights into ecosystem health: comparative characterisation of dietary ecology and spatial biodiversity patterns through DNA metabarcoding of threatened predator species in Aotearoa New Zealand.

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by

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THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

Whatungarongaro te tangata, toitū te whenua.

As people disappear from sight, the land remains.

“I know of no pleasure deeper than that which comes from contemplating the natural world and trying to understand it.”

-Sir David Attenborough

Abstract

Predator species play critical ecological roles, influencing food web dynamics, regulating prey populations, and reflecting the health of their surrounding ecosystems. In Aotearoa New Zealand, many threatened predators inhabit environments under pressure from habitat modification, invasive species, and climate change. Understanding their dietary ecology and the biodiversity of their habitats is essential for effective conservation management. Environmental DNA (eDNA) metabarcoding, which uses genetic material shed into the environment to identify taxa, offers a non-invasive and highly sensitive approach to investigating both diet and broader biodiversity patterns. My thesis aimed to compare the dietary ecology and incidental biodiversity detection of two threatened predators inhabiting contrasting ecosystems, and to evaluate how ecological context shapes the information recovered through molecular analyses.

The first analysis (Chapter 2) examined the diet of the long-tailed bat (*Chalinolobus tuberculatus*) across multiple roosting locations within a single plantation forest over the summer season. This was the first study of its kind at this scale for the species, generating an unprecedented molecular dataset. Metabarcoding revealed a diet dominated by arthropods from multiple insect orders, encompassing both native and introduced taxa. Differences linked to reproductive status suggested shifts in prey choice driven by energetic demands, while variation between pre- and post-harvest periods indicated sensitivity to forestry management cycles. These results highlight both the potential role of this species in regulating introduced insects and its value as a bioindicator in managed forest systems.

The second analysis (Chapter 3) investigated little blue penguins (*Eudyptula minor*) from an island breeding colony within a sheltered harbour, focusing on both dietary and non-diet biodiversity signals. While direct dietary resolution was limited, eDNA recovered a rich record of terrestrial and nearshore taxa, reflecting seasonal and spatial variation in colony-adjacent biodiversity. These patterns were potentially influenced by seasonal ocean productivity, prey migration, microhabitat heterogeneity, and anthropogenic disturbance, demonstrating the capacity of faecal eDNA to capture wider ecosystem information.

Together, these studies demonstrate that predator faecal metabarcoding can provide complementary insights into species ecology and environmental condition, even when direct dietary resolution is constrained. By integrating trophic and incidental biodiversity data, this research contributes molecular baselines for long-term monitoring, enables the characterisation of hard-to-study or cryptic species that are otherwise difficult to observe directly, underscores the influence of ecological context on eDNA recovery, and supports the use of non-invasive molecular tools to inform evidence-based management and the conservation of threatened species and their habitats in Aotearoa New Zealand.

Acknowledgements

This thesis marks the culmination of a journey that has been both academically challenging and personally transformative, and it would not have been possible without the support, encouragement, and generosity of so many people.

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in me never wavered, your love has been one of my greatest sources of strength, and you always made sure I knew how proud you were of me. I'm so grateful to have walked this journey with you by my side, and I look forward to every road we have yet to travel together.

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wavered, and the nights when I questioned whether I was on the right path at all. There was a time when I truly didn't think I would make it this far, but you never once stopped believing in me. You reminded me why I started, encouraged me to take each step forward even when it felt impossible, and quietly carried some of the weight when I couldn't carry it myself. You have been there through every tearful phone call, every moment of frustration, and every quiet victory. You've celebrated each milestone with genuine pride, no matter how small, and found ways to lift me when the road ahead felt overwhelming. Without your unwavering love, your encouragement, and the countless sacrifices you've made to give me the space and support to follow this path, none of this would have been possible. This thesis may bear my name, but it carries your fingerprints in every page, every idea, and every step it took to get here.

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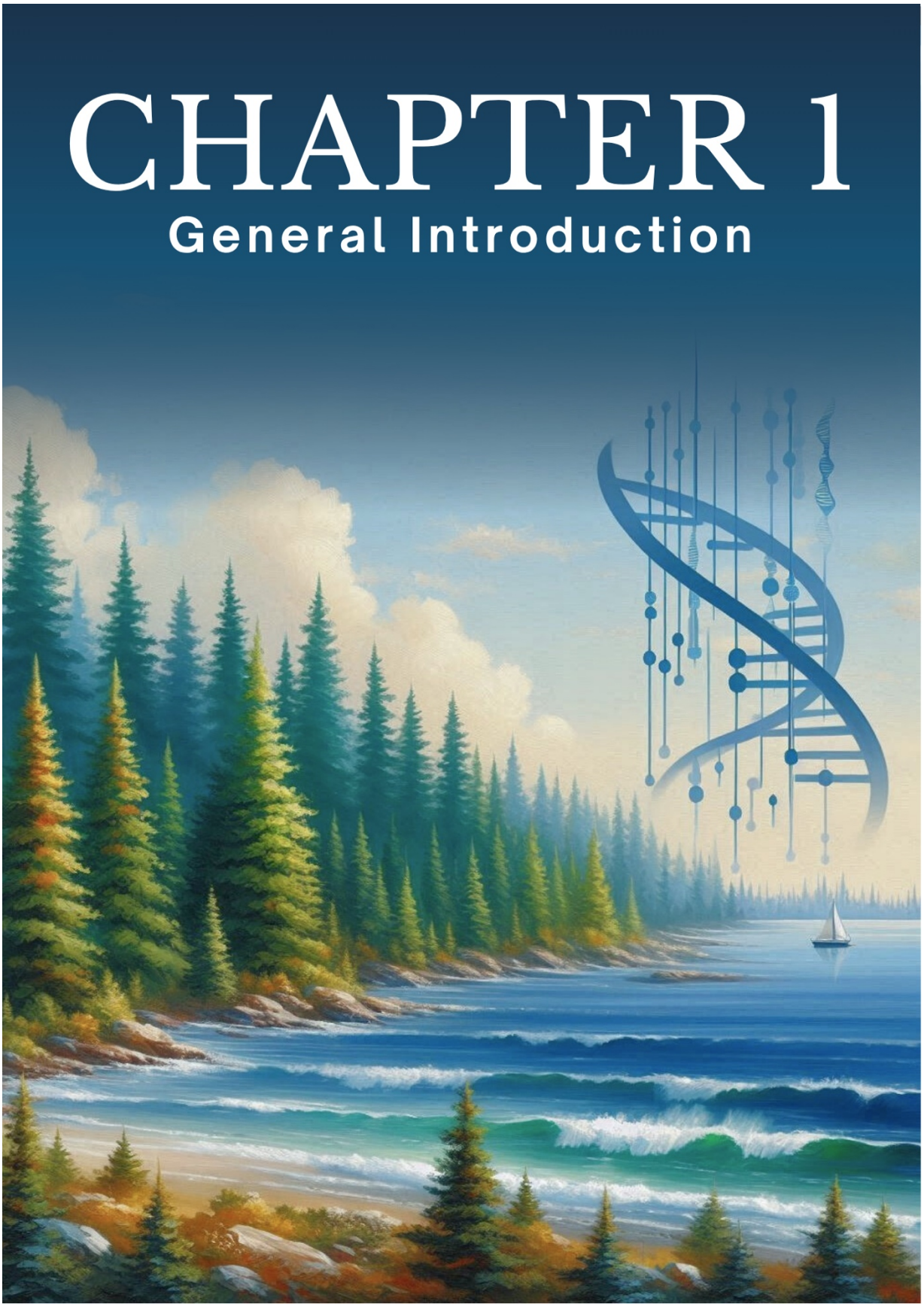
CHAPTER 1

General Introduction



1.1. Ecosystem health, structure and services

Ecosystems represent complex, interdependent networks where biotic components such as animals, plants, fungi, and microorganisms interact with each other and with abiotic factors (e.g., temperature, nutrient availability, salinity, and light) within defined geographic regions (Wang et al., 2021). These diverse ecosystems, including deserts, forests, and oceans, are fundamental to biodiversity and biosecurity globally. They are arranged across different levels of biological organisation, from individual organisms to populations and communities (Weathers et al., 2013). Ecosystems are sustained by essential ecological processes such as primary productivity, decomposition, nutrient cycling, and energy flow, which drive ecosystem functioning and maintain the cycling of matter and energy through living and non-living components (Fu et al., 2013; Lambers et al., 1998).

Organisms within ecosystems are classified into trophic levels based on their feeding relationships. These include primary producers (photosynthetic plants and phytoplankton), primary consumers (herbivores), and secondary and tertiary consumers (omnivores and carnivores) (Lindeman, 1942; Pimm & Lawton, 1977). Energy flows through these trophic interactions, supporting essential ecosystem services that are categorised into four main types: provisioning, regulating, cultural, and supporting (Aznar-Sánchez et al., 2018; Balvanera et al., 2017). Provisioning services deliver critical resources like food, water, timber, and medicinal plants necessary for survival (Ala-Hulkko et al., 2019). Regulating services maintain environmental stability through factors such as climate, water quality, and disease, with carbon sequestration by forests and wetlands helping mitigate climate change (Adhikari & Hartemink, 2016; Daba & Dejene, 2018). Cultural services provide non-material benefits, including recreation,

spiritual value, and aesthetic enjoyment, which contribute to human well-being and identity (Pleasant et al., 2014). Supporting services underpin all other ecosystem functions, providing foundational processes like soil formation, nutrient cycling, and photosynthesis (Naeem & Bunker, 2009; Tallis & Kareiva, 2005).

Ecosystem functions and services are underpinned by biodiversity, which provides functional redundancy and supports adaptive responses to environmental change (Elmqvist et al., 2003; Naeem et al., 2012). While some aspects of biodiversity may appear stochastic, the overall composition and diversity of ecosystems are often shaped by interacting abiotic and biotic factors across spatial and temporal scales (Gottschall et al., 2022). Abiotic factors such as temperature, precipitation, and nutrient availability influence species distributions and productivity, while biotic interactions including competition, mutualism, and predation further structure community dynamics (Han et al., 2007). Habitat heterogeneity, microclimate, and historical land use also play key roles in shaping local biodiversity (Stein et al., 2014; Siefert et al., 2013). Over time, biodiversity may respond to seasonal cycles, successional dynamics, species migrations, or phenological events, creating fine-scale temporal variation within ecosystems (Wolkovich et al., 2014; Vellend et al., 2007). Together, these drivers underpin the spatial and temporal complexity of biodiversity, influencing ecosystem resilience and function.

Human activities such as deforestation, pollution, and urbanisation, are major drivers of ecosystem degradation, undermining the delivery of essential services including food provision, freshwater availability, and environmental regulation (Sala et al., 2000; Vitousek et al., 1997). These disruptions not only affect ecological integrity but have far-reaching consequences for human health and economic stability (Rockström et al., 2009; Folke et al., 2010). In addition,

global change pressures such as invasive species, habitat fragmentation, and climate change, further destabilise ecosystems and can push them beyond critical thresholds or tipping points, where recovery becomes increasingly difficult or even irreversible (Allen et al., 2016; Folke et al., 2010). Recognising the value of ecosystem services is therefore crucial for guiding land-use and conservation decisions, supporting sustainable practices that aim to maintain and restore these services (Rockström et al., 2009; Folke et al., 2010).

In light of escalating pressures, understanding ecosystem structure and monitoring ecological health are essential to sustaining ecosystem functionality and resilience (Folke et al., 2010; Allen et al., 2016). Ecosystems operate across a range of spatial and temporal scales, and their ability to absorb disturbance while maintaining core functions is strongly influenced by their heterogeneity and connectivity (Chuang et al., 2018). High levels of spatial diversity, ecological connectivity, and functional redundancy contribute to ecosystem persistence, whereas fragmentation and loss of complexity reduce resilience and increase vulnerability to abrupt changes (Chuang et al., 2018; Allen et al., 2016; Folke et al., 2010).

As ecosystems continue to degrade, assessing their "health" has become increasingly crucial. An ecosystem's health is a holistic concept that defines its environmental state, particularly in terms of how human activities affect its structure, composition, and functioning (Kruse, 2019). Effectively measuring ecosystem health requires obtaining a range of biological, chemical, physical, and ecological indicators that provide a comprehensive and accurate measure of an ecosystem's condition and resilience (Hernández-Blanco et al., 2022). Key biological indicators—such as species abundance, diversity, and community composition—are thought to reflect ecosystem stability and functionality (Burkhard et al., 2008). Chemical indicators, like nutrient

concentrations and pollutant levels, help assess degradative stressors such as eutrophication and contamination (Hwang, 2020). Physical indicators, including habitat quality, water flow, and soil integrity, offer insight into ecosystem structure (Jørgensen et al., 2016; Thomsen et al., 2012). Together, these indicators provide a multi-faceted approach to assessing ecosystem health (Fig. 1.1).

Ecosystem monitoring must be adaptive and must incorporate an understanding of feedback mechanisms and socio-ecological interactions if managers are to effectively anticipate and respond to environmental change (Garmestani et al., 2023). However, ecosystems are dynamic and constantly undergoing cycles of stabilisation and destabilisation (Cortez et al., 2020). Therefore, monitoring must remain adaptive to capture both current conditions and potential future changes (Allesina & Tang, 2012). This flexibility ensures that ecosystem management and conservation strategies can respond to emerging threats and long-term ecological shifts (Auld & Keith, 2009).

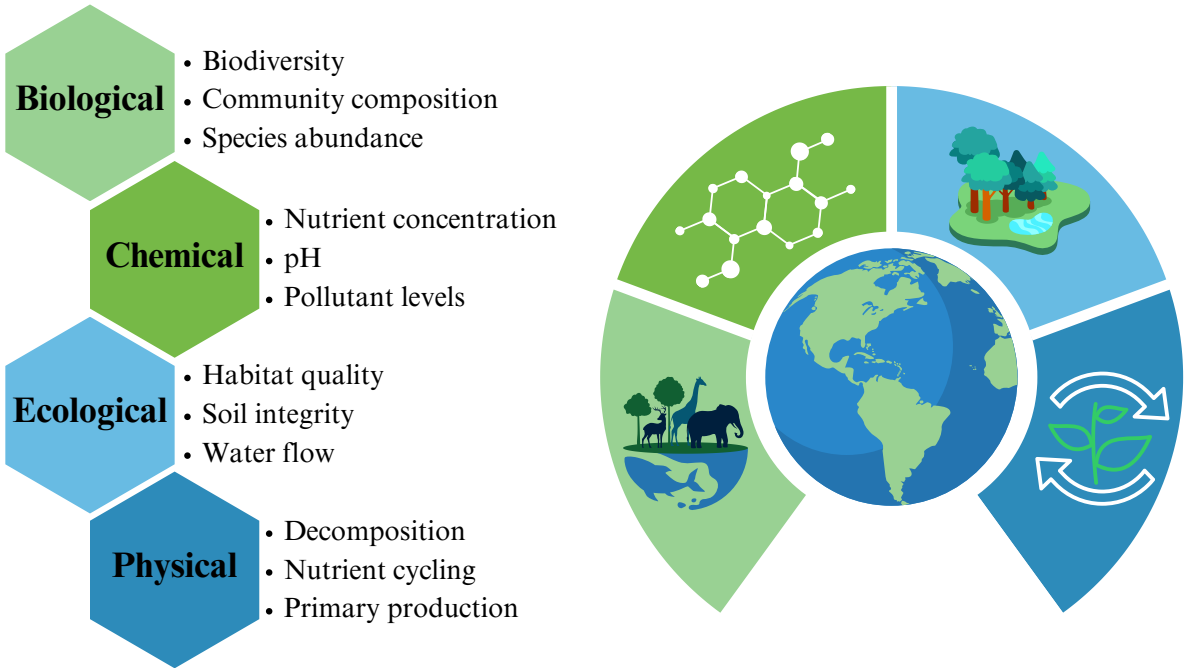


Figure 1.1. Examples of biological, chemical, ecological, and physical indicators (left) used to assess ecosystem health (depicted graphically on the right).

1.2. Aotearoa New Zealand ecology and biodiversity

New Zealand (Aotearoa) represents a globally unique and ecologically isolated system that has evolved independently for over 80 million years following its separation from the ancient supercontinent Gondwana (Gibbs, 2006). Protracted geographic isolation has fostered extraordinarily high levels of endemism across multiple taxonomic groups, including vascular plants, avifauna, reptiles, and invertebrates (de Lange et al., 2009; Gibbs, 2006). Notably, New Zealand's native terrestrial mammal fauna is restricted to only two endemic bat species, the long-tailed bat (*Chalinolobus tuberculatus*) and the lesser short-tailed bat (*Mystacina tuberculata*), which have evolved distinct ecological roles in the absence of other mammalian competitors (O'Donnell et al., 2010). This absence has also enabled birds and reptiles to occupy ecological niches typically filled by mammals in other regions, resulting in highly distinctive community assemblages and evolutionary trajectories (King & Forsyth, 2021).

Since the initial human colonisation by Polynesian ancestors approximately 700 years ago, New Zealand's landscapes and biodiversity have undergone significant transformation (Holdaway et al., 2019; McWethy et al., 2010). Early Māori practices, such as intentional burning, hunting, and the introduction of commensal species including the kurī (Polynesian dog) and kiore (Pacific rat), began altering native species assemblages and ecosystem structures (McGlone, 1989). These impacts were compounded dramatically following European colonisation in the 18th and 19th centuries, which brought about large-scale deforestation and agricultural conversion, and introduced invasive mammalian predators—particularly rats (*Rattus* spp.), stoats (*Mustela erminea*), possums (*Trichosurus vulpecula*), and cats (*Felis catus*)—that have contributed to

widespread declines and extinctions of native fauna and flora (Innes et al., 2010; King & Forsyth, 2021).

The country's native forests, primarily composed of podocarps (e.g., *Dacrydium cupressinum*), southern beech (*Nothofagus* spp.), and diverse broadleaf species, sustain complex ecological networks supporting numerous endemic vertebrates and invertebrates (Cumberland, 1941; Wardle, 1991). These include iconic avian species such as the nocturnal, flightless kiwi (*Apteryx* spp.), the canopy-dwelling kākā (*Nestor meridionalis*), and the highly intelligent alpine kea (*Nestor notabilis*), which fulfil crucial ecosystem functions including seed dispersal, pollination, and insect population regulation (Robertson et al., 2021). Native herpetofauna, comprising approximately 100 species of geckos and skinks, are integral components of food webs, functioning as both predators and prey (Hitchmough et al., 2021). They display unique life history traits, such as viviparity and nocturnality, which enable their survival in diverse microhabitats ranging from forest floor leaf litter to rocky alpine environments (Bickford et al., 2024; Hare et al., 2016).

New Zealand's extensive and varied coastlines further contribute to its ecological distinctiveness. Coastal and marine habitats, including wetlands, estuaries, saltmarshes, dunes, and offshore islands, support native and endemic seabirds such as the yellow-eyed penguin (*Megadyptes antipodes*), Fiordland crested penguin (*Eudyptes pachyrhynchus*), and little blue penguin (*Eudyptula minor*) (Mattern & Wilson, 2018). These species rely on a combination of terrestrial nesting sites and marine foraging grounds, rendering them potentially sensitive indicators of both land-based and marine ecosystem health (Mattern & Wilson, 2018; Muller et al., 2024). Many of these seabirds are vulnerable or endangered due to predation, habitat

degradation, and human disturbance, highlighting the critical need for integrated conservation management across ecological boundaries (Whitehead et al., 2019).

Despite New Zealand's ecological richness, its biodiversity faces persistent threats from habitat loss, introduced species, and global environmental change, including climate change (Innes et al., 2010, 2024; Wilson, 2004). Efforts to conserve and restore native ecosystems increasingly incorporate Māori concepts of *kaitiakitanga* (guardianship and protection) and *mātauranga (knowledge and understanding)*, fostering partnerships that blend Indigenous knowledge with scientific methodologies to enhance ecosystem resilience and biodiversity outcomes (Harmsworth & Awatere, 2013). These collaborative approaches are vital for addressing the complex socio-ecological challenges facing New Zealand's ecosystems and ensuring the protection of its irreplaceable natural heritage.

1.3. Biodiversity

Biological diversity, commonly referred to as biodiversity, encompasses the vast variety of living organisms that exist within the natural world (Rawat & Agarwal, 2015). This diversity can be understood through multiple lenses, notably evolutionary (phylogenetic) and ecological (functional) (Cadotte et al., 2009; Srivastava et al., 2012). From an evolutionary standpoint, biodiversity reflects the differences in genetic lineage and ancestral relationships among species, while ecologically it pertains to the roles and functions organisms play within their environments (Colwell, 2009; Faith, 1992). Biodiversity thus includes the full spectrum of life's genetic makeup, the range of species inhabiting a region, and the variety of ecosystems they form and interact within (Swingland, 2013).

The significance of biodiversity extends beyond mere cataloguing of species; it plays a fundamental role in maintaining ecosystem stability and resilience (Eisenhauer et al., 2024). Ecosystems that harbour a rich array of species and genetic variability are more capable of surviving and adapting to environmental changes and disturbances (Banks et al., 2013). This resilience is derived from the presence of diverse species with different functional traits and ecological niches, allowing ecosystems to buffer against stressors, such as climate fluctuations, invasive species, and natural disasters (Odum, 1999). Consequently, biodiversity enhances ecosystem functions such as nutrient cycling, energy flow, and productivity, helping these systems remain functional even when challenged (Tilman et al., 2014).

Adaptive capacity is especially crucial in the context of accelerating anthropogenic pressures, including habitat destruction, pollution, climate change, and overexploitation of natural

resources (Frietsch et al., 2023; Pelletier & Coltman, 2018). Human activities are rapidly transforming landscapes and disrupting ecological processes, often reducing biodiversity and, with it, the resilience of ecosystems (Haddad et al., 2015; Jaureguiberry et al., 2022; Reader et al., 2023). In response, ecosystems with higher biodiversity are generally better equipped to maintain essential ecological processes, recover from disturbances, and continue providing vital services such as carbon sequestration, soil formation, and water purification (Hughes et al., 2008). These functions support not only the natural environment but also human well-being and economic livelihoods, underscoring the broader value of conserving biodiversity (Hausmann et al., 2016).

To quantify biodiversity, ecologists use various metrics, with *species richness* being one of the most straightforward and widely employed (Roswell et al., 2021). Species richness counts the number of distinct species present within a given ecosystem or geographic area, offering a simple snapshot of biological variety (Waide et al., 1999). However, this measure alone does not capture the full complexity of biodiversity (Magurran, 2013). It overlooks how abundant or rare each species is, and how species are distributed across the community (Magurran, 2013). For example, an ecosystem with many species but dominated by a few individuals of one or two species would have high species richness but an uncaptured view of species abundance (Whittaker et al., 2001).

Species evenness complements richness by measuring the relative abundance of each species within the community (Buzas & Hayek, 2005). It reflects how evenly individuals are distributed among the species present, providing insight into the community's structure and balance (Buzas & Hayek, 2005). However, accurately assessing species evenness requires comprehensive and precise data on species abundance, which can be challenging to obtain,

especially in diverse or cryptic communities (Wang et al., 2021). When considered together, species richness and evenness form the broader concept of *species diversity*, which also accounts for other factors such as the various species' ecological roles, body sizes, or trophic positions (Terborgh, 2015).

Biodiversity metrics are not merely descriptive; they are deeply connected to ecological and evolutionary processes (Purvis & Hector, 2000). Variations in species diversity influence population dynamics, competitive interactions, community assembly, and ecosystem productivity (Fukano et al., 2022; Lehman & Tilman, 2000; Meng et al., 2024). They also affect evolutionary phenomena, such as adaptive radiation, speciation, and the evolution of traits within populations (Kiester, 2013). Understanding species diversity is therefore essential for interpreting higher-level biological organisation, including communities, ecosystems, and biomes, all of which shape the broader patterns and processes of life on Earth (Loreau, 2010; Ricklefs & Jenkins, 2011).

Despite its critical importance, measuring biodiversity presents numerous challenges. Sampling is often incomplete due to logistical constraints, such as difficult terrain, limited funding, or restricted access to remote or protected habitats (Schuette et al., 2018). Many species remain cryptic or rare, making detection difficult without specialised expertise or advanced molecular tools (Hending, 2025). Additionally, taxonomic knowledge is unevenly distributed, with many groups poorly studied or described, complicating accurate identification and classification (Rousseau & Van Hecke, 1999). Biodiversity is inherently dynamic, fluctuating over time and across spatial scales to further complicate assessments. Human-induced environmental changes can cause rapid shifts in species composition, distribution, and abundance, necessitating long-term monitoring to capture these trends (Van Dyke, 2008).

To overcome these challenges, researchers employ a variety of methods, including remote sensing, molecular techniques such as environmental DNA (eDNA) metabarcoding, and citizen science initiatives (Larson et al., 2020). Long-term ecological studies are particularly valuable for tracking biodiversity changes and understanding ecosystem responses to environmental pressures. One effective strategy is the use of bioindicator species: organisms whose presence, absence, or abundance reflect the health and integrity of ecosystems (Hilty & Merenlender, 2000; Siddig et al., 2016). These species can provide critical, cost-effective insights into biodiversity patterns and ecosystem functioning, serving as early warning signals for environmental degradation or recovery (Burger, 2006).

1.3.1 Bioindicators

Bioindicator species are living organisms that serve as vital tools for assessing ecosystem health (Chowdhury et al., 2023). Their function, population dynamics, and overall status provide key qualitative insights into environmental quality and ecosystem integrity because they reveal the impacts of pollution, habitat destruction, and climate change, highlighting both positive and negative trends in ecosystem health (Fig. 1.2) (Taheri et al., 2021). Bioindicators also act as sensitive monitors of biogeographic changes, including shifts in species distribution and community composition, which are vital for understanding ecological dynamics and forecasting potential ecosystem shifts (Siddig et al., 2016). Additionally, bioindicators can reveal alterations in food web interactions, resource competition, and habitat changes due to invasive species (Kumar Rai & Singh, 2020). For instance, copepods, with their short life cycles and rapid reproductive rates, quickly show measurable responses to changes in temperature, salinity, oxygen levels, and pollution—particularly from heavy metals and organic contaminants (Drira et al., 2018). Likewise, lichens, which absorb nutrients and water from atmospheric deposition, are highly sensitive to

nitrogen pollution, making them effective indicators of environmental shifts (Conti & Cecchetti, 2001).

While copepods and lichens are valuable indicators for aquatic and terrestrial ecosystems, numerous other species have the potential to serve as bioindicators. For example, various plants, animals, and microorganisms across different environments can provide unique insights into environmental changes, pollution levels, and overall ecosystem health (Sentenac et al., 2022). Exploring and validating the bioindicator potential of these additional species can not only enhance our understanding of diverse ecosystems but also improve the sensitivity and accuracy of environmental monitoring programmes (Carew et al., 2013; Cordier et al., 2021). This, in turn, can inform conservation efforts and policy decisions, aiding the development of more effective strategies to protect, manage, and restore ecosystems in the face of ongoing environmental change (Parmar et al., 2016).

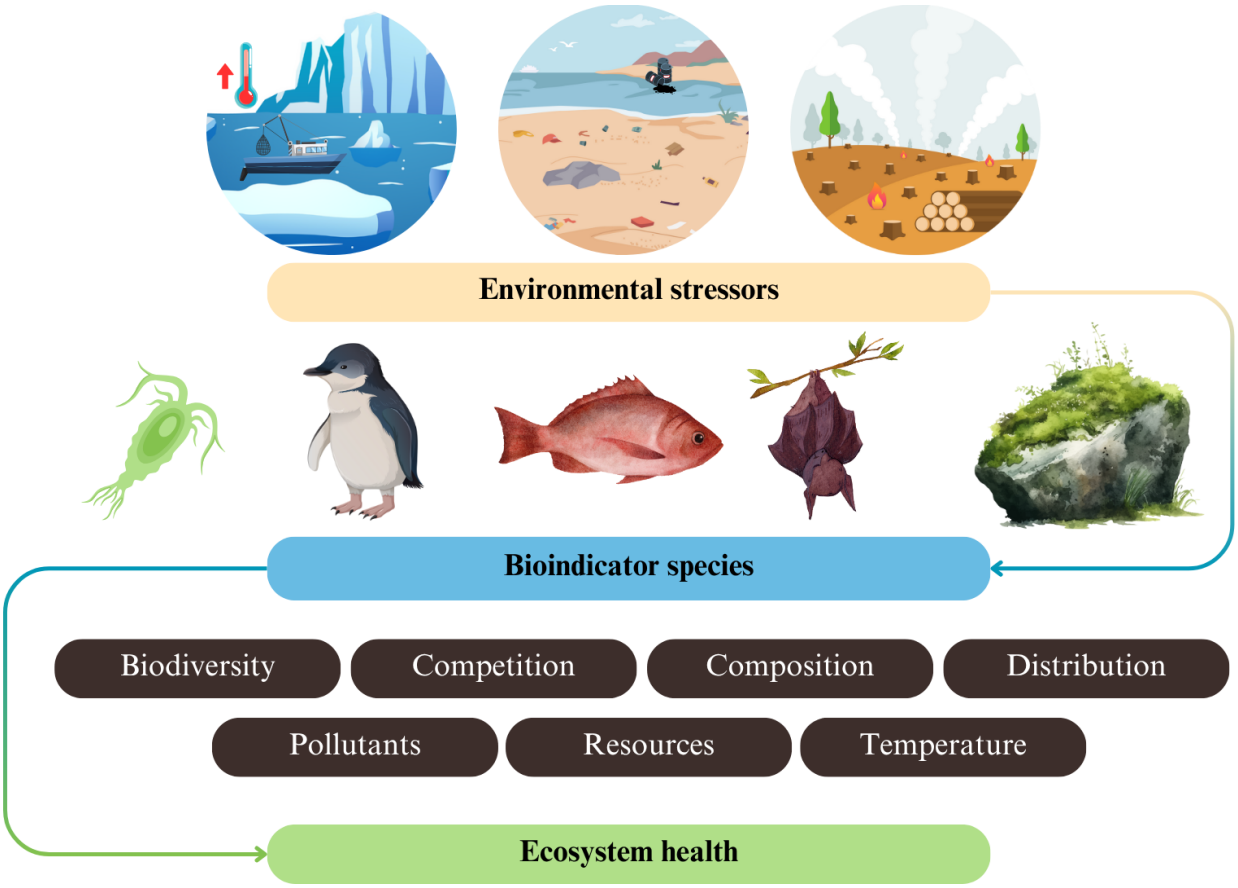


Figure 1.2. Environmental stressors, bioindicator species, and components used to measure and assess ecosystem health.

1.4. eDNA and metabarcoding

Environmental DNA (eDNA) refers to genetic material that is obtained directly from the environment, by sampling substrates such as soil, water, air, and faecal material, rather than through direct sampling of specific organisms via blood or tissue (Bohmann et al., 2014). As organisms interact with their environments, they shed DNA via sloughed cells, gametes, excretions/waste, and decomposing carcasses, which accumulates in their surrounding environment (Stewart, 2019). This accumulated DNA can then be captured through filtration or collection of air, water, and other substrates and extracted in the laboratory (Fig. 1.3).

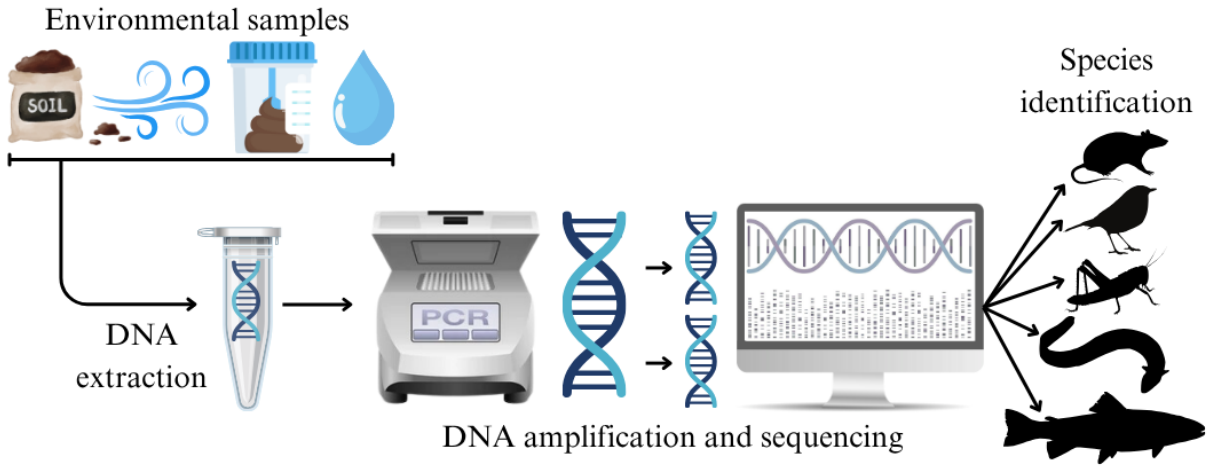


Figure 1.3. Workflow of DNA metabarcoding: from environmental sample collection to DNA extraction, amplification, sequencing, and species identification.

The process of identifying species from environmental samples through molecular approaches begins with DNA extraction. Primers, short nucleotide sequences designed to bind to conserved regions flanking the target barcode regions, are then used in polymerase chain reactions (PCR) to amplify specific segments of genetic material (Taberlet et al., 2012). This targeted amplification increases the abundance of barcode regions, making the DNA easier to detect and analyse (Taberlet et al., 2018). Once amplified, the DNA is subjected to high-throughput

sequencing (HTS) technologies, such as Illumina or Oxford Nanopore platforms, which can generate millions of short DNA sequences (reads) from a single environmental sample (Reuter et al., 2015). These sequencing reads are processed using bioinformatics pipelines that perform quality control to remove sequencing errors and contaminants. The cleaned sequences are then clustered into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs), which represent distinct taxa in the sample (Callahan et al., 2016). These sequence clusters are compared against large DNA reference databases, such as GenBank, to assign taxonomic identities by matching the barcode sequences with those of known species (Creer et al., 2016; Benson et al., 2013). This molecular identification allows researchers to determine the presence and diversity of organisms within the sampled environment. Resulting data can then be visualised and analysed to infer ecological interactions, assess biodiversity patterns, and monitor ecosystem health over time (Fig. 1.3).

The effectiveness of the overall eDNA process depends heavily on the choice of barcode region. An ideal genetic marker must be variable enough to distinguish between species while remaining conserved across broader taxonomic groups (Antil et al., 2023). Commonly used barcode regions include mitochondrial cytochrome c oxidase subunit I (COI) for animals (Hebert et al., 2003), internal transcribed spacer (ITS) regions for fungi (Schoch et al., 2012), and the 16S rRNA gene for bacteria. These markers, when used alongside HTS and robust bioinformatics pipelines, provide a powerful toolkit for molecular biodiversity assessments (Porter & Hajibabaei, 2018).

eDNA has diverse applications, including species detection, diet analysis, biodiversity assessments, and conservation (Fig. 1.4). Its key strengths include the non-invasive nature of

sample collection, especially in remote or dynamic environments—such as aquatic habitats or difficult-to-access terrestrial areas (Foote et al., 2012)—and the ability to simultaneously detect multiple taxa from a single environmental sample (Deiner et al., 2017). For diet analysis, eDNA from faecal material can reveal feeding habits and trophic relationships without direct contact with consumer species, offering less intrusive alternatives to traditional methods that require taxonomic expertise (Banerjee et al., 2022; Nørgaard et al., 2021). eDNA also enhances biodiversity and biosecurity assessments by detecting cryptic or rare species often overlooked by conventional surveys, improving species distribution data critical for conservation and management (Beng & Corlett, 2020; Hending, 2024).

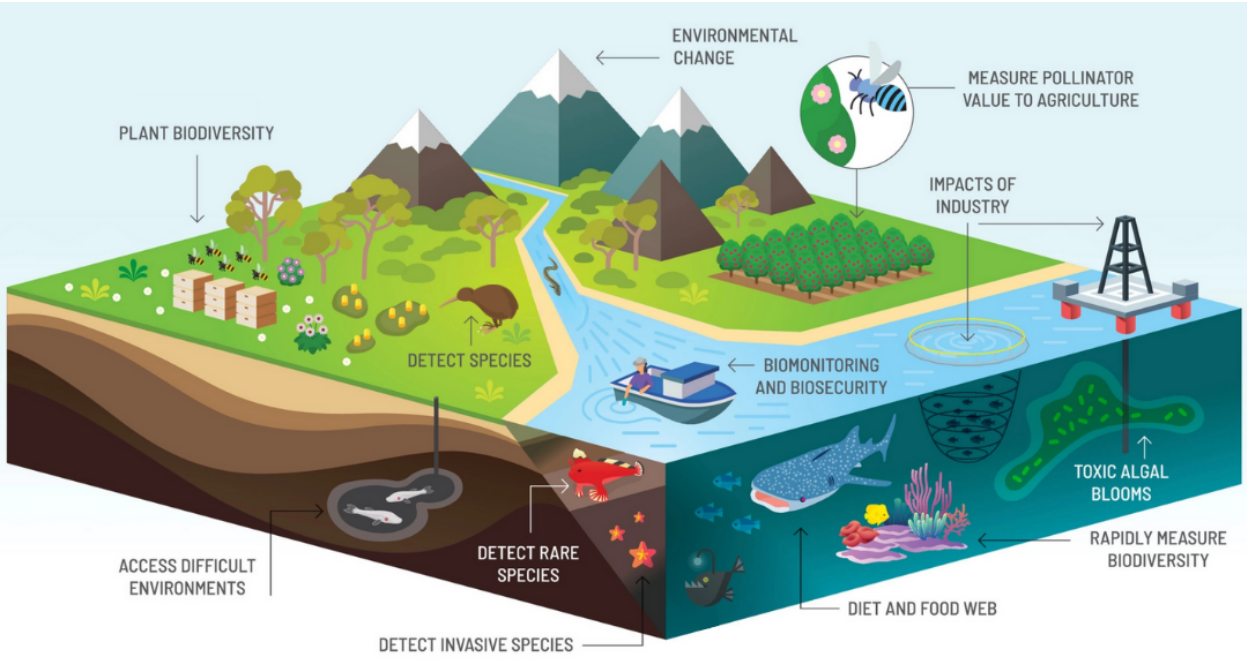


Figure 1.4. Applications of environmental DNA (eDNA) across ecological and biodiversity studies (adapted from McFarlane 2021).

It is important to note that eDNA is often highly fragmented and subject to degradation over time due to environmental factors, such as ultraviolet radiation, temperature fluctuations, microbial activity, and water chemistry (Çevik & Çevik, 2025). This degradation limits the

temporal detection window and affects spatial interpretation, requiring careful consideration when inferring species presence or absence (Barnes & Turner, 2016). In addition, eDNA analyses face challenges from contamination, PCR errors, sequencing biases, and gaps in the reference database, which can cause false positives or negatives and must be carefully evaluated (Goldberg et al., 2015). Distinguishing DNA from living organisms versus residual or transported sources also complicates data interpretation. Thus, to ensure reproducibility and comparability, standardised protocols for sample collection, preservation, and DNA extraction are critical to reduce variability from environmental and procedural factors (Hinlo et al., 2017; Deiner et al., 2017). Additionally, ethical and legal considerations surrounding eDNA sampling are gaining prominence, as permissions may be needed for collections in protected areas or endangered species, and concerns about data privacy arise when detecting species of cultural or economic importance (Roberts & Montoya, 2023; Whitmore et al., 2023). Responsible eDNA research must balance scientific progress with respect for ecological and social values (Ruppert et al., 2019).

Despite some challenges, eDNA offers powerful metrics for ecosystem health assessments. For instance, the Taxon Independent Community Index (TICI) utilises eDNA data to assess freshwater ecosystem health in New Zealand (Wilkinson et al., 2024). This approach assigns health indicator values to species detections (i.e., ASVs representing a broad range of taxa including bacteria, fungi, plants, and animals), removing the need for full taxonomic identification. The TICI model was trained using eDNA data correlated with macroinvertebrate-based ecological assessments across multiple freshwater sites, producing an accessible ecological health score that aids environmental managers and decision makers (Wilkinson et al., 2024). This exemplifies how integrating standardised barcoding, HTS, and bioinformatics extends eDNA's utility from biodiversity inventories to robust ecosystem health evaluations.

1.5.1. Chalinolobus tuberculatus—New Zealand long-tailed bats

Forests are complex networks that cover approximately 31% of the Earth's land area and are among the most diverse terrestrial ecosystems (United Nations, 2024). Forests are estimated to support 80% of Earth's terrestrial biodiversity, harbouring 68%, 75%, and 80% of mammal, bird, and amphibian species, respectively (Reid et al., 2005; Vié et al., 2009). Beyond biodiversity, they play crucial roles in carbon storage, water cycle regulation, and soil fertility while also providing essential resources for human society (e.g., supplying timber, fuel, and a range of non-timber products to global communities; Aznar-Sánchez et al., 2018; Krieger, 2001).

New Zealand forests are particularly significant due to their unique biodiversity, characterised by high levels of plant, avian, reptilian, amphibian, and insect endemism (Barnsley, 2021; de Lange et al., 2009; Doronin, 2023; Macinnis-Ng et al., 2021; Robertson et al., 2021). Approximately 38% of New Zealand's landmass is covered by forests, including indigenous and exotic plantation forests, with the latter predominantly composed of introduced species like *Pinus radiata* (radiata pine) (Ministry for Primary Industries, 2022). While indigenous forests support a range of native species, exotic plantations harbour a range of flora and fauna while also accounting for most of New Zealand's timber production (Pawson et al., 2010). However, both forest types are subject to environmental pressures, including land-use change, logging, pollution, and climate change (Ridley, 2000; Villamor et al., 2024; Watt et al., 2019). These pressures threaten biodiversity and ecosystem health, making it imperative to assess and monitor forest ecosystems to maintain their ecological integrity and sustainability (Tierney et al., 2009).

Integral to forest ecosystems worldwide, bats occupy tropical rainforest and temperate forests on all continents except Antarctica, displaying diverse behaviours and physiological traits (Altringham, 2011). As the only mammals capable of true flight, bats fulfil key roles in insect control, pollination, and seed dispersal, supporting the health and diversity of forest ecosystems (Anderson & Ruxton, 2020; Maslo et al., 2022). New Zealand is home to two endemic bat species: the New Zealand long-tailed bat (pekapeka; *Chalinolobus tuberculatus,* Forster, 1844) and the New Zealand lesser short-tailed bat (pekapeka; *Mystacina tuberculata,* Gray, 1843), each of which contribute to the country's unique ecological dynamics (O'Donnell et al., 2010).

Populations of long-tailed bats show ecological flexibility, inhabiting native and exotic plantation forests (Borkin & Parsons, 2010; Pryde et al., 2005). This species has a small body, with a wingspan of approximately 25–30 cm and weighs just 8–12 grams. It is distinguished by its long tail, which is nearly equal in length to its body and is fully enclosed within the wing membrane, facilitating agile flight and manoeuvrability in dense vegetation (Fig. 1.5) (O'Donnell & Borkin, 2021). The long-tailed bat is thought to be an insectivorous secondary consumer and is nocturnal, relying on echolocation to navigate and hunt. During daylight, it exhibits flexible roosting behaviours, primarily in tree cavities, where it forms small communal roosts ranging from a few to several dozen individuals (Sedgeley & O'Donnell, 1999).

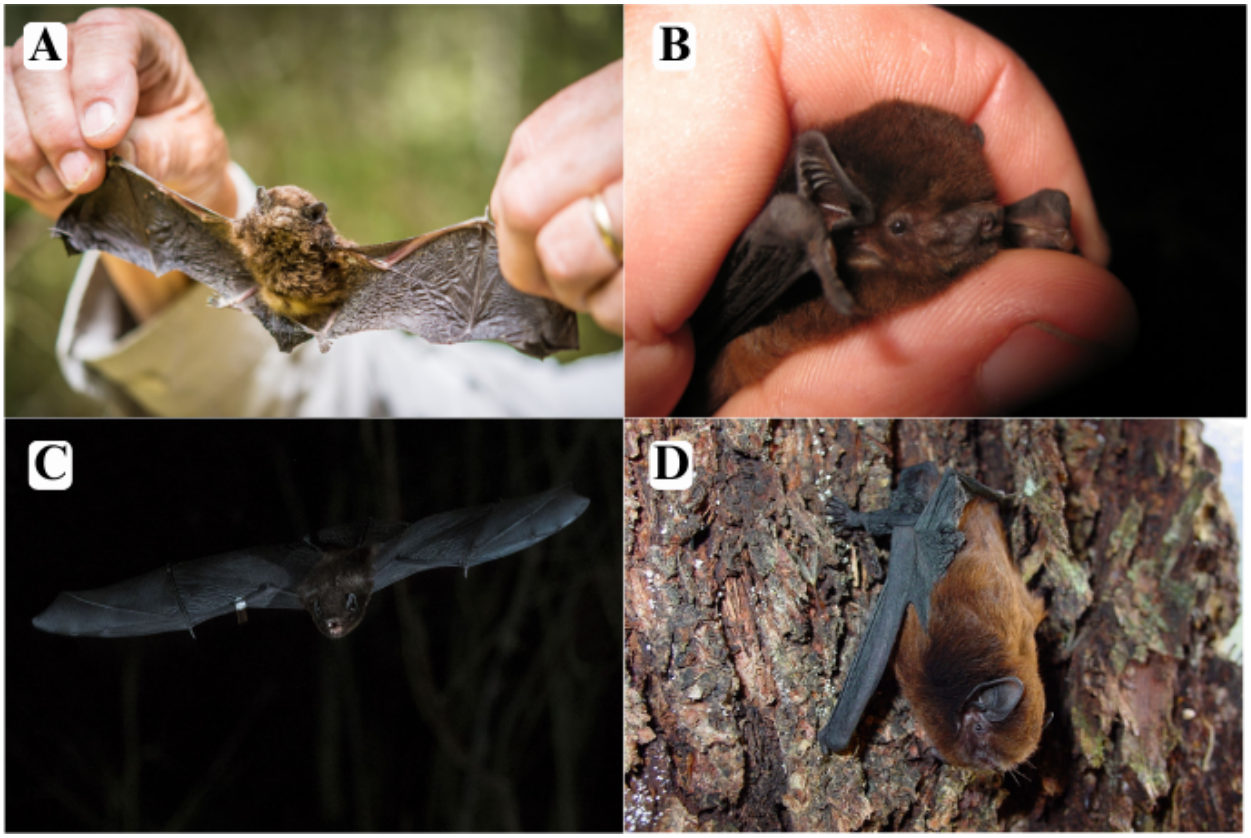


Figure 1.5. Images illustrating the wingspan, size, flight, and resting behaviours of *Chalinolobus tuberculatus*: **A** Wingspan of an individual at Piano Flat, Southland (Shellie, 2017); **B** An individual caught in the Waikato region (DOC, 2024a); **C** An individual in flight in Piopio, Waitomo (Hillock, 2017); **D** An individual resting on the side of a tree, Waikato (Webb, 2024).

Like many endemic species in New Zealand, the long-tailed bat evolved without land-based mammalian predators, making it especially vulnerable to introduced threats such as rats, possums, stoats, and cats (O'Donnell, 2000; O'Donnell et al., 2017). In addition to these predation pressures, anthropogenic activities, including habitat loss and environmental pollution, have led to significant declines in many native species (Craig et al., 2000; Proce et al., 2006). As a result, the long-tailed bat is classified as "Nationally Critical" on the International Union for Conservation of Nature (IUCN) 'Red List' (IUCN, 2020; O'Donnell, 2021), highlighting an urgent need for conservation efforts aimed at protecting not only the bat but also the broader forest ecosystems it inhabits. The bats' sensitivity to environmental changes means that monitoring the diet and

ecological roles of long-tailed bats within New Zealand may also enable assessment of the impacts of habitat degradation, climate change, and human activities on forest health.

1.5.2. Eudyptula minor—little blue penguins

Coastal ecosystems are increasingly threatened by a combination of human activities and climate-driven stressors (He & Silliman, 2019). Development, recreation, and infrastructure expansion have significantly altered shoreline and island environments, disrupting natural processes and degrading habitat quality (Bulleri & Chapman, 2010; Nichols et al., 2019). These impacts have reduced the availability of foraging and refugial habitats for native species, leading to cascading effects on biodiversity and ecosystem function (Riera et al., 2016; Sahavacharin et al., 2022; Zhai et al., 2020). Coastal development, including vegetation clearance, seawall construction, and beach grooming, simplifies complex landscapes and disrupts sediment dynamics (Nordenson et al., 2018; Sahavacharin et al., 2022). On islands, these pressures are often intensified by the introduction of invasive species and the loss of native vegetation (Houghton et al., 2019). Climate-related stressors, such as sea-level rise, increased storm intensity, pollution, and ocean warming compound these challenges by altering habitat availability, shifting species distributions, and disrupting critical life-history events like moulting or nesting (Carroll et al., 2015; Von Holle et al., 2019). Due to the narrow, open nature of many coastal zones, even small-scale disturbances can result in major ecological consequences (Costanza et al., 1993).

New Zealand's coastal ecosystems vividly illustrate these challenges. Spanning over 15,000 km of dynamic coastline, they support a rich array of biodiversity, much of which is endemic to the region (Bell & Gibb, 1996). Around 30% of New Zealand's native birds and over 80% of its fish species depend on these coastal and marine habitats, including estuaries,

mangroves, coral reefs, and kelp forests (DOC, 2024). These ecosystems also contribute to essential ecological functions, such as carbon sequestration, nutrient cycling, and provision of nursery grounds for marine life (Joseph & Pradeepkumar, 2024). Beyond their ecological value, they underpin key fisheries and tourism sectors and hold deep cultural importance for indigenous communities (Bax et al., 2022; Brown & Hausner, 2017; Eddy et al., 2015). As in other regions, however, New Zealand's coastal environments are increasingly vulnerable to threats, including pollution, overfishing, invasive species, and climate change (MacDiarmid et al., 2012; Macinnis-Ng et al., 2021).

Monitoring biodiversity New Zealand's dynamic and threatened coastal ecosystems is both essential and challenging (Hewitt et al., 2022; McGlone et al., 2020). Islands in particular pose logistical difficulties for traditional ecological surveys, which often rely on direct observation, trapping, or acoustic methods (Bombaci & Pejchar, 2018; Graham et al., 2017). These approaches can be labour-intensive, biased toward conspicuous species, and ineffective for detecting cryptic, nocturnal, or burrow-dwelling organisms—many of which play crucial ecological roles (Brown et al., 2013; Owens et al., 2024).

The burrow-nesting little blue penguin (*Eudyptula minor*) is the smallest penguin species in the world, standing at just 30–40 cm tall and weighing around 1 kg (Dann, 2013; Kooyman, 1996). It is easily recognised by its slate-blue plumage and compact, streamlined body adapted for diving (Fig. 1.6.). With short flippers and dense waterproof feathers, *E. minor* is a highly efficient forager, feeding on small fish, squid, and crustaceans in temperate marine waters (Dann, 2013; Kooyman, 1996). *E. minor* plays an important role in marine food webs as a secondary consumer, while also serving as prey for higher trophic level predators (Marchant & Higgins, 1990; Chilvers,

2017). Despite population declines in some areas, *E. minor* is currently listed as "Least Concern" on the IUCN Red List (IUCN, 2020b). However, its coastal distribution and proximity to urban areas expose it to numerous anthropogenic threats, including habitat degradation, light pollution, human disturbance, and predation by introduced mammals (Braidwood et al., 2011; Hocken, 2000; Ratz et al., 1999). Beyond its ecological role, *E. minor* holds cultural significance in te ao Māori (the Māori worldview) as a taonga (treasured) species. Its presence along the coastline symbolises the connection between land and sea and its resilience is seen as a reflection of both environmental conditions and guardianship responsibilities (Te Aka, 2024).

Figure 1.6. Images illustrating the burrowing, moulting, and swimming behaviours of the *Eudyptula minor*: **A** Individuals in a natural burrow, Melbourne, Australia (Veronesi, 2014); **B** Holding a young chick, prior to release, Matiu/Somes Island, New Zealand (Photo: Bishop, 2024); **C** A moulting chick, Matiu/Somes Island, New Zealand (Photo: Stanners, 2024); **D** Parent protecting chick in nest box, Matiu/Somes Island, New Zealand (Photo: Stanners, 2024).

Despite their wide distribution along the coasts of New Zealand and southern Australia, several *E. minor* populations are in decline due to escalating environmental pressures (Cargill et al., 2022; Stevenson & Woehler, 2007). These penguins are particularly susceptible to habitat degradation and urban encroachment because of their preference for nesting in coastal sand dunes, rock crevices, and vegetated margins areas increasingly impacted by development (Braidwood et al., 2011; Reina, 2015). Coastal construction, housing, roads, and recreational infrastructure have reduced the availability of suitable nesting sites, forcing penguins into suboptimal environments inside stormwater drains, under buildings, or in backyards (Cannell et al., 2016; Dann & Chambers, 2013). These settings expose them to elevated levels of noise, light pollution, and human disturbance, which can disrupt natural behaviour (Costello & Colombelli-Négrel, 2023). Light pollution, in particular, disorients penguins during nocturnal foraging and delays nest returns, increasing the risk of predation and exhaustion (Iasiello & Colombelli-Négrel, 2025). Introduced predators also pose a major threat. Off-leash dogs are a leading cause of adult penguin mortality in accessible colonies, while stoats, cats, and ferrets prey on penguin chicks and eggs, often with devastating consequences for small or isolated populations (Braidwood et al., 2011; Hocken, 2000; Ratz et al., 1999). During moulting periods, when penguins are confined to land and cannot forage, they are particularly vulnerable to predation (Ancel et al., 2013).

The nesting ecology of *E. minor* offers a valuable opportunity for non-invasive biodiversity monitoring. Burrows accumulate faeces and organic debris, which contain eDNA not only from prey but also from co-occurring soil invertebrates, parasites, fungi, and microbial communities (Valentini et al., 2016; Wang et al., 2022). The soil around nest entrances also serves as a passive sampler, enriched with guano and biologically active substrates. These deposits provide a window

into the local ecological context—capturing data on trophic interactions, habitat quality, and changes in surrounding biota.

1.6. Thesis questions and objectives

My thesis aims to use eDNA metabarcoding of faecal samples to assess ecosystem health and dietary and local biodiversity patterns using two iconic and threatened New Zealand species: *Chalinolobus tuberculatus*—New Zealand long-tailed bats, and *Eudyptula minor*—little blue penguins. DNA metabarcoding offers a powerful method to identify diet composition while also capturing non-target ('bycatch') DNA that can provide broader insights into local biodiversity.

My research focuses on the following broad questions:

- How does diet vary with physiological state and plantation status in *C. tuberculatus*?

- How does dietary composition and biodiversity bycatch vary across time and space in *E. minor*?

By connecting individual diet and bycatch signals with broader measures of ecosystem health, my research further seeks to evaluate each species as a potential bioindicator, offering new insights into ecological dynamics and conservation management in diverse and often remote environments.

1.7. Thesis structure

Following this introductory chapter (**Chapter 1**), **Chapter 2** uses a fine-scale approach to examine *C. tuberculatus* dietary variation, highlighting links between bats, invertebrate communities, and forest ecosystem health. **Chapter 3** then shifts focus to *E. minor*, demonstrating how faecal DNA can reveal local microhabitat signals and the potential for seabird colonies to act as passive bioindicators of coastal ecosystems. **Chapters 2** and **3** are both written in publication format. **Chapter 4** integrates findings across both empirical chapters, providing a critical comparison and discussing broader ecological implications.

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CHAPTER 2

Bats, bugs, and biodiversity: dietary DNA metabarcoding of New Zealand long-tailed bats (*Chalinolobus tuberculatus*)

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Contributions: K.B. and S.P. collected the faecal samples. A.S. performed the DNA extractions, PCR, and data analysis. A.M. and A.S. co-wrote the manuscript with contributions and input from K.B.

Data accessibility to be added.

2.1. Abstract

Dietary studies in bats offer critical insights into their ecological roles, foraging behaviour, and potential as bioindicators of ecosystem health. As insectivorous predators, bats may play an important role in regulating insect populations, including forestry pests. However, our understanding of the diet of New Zealand's endemic long-tailed bat (*Chalinolobus tuberculatus*) remains limited, particularly within exotic plantation forests where forestry practices may influence foraging patterns. Given the widespread presence of plantation forestry across Aotearoa New Zealand and its importance as roosting and foraging habitat, understanding bat dietary ecology in these modified landscapes is vital.

We used DNA metabarcoding to analyse 91 *C. tuberculatus* faecal samples collected from Kinleith Forest, an exotic plantation forest in the central North Island, examining dietary composition in relation to sex, reproductive status, and forestry harvest (pre- and post-harvest). We identified 3,097 arthropod taxa, including 1,027 species across six classes, with insects (particularly Lepidoptera, Diptera, and Coleoptera) comprising approximately 85% of the diet. Non-native species made up around 40% of total prey, suggesting that *C. tuberculatus* may play a role in controlling exotic and native insect populations within plantation forests. Among our samples, lactating females showed the highest dietary diversity, likely reflecting elevated nutritional requirements, while pregnant females consumed fewer and more sedentary prey types, potentially due to reduced flight agility. Subtle but significant dietary shifts were also observed between pre- and post-harvest periods, suggesting that forestry operations may alter prey availability and bat foraging behaviour.

Collectively, we demonstrate that *C. tuberculatus* exhibits considerable dietary breadth and ecological adaptability within managed forest landscapes. Our results underscore the potential role of *C. tuberculatus* in pest suppression and highlight its value as a potential bioindicator for monitoring the ecological impacts of forestry operations. Supporting diverse and abundant arthropod communities, particularly to meet the heightened energetic demands of reproductive females, will be essential for conserving *C. tuberculatus* and sustaining the ecosystem services it provides within New Zealand's plantation-dominated environments.

2.2. Introduction

Habitat fragmentation is a major ecological concern, influencing species distributions and community composition globally (Mullu, 2016). Plantation forests contribute to fragmentation by replacing native ecosystems with managed, often exotic monocultures, thereby altering habitat structure, microclimate, and resource availability (Barsoum et al., 2016; Brockerhoff et al., 2008; Clout & Gaze, 1984; Stephens & Wagner, 2007). However, in highly modified landscapes, plantation forests may also function as habitat refuges or ecological corridors by facilitating physical movement and genetic connectivity between isolated native forest remnants (Pliscoff et al., 2020).

Despite their potential ecological value, the effects of intensive forestry practices such as harvesting, replanting, and chemical treatments on native species can be difficult to assess, particularly when species are cryptic. Such species often display elusive behaviour, low detectability, and irregular activity patterns, which complicates conventional survey methods and may result in false negatives or overlooked population declines (Bennett et al., 2024; Martin et al., 2022). Consequently, cryptic taxa are frequently underrepresented in ecological assessments, leaving gaps in our understanding of how plantation forestry influences native biodiversity and ecosystem functioning (Pawson et al., 2013).

In addition to reducing biodiversity, forestry practices can alter species interactions and behaviours, with consequences manifesting both within and between species (Brockerhoff et al., 2008). At the intra-specific level, modifications to habitat structure and resource availability can differentially affect individuals depending on physical state, such as reproductive status or sex, by

altering the energetic costs associated with foraging (Clinchy et al., 2013). Inter-specific effects may include disruptions to trophic interactions, such as predator-prey dynamics, resulting from changes in landscape connectivity and structural complexity associated with plantation development and harvest (Manel et al., 2003). For example, the tiger spotted-tailed quoll (*Dasyurus maculatus*) in southeastern Australia avoids recently logged areas, likely due to the impacts of reduced prey abundance and protective cover on limiting foraging opportunities and increasing predation risk (Belcher et al., 2007; Belcher & Darrant, 2006). In New Zealand, the foraging behaviour of the endemic New Zealand falcon (*Falco novaeseelandiae*) exhibits sex-specific patterns within plantation forests. During the breeding season, females preferentially use the interiors of young pine stands (< 4 years old), likely due to nesting responsibilities and proximity to nest sites, whereas males predominantly forage along the edges of these stands, possibly to maximise prey capture efficiency (Seaton, 2007; Seaton et al., 2008, 2013). These examples illustrate how forestry-driven habitat modification can influence foraging behaviour and resource acquisition, particularly for individuals with elevated energetic demands. To better understand how such habitat-driven changes influence animal behaviour, it is essential to consider the broader ecological principles underpinning predator-prey interactions and foraging strategies.

Predator-prey energetics, foraging behaviour, and prey selection are fundamental components of ecological interactions that influence food web dynamics and drive evolutionary adaptations across taxa (Pyke et al., 1977; Stephens & Krebs, 1986). These processes govern the survival and reproductive success of individual organisms while also contributing to broader patterns of species distribution, community composition, and ecosystem functioning (Lazzaro et al., 2009; Schilling et al., 2022). Central to the energetics of predation is the balance between the energy expended in searching for, capturing, and handling prey, and that gained from its

consumption (Giacomini, 2022; Norberg, 2021). This cost-benefit trade-off is formalised in optimal foraging theory, which proposes that natural selection favours foraging strategies that maximise net energy intake per unit of time (Pyke et al., 1977). Accordingly, predators are predicted to exhibit prey preferences and foraging behaviours that enhance energy efficiency, especially under conditions of resource limitation or environmental stress (Giacomini, 2022; Schilling et al., 2022).

Within the optimal foraging theoretical framework, prey selection emerges as a multifactorial process shaped by the internal physiological state of the predator**,** such as its current energetic demands, hunger levels, or reproductive status**,** and by the external environmental conditions (Stephens & Krebs, 1986). Prey characteristics, including size, caloric content, spatial and temporal availability, and the risk of injury or failure during capture, further influence foraging decisions (Holmes & McCormick, 2009; Murphy & Ruth, 2009; Olson et al., 2015). Predators may switch between prey types or foraging strategies in response to changes in prey abundance, competition, or habitat structure, illustrating the plasticity of foraging behaviour in dynamic environments (Pyke et al., 1977). Foraging strategies vary from low-energy approaches, such as ambush or sit-and-wait tactics, to energetically costly modes like active pursuit or aerial hawking – each suited to different ecological contexts and predator morphologies (Luttbeg et al., 2020; Quinn & Cresswell, 2004). These behaviours are further shaped by the sensory and locomotor adaptations of the predator, which influence prey detection and capture efficiency (Ruxton et al., 2004). Prey species simultaneously exhibit a suite of defensive traits, including crypsis, mimicry, agility, and chemical deterrents, that reduce predation risk and contribute to co-evolutionary dynamics (Brakefield, 2009; Sherratt, 2008; Xu et al., 2016).

The New Zealand long-tailed bat (*Chalinolobus tuberculatus*; Fig. 2.1A,B), one of the country's two extant bat species, is classified as '*Threatened – Nationally Critical'* due to widespread habitat loss and predation (IUCN, 2020; O'Donnell, 2021). A member of the family Vespertilionidae, commonly known as vesper or evening bats, *C. tuberculatus* is nocturnal and insectivorous, typically roosting in tree cavities, beneath peeling bark, or within human-made structures during the day. These small, agile fliers weigh between 8 to 12 grams and are known for their fast, erratic flight patterns (O'Donnell & Borkin, 2021). *C. tuberculatus* occupies a range of environments across Aotearoa New Zealand, including native forests and exotic plantation forests. While its presence in these landscapes is well documented, its role as a predator of forestry pests, had not been confirmed prior to this study (Borkin & Parsons, 2010; O'Donnell & Borkin, 2021). Because changes in diet may reflect shifts in prey availability or habitat quality, *C. tuberculatus* has potential as a bioindicator species, improving understanding of ecosystem dynamics and the effects of forest management of biodiversity. However, logistical challenges, such as the difficulty of locating roosts and collecting stomach content or faecal samples, currently limit long-term monitoring and broad-scale surveillance efforts.

Traditional diet studies in bats have primarily relied on visual identification of prey remains in faecal samples, a method with notable challenges. First, visual identification depends on the detection of undigested fragments, such as exoskeletons, wings, or other hard parts, which biases results towards species with robust structures that survive digestion (Arkins et al., 1999; Klare et al., 2011). Second, soft-bodied prey, which are more easily digested and leave little to no trace, are often underrepresented (Deagle et al., 2014). Third, visual identification typically provides coarse taxonomic resolution, which can obscure more detailed dietary patterns, particularly in cases where bats consume rare or cryptic prey (Foran et al., 1997; Putman, 1984). Despite these

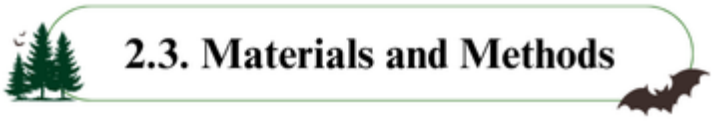
challenges, visual methods have provided some insight into bat diets, identifying high proportions of Diptera (flies), Coleoptera (beetles), and Lepidoptera (moths), with two studies finding that these groups contribute to 29–40%, 18–25%, and 17–24% of the total diet, respectively (Gillingham, 1996; Gurau, 2014). Other taxa, such as Ephemeroptera (mayflies), Hymenoptera (bees, ants), Acari (mites), and Trichoptera (caddisflies), were found in lower percentages, with up to 26% of prey items unidentified (Gillingham, 1996; Gurau, 2014).

DNA metabarcoding of faecal material is a non-invasive method of diet analysis that circumvents the need for visual prey identification (Valentini et al., 2016; Wang et al., 2022). This approach involves extracting total DNA from faecal samples, amplifying taxonomically informative gene regions using universal primers, and sequencing the resulting amplicons via high-throughput sequencing platforms. Taxonomic assignment is then performed by comparing sequence reads to curated reference databases (De Barba et al., 2014; Pompanon et al., 2012; Valentini et al., 2016; Wang et al., 2022). A key advantage of DNA metabarcoding is that it does not require specialised taxonomic or entomological expertise to identify species based on morphological traits, thereby enabling more efficient, accurate, and standardised dietary analyses across diverse taxa when compared to traditional techniques (Stoeckle, 2003).

Recent research by Ling et al. (2023) on the diet of *C. tuberculatus* used DNA metabarcoding of faecal samples from six individuals in Whirinaki Forest Park (a native forest in New Zealand's central North Island), and revealed that aquatic insects, particularly mayflies and caddisflies, comprised 62% of the diet, with Lepidoptera, including leafroller and grass moths also observed. While these findings demonstrate the potential of DNA metabarcoding to contribute to our understanding of bat diets, further research with larger sample sizes and broader geographic

and habitat coverage is needed to fully characterise the dietary diversity and ecological role of *C. tuberculatus*, as well as to assess how habitat modification influences its diet.

In this study, we used DNA metabarcoding of *C. tuberculatus* faecal samples collected from roosts dominated by males and lactating and pregnant females to investigate how plantation forestry practices and bat physiology influenced ecosystem biodiversity and bat foraging behaviours, respectively. We hypothesised that harvest activities would decrease overall prey diversity due to the associated habitat disturbance. Further, we expected to observe: (i) sexual dichotomy in dietary composition, with males and females differing in foraging behaviour and prey selection due to variation in their energy requirements and ecological roles (Racey & Speakman, 1987); and (ii) differences in female diets, with lactating females exhibiting greater diet diversity than pregnant females, reflecting the elevated nutritional demands of lactation and a potentially broader prey spectrum associated with their greater mobility and reduced weight burden.



2.3.1. Study area and sample collection

Faecal samples from long-tailed bats were collected during the summer periods of 2007-2008 and 2008-2009 (December-February) from Kinleith Forest, Waikato, New Zealand – a 96652-ha plantation forest dominated by exotic monocultures, primarily radiata pine (*Pinus radiata*) (Table 2.1; Fig. 2.1.C,D). Bats were captured and samples were collected with permission from Ngāti Raukawa iwi, the Department of Conservation (Low Impact, Research and Collection Permit BP-18899-RES under Section 53, Wildlife Act 1953), and the University of Auckland Animal Ethics Committee (AEC 08/2004/R282) (Borkin & Parsons, 2014). Pre- and post-harvest sites were classified based on forest stand age, silvicultural treatments, and structural attributes including tree status (alive, recently dead, long dead, defoliated), canopy cover, thinning or pruning, and live versus dead tree abundance within 10 m plots (Borkin & Parsons, 2011, 2014). These factors reflect plantation successional stages, with pre-harvest sites having intact canopies and live trees, and post-harvest sites showing increased mortality, reduced canopy, and altered understorey (Borkin & Parsons, 2011, 2014). Sampling was undertaken during the summer to capture pregnant and lactating females for comparison, as well as males (Fig. 2.1E).

Table 2.1. Number of *Chalinolobus tuberculatus* faecal samples collected by reproductive status and harvest stage. See also Table S2.1.

Reproductive status	Harvest stage	No. of samples
Male	Pre	35
Male	Post	1
Female lactating	Pre	19
Female lactating	Post	22
Female pregnant	Post	14
Total		**91**

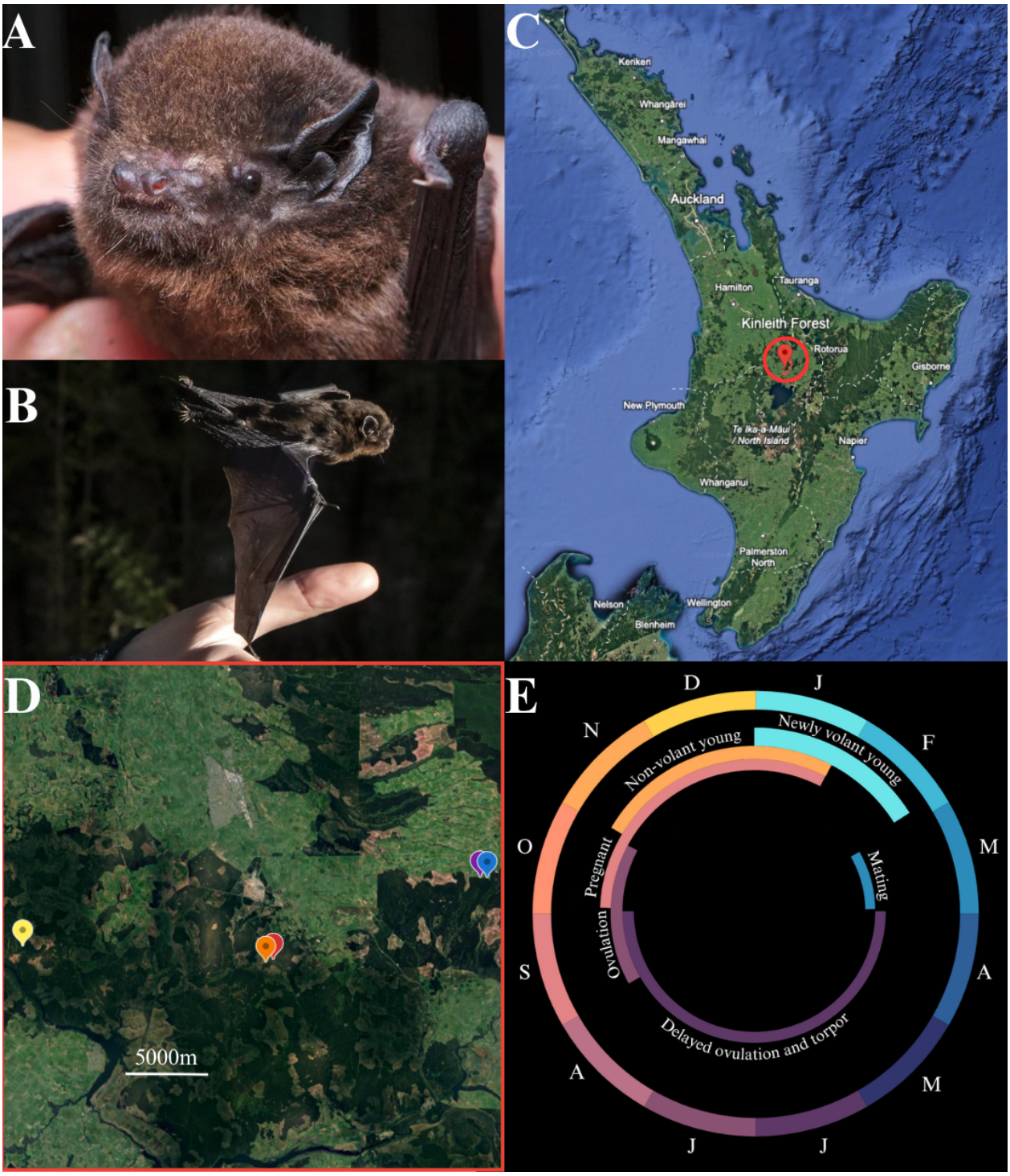


Figure 2.1. A *Chalinolobus tuberculatus* being held for identification (Image: Department of Conservation, 2024); **B** *C. tuberculatus* takes flight in Fiordland (Image: Iain Mcgregor, 2021); **C** Map of New Zealand showing the location of Kinleith Forest; **D** Satellite image of Kinleith Forest indicating roost sites: B Roost 1 (red), B Roost 3 (orange), Carter 2 (yellow), Kinleith Roost 1 (blue), and Kinleith Roost 3 (purple); **E** Annual reproductive cycle of *C. tuberculatus*, illustrating key stages (adapted from O'Donnell, 2002).

Roosts were located via radiotelemetry and bats were also captured using mist nets set across forest roads, harp traps, or hand nets at roost emergence sites (Borkin & Parsons, 2011). Faecal samples were collected from individual bats during capture by retrieving droppings deposited in sterile holding bags, with additional samples obtained non-invasively from communal and solitary roosts by collecting faeces directly from roost structures or from the bases of roost trees, following established protocols to minimise contamination (Borkin & Parsons, 2011). All faecal samples were immediately placed in sterile microcentrifuge tubes and stored at −20 °C until further processing to prevent degradation (Borkin & Parsons, 2011).

Distances from roost or reference trees to key landscape features including forest edges, waterways, roads, and artificial waterpoints (e.g., firefighting ponds) were measured using a Vertex III and Transponder T3 laser rangefinder when features were visible; when direct measurement was not possible, spatial data were extracted using ArcView GIS software (ESRI) with geospatial layers provided by Hancock Forest Management and Carter Holt Harvey Forests (Borkin & Parsons, 2021).

Table 2.2. Euclidean distances from roost locations (Fig. 2.1D) to key landscape features, including water source, roads, and artificial waterpoints. "Distance to water source" refers to the nearest natural waterbody (e.g., stream, river, lake), while "distance to waterpoint" refers to the nearest artificial or designated water source (e.g., trough, tank, pond).

Site name	Map ID (Fig. 2.1)	Sex	Distance to water source (m)	Distance to road (m)	Distance to waterpoint (m)
B Roost 1	Red	Female	145	74.2	485
B Roost 3	Orange	Female	90.0	57.8	119
Carter 2	Yellow	Male	152	9.81	1654
Kinleith Roost 1	Blue	Male	346	175	1161
Kinleith Roost 3	Purple	Male	463	52	1006

2.3.2. DNA extraction and metabarcoding

Genomic DNA was extracted from faecal samples using the Geneaid© Soil Presto DNA Extraction Kit, according to the manufacturer's instructions. The final elution was performed in nuclease-free water to minimise the risk of PCR inhibition. DNA concentration was quantified using a Qubit© fluorometer, which employs fluorescence-based detection for accurate measurement, with calibration against appropriate standards to ensure precise and reliable quantification while minimising potential interference from other substances in the samples.

DNA metabarcoding targeted the mitochondrial cytochrome c oxidase subunit I (COI) gene region, selected for its broad taxonomic coverage across eukaryotic organisms (Folmer et al., 1994; Hebert et al., 2003). PCR amplification was carried out using the mlCOIintF (GGWACWGGWTGAACWGTWTAYCCYCC; Leray et al., 2013) and jgHCO2198 (TAAACTTCAGGGTGACCAAAAAATCA; Geller et al., 2013) primers.

Each 25 µL PCR reaction consisted of 5 µL of MyFi© 5× reaction buffer, 1 µL each of 10 µM forward and reverse primers, 1 µL of 20 mg/mL bovine serum albumin (BSA), 1 µL of 2 U/µL MyFi© DNA polymerase, 15 µL of nuclease-free water, and 2 µL of extracted DNA. The thermal cycling conditions included an initial denaturation at 95 °C for 4 mins, followed by 40 cycles consisting of denaturation at 95 °C for 20 s, annealing at 46 °C for 45 s, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 5 mins. Negative controls, where PCR-grade water was used in place of the DNA template, were included in all amplification runs to monitor for potential contamination.

Following amplification, DNA yield and quality were assessed using Qubit fluorometry and 1% agarose gel electrophoresis, with visualisation facilitated by RedSafe Nucleic Acid Staining Solution. IDT unique dual indices (UDI) were incorporated at index level 7. Post-amplification, products were cleaned and size-selected using solid-phase reversible immobilisation (SPRI) beads according to the supplier's protocol, applying a 2:1 bead-to-template ratio and two 80% ethanol washes to isolate fragments in the ~300–400 bp range. Sequencing of the COI amplicons was carried out by SeQuench Ltd (Nelson, New Zealand) using an Illumina MiSeq platform with a v2 500-cycle kit (2 × 251 bp paired-end reads) and Nextera XT v2 Index Kit Set C dual indices. Prior to sequencing, amplified products were purified and size-selected with solid-phase reversible immobilisation (SPRI) beads following the manufacturer's protocol, retaining fragments of approximately 300–400 bp.

2.3.3. Data analysis

Individual FASTQ read files were processed using R v4.2.1 (R Core Team, 2024) and the DADA2 pipeline v1.34.0 (Callahan et al., 2016). Initially, reads were truncated to remove primer sequences, followed by trimming based on quality score profiles, with up to 30 bp removed from each read end depending on the sequencing quality (Q score). Reads containing ambiguous bases (maxN = 0) or more than two expected errors (maxEE = c(2,2)) were excluded. Error rates for the COI amplicon were estimated, after which the reads were dereplicated and denoised. Chimeras were removed from merged reads de novo, resulting in a high-resolution table of amplicon sequence variants (ASVs).

Taxonomic classification of the ASVs was performed using a custom reference database built with CRABS v1.7.6 (Jeunen et al., 2022), according to the methods described at

https://github.com/gjeunen/reference_database_creator. The reference database was constructed using sequences obtained from the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/) on 17 March 2025, with the following search terms: COI[All Fields] OR COXI[All Fields] OR CO1[All Fields] OR COX1[All Fields] OR cytochrome oxidase subunit i[All Fields] OR cytochrome oxidase subunit 1[All Fields] OR cytochrome oxidase subunit I[All Fields] AND (mitochondrion[filter] AND ("200"[SLEN] : "50000"[SLEN])). Sequences were retrieved, dereplicated, and filtered using CRABS default settings. The COI amplicon region was extracted, primer regions were removed via pairwise global alignment, and sequences were dereplicated and filtered (Supplementary Table S2.2). The final reference database was exported in a format compatible with DADA2.

Following the initial taxonomic assignment in DADA2, some ASVs were further identified to the species level using BLAST searches against the NCBI nucleotide database, during which only ASVs with strong matches (e-value > $1e^{-50}$) were retained. Per-sample ASV counts, taxonomic assignments, and associated metadata were imported into R v4.4.2 for further analysis using the phyloseq package v1.50.0 (McMurdie & Holmes, 2013). Raw read counts per sample ranged from 280 to 309,837 (Supplementary Table S2.2). Samples were initially filtered to include only invertebrate taxa (Annelida, Arthropoda, Cnidaria, Gastrotricha, Mollusca, Nematoda, Platyhelminthes, Porifera, Rotifera, Tardigrada), reflecting the known insectivorous diet of *C. tuberculatus* (Gillingham, 1996; Gurau, 2014; O'Donnell & Borkin, 2021). The dataset was then further refined to include only sequences classified as Arthropoda, so that the most predominant prey (see Results) could be explored in more detail. To ensure data quality and reduce noise from potential sequencing errors or environmental contaminants, samples with fewer than five total reads and amplicon sequence variants (ASVs) lacking phylum-level classification were excluded.

This filtering process resulted in a final dataset comprising 3,097 unique taxa corresponding to 1,027 distinct species across 91 analysed samples.

During preliminary analyses, control samples comprising 1.91% of total reads were included in principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarities alongside biological samples (Supplementary Table S2.3). The resulting ordination (Supplementary Fig. S2.1) showed that control samples clustered tightly near the centroid, indicating a lack of structured or unique taxonomic composition. The extraction control was lost during rarefaction due to low read depth. As such, control samples were determined to have negligible influence on observed biodiversity patterns and were subsequently excluded from final diversity and composition assessments.

Data exploration steps included plotting the relative abundance of the top 20 taxa within each group. Subsequently, analyses on the full filtered dataset included alpha diversity (Shannon's diversity index), and taxonomic richness (ASV count) for each harvest status (pre-, post) and reproductive status (lactating female, pregnant female, male). Rarefaction and species accumulation curves were generated using the rarecurve (100 steps) and accu_plot (10 steps) functions from the vegan and MiscMetabar v0.14.2 (Taudière, 2023) packages, respectively. Following standardisation of read depth across samples (using rarefaction, normalising to 300 reads; resulting in 1,477 taxa and 89 samples), Bray-Curtis dissimilarities were used for multivariate analyses to examine patterns in community composition. Ordinations were performed using PCoA using the ordinate function in phyloseq. PERMANOVA was carried out using the adonis2 function from vegan v2.6-8 (Oksanen et al., 2018), with 999 permutations and $\alpha = 0.05$. Beta dispersion was then calculated using the betadisper function from vegan to assess variance

within groups. To evaluate differences in taxon recovery across reproductive and harvest statuses, clamtest analysis was performed on rarefied data using the clamtest function from vegan. This method identified specialist prey taxa occurring exclusively within each comparison group based on two categorical axes: reproductive state (lactating versus pregnant females) and forestry treatment period (pre-harvest versus post-harvest).

Relationships between dietary diversity and proximity to environmental features were tested using correlation analysis between alpha (Shannon's) diversity and the distance of roosts to roads, open water, and waterpoints via Spearman tests (citation), with trends visualised using LOESS-smoothed plots in ggplot2 v3.5.1 (Wickham, 2016).

The biological status (e.g., native, introduced, or pest species) of each taxon was confirmed through online searches using iNaturalist and/or the Farm Forestry Association of New Zealand's pest database (https://www.nzffa.org.nz/farm-forestry-model/the-essentials/forest-health-pests-and-diseases/Pests/) based on the provided genus or species name. Those that could not be determined were labelled as "biostatus-unclassified".

All visualisations were generated in base R or with ggplot2.

2.4. Results

2.4.1. Overall dietary diversity and composition

The complete invertebrate dataset, compiled from all faecal samples, comprised 3,759 unique taxa corresponding to 1,223 distinct species. These taxa were distributed across ten phyla, with Arthropoda accounting for 83.3% (n = 4,778) of the total occurrences. Other phyla included Nematoda (n = 348; 6.1%), Rotifera (n = 247; 4.3%), Annelida (n = 168; 2.9%), Cnidaria (n = 89; 1.6%), Mollusca (n = 43; 0.8%), Platyhelminthes (n = 33; 0.6%), Porifera (n = 20; 0.3%), Tardigrada (n = 8; 0.1%), and Gastrotricha (n = 1; < 0.1%).

When focusing only on phylum Arthropoda (3,097 taxa, 1,027 species), seven classes were identified, the majority of which (n = 2,633; 85%) were Insecta. Other arthropod classes included Arachnida (n = 382; 12.4%), Malacostraca (n = 48; 1.6%), Collembola (n = 22; 0.7%), Hexanauplia (n = 3; 0.1%), Protura (n = 2; 0.06%), and Symphyla (n = 1; 0.03%) (Table 2.3).

The 18 identified most abundant arthropod taxa were exclusively insects (Fig. 2.2A). Lepidoptera dominated the assemblage, with seven taxa accounting for 51.1% of occurrences, followed by Coleoptera (four taxa; 12.8%) and Diptera (three taxa; 10.9%). Other taxa with < 5% of occurrences included Hemiptera and Siphonaptera, while three taxa (18.6%) were unclassified at the order level (Fig. 2.2B). Classification by biological status revealed that 40.0% of taxa were non-native and 19.9% were native or endemic to Aotearoa New Zealand; 40.1% were unable to be assigned (Fig. 2.2C).

Table 2.3. Overall Arthropoda taxonomy detected in faecal samples of *Chalinolobus tuberculatus*, including their corresponding Class, Order, common names, and proportions of total prey detections represented.

Class	Order	Common Name	Proportion (%)
Insecta	Lepidoptera	Butterflies and moths	26.6%
Insecta	Diptera	Flies, mosquitoes, midges	23.2%
Insecta	Coleoptera	Beetles	16.0%
Insecta	Hemiptera	True bugs (e.g., aphids, cicadas)	3.8%
Arachnida	Sarcoptiformes	Mites (e.g., oribatid mites)	2.3%
Arachnida	Araneae	Spiders	2.1%
Insecta	Trichoptera	Caddisflies	1.9%
Malacostraca	Cumacea	Cumaceans (hooded shrimp)	1.6%
Arachnida	Trombidiformes	Mites (e.g., velvet mites, chiggers)	1.5%
Insecta	Hymenoptera	Bees, wasps, ants	1.2%
Arachnida	Mesostigmata	Predatory mites	1.1%
Insecta	Psocoptera	Booklice and barklice	< 1%
Malacostraca	Amphipoda	Amphipods	< 1%
Hexanauplia	Calanoida	Calanoid copepods	< 1%
Malacostraca	Decapoda	Crabs, lobsters, shrimp	< 1%
Entognatha	Entomobryomorpha	Springtails (elongate-bodied)	< 1%
Insecta	Ephemeroptera	Mayflies	< 1%
Malacostraca	Isopoda	Isopods	< 1%
Arachnida	Ixodida	Ticks	< 1%
Insecta	Neuroptera	Lacewings and relatives	< 1%
Insecta	Odonata	Dragonflies and damselflies	< 1%
Insecta	Orthoptera	Grasshoppers, crickets, wētā	< 1%
Insecta	Plecoptera	Stoneflies	< 1%
Entognatha	Poduromorpha	Springtails (globular-bodied)	< 1%
Arachnida	Pseudoscorpiones	Pseudoscorpions	< 1%
Arachnida	Scorpiones	Scorpions	< 1%
Insecta	Siphonaptera	Fleas	< 1%
Insecta	Thysanoptera	Thrips	< 1%
Unclassified	Unclassified	Unclassified	10.2%

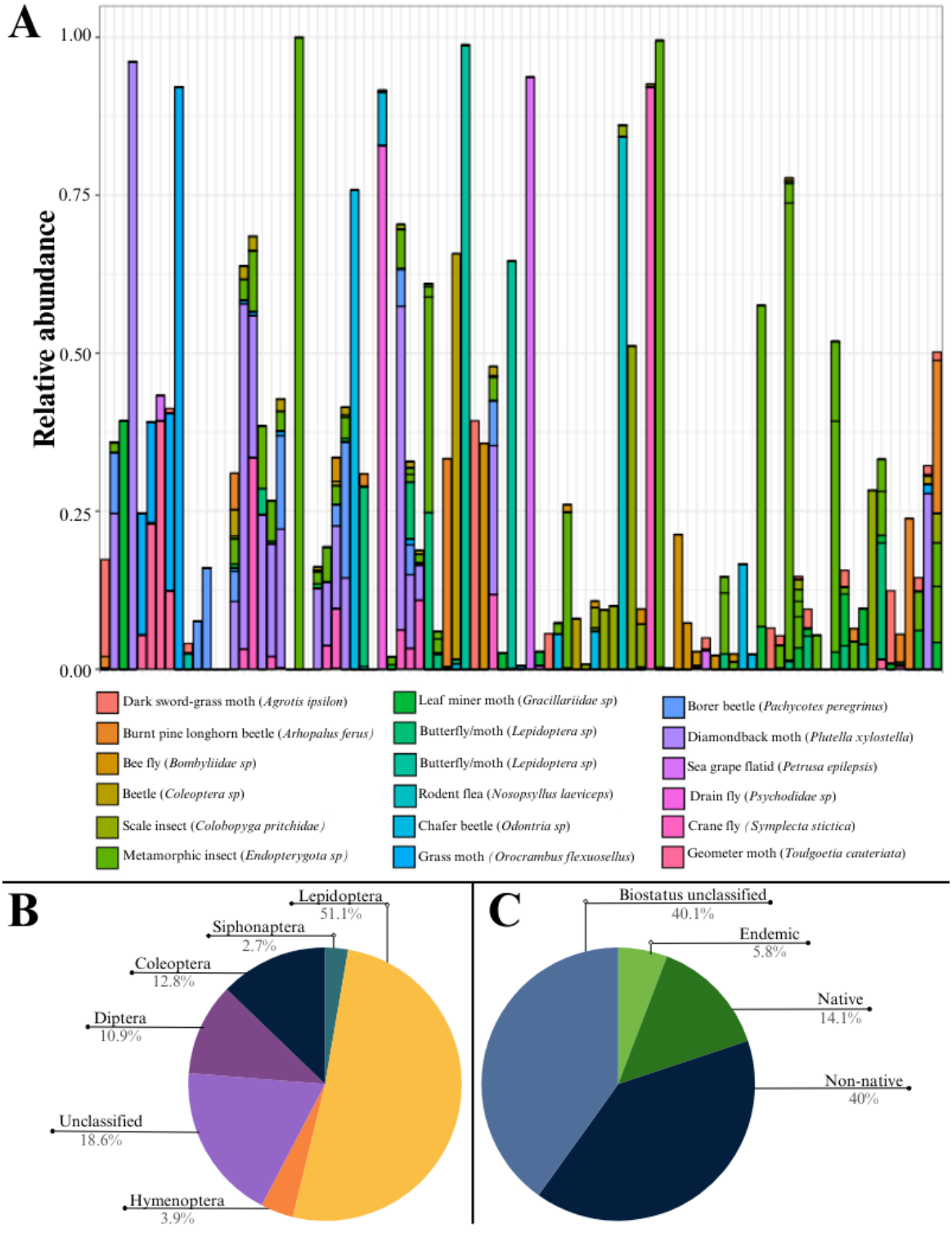


Figure 2.2. A Relative abundance of the top 18 most abundant taxa across individual *Chalinolobus tuberculatus* faecal samples, with each bar representing a single sample. Taxa are colour-coded according to the legend, and both common and scientific names are provided for each species; **B** Taxonomic distribution of the top 18 most abundant taxa, shown at the order level; **C** New Zealand biological status classification of the top 18 most abundant taxa.

2.4.2. Insect species of economic and ecological concern

DNA metabarcoding of faecal samples from *C. tuberculatus* detected multiple arthropod taxa identified as pest species across ecological, horticultural, agricultural, and forestry contexts in Aotearoa New Zealand. Among horticultural and agricultural pests, non-native species such as *Acalitus vaccinii* (blueberry bud mite), *Agrotis ipsilon* (dark swordgrass moth/greasy cutworm), *Bemisia tabaci* (silverleaf whitefly), and *Plutella xylostella* (diamondback moth) were detected. Forestry pests included both non-native species, such as *Arhopalus ferus* (burnt pine longhorn beetle), and native or endemic species (e.g., *Planotortrix notophaea,* blacklegged leafroller; *Ctenopseustis obliquana,* brownheaded leafroller; *Pachyodes peregrinus,* borer beetle). The endemic species *Orocrambus flexosellus* (common grass moth) was also present in 21 samples. Additionally, mosquitoes of the genus *Anopheles*, which are of biosecurity concern and considered non-native, were detected in 50 samples.

2.4.3. Influence of reproductive and harvest states on dietary composition

We identified marginal differences in dietary richness between sex and within reproductive categories, with lactating and pregnant females showing slightly higher Shannon's alpha diversity compared to males (Fig. 2.3A). Harvest status (pre- versus post-) had a minimal effect on the variability of within-sample alpha diversity (Fig. 2.3A). nMDS plots showed some separation in dietary composition among reproductive categories, particularly for pregnant females, which formed a distinct cluster compared to males and lactating females (PERMANOVA: $F_{2,88} = 1.5864$, $p = 0.001$; BETADISPER: $F_{2,88} = 5.7654$, $p = 0.008$) (Fig. 2.3B, Supplementary Fig. S2.2). Pairwise comparisons indicated that these patterns were driven by significant differences between pregnant females and both males ($p = 0.0164$) and lactating females ($p = 0.003$), while no

significant difference was found between lactating females and males ($p = 0.282$). Compositional diet differences due to harvest were more subtle, giving an overall signal for significant differences (PERMANOVA: $F_{1,89} = 1.4892$, $p = 0.004$; BETADISPER: $F_{1,89} = 0.3036$, $p = 0.591$) (Supplementary Fig. S2.2), but no significant difference at the pairwise level ($p = 0.597$).

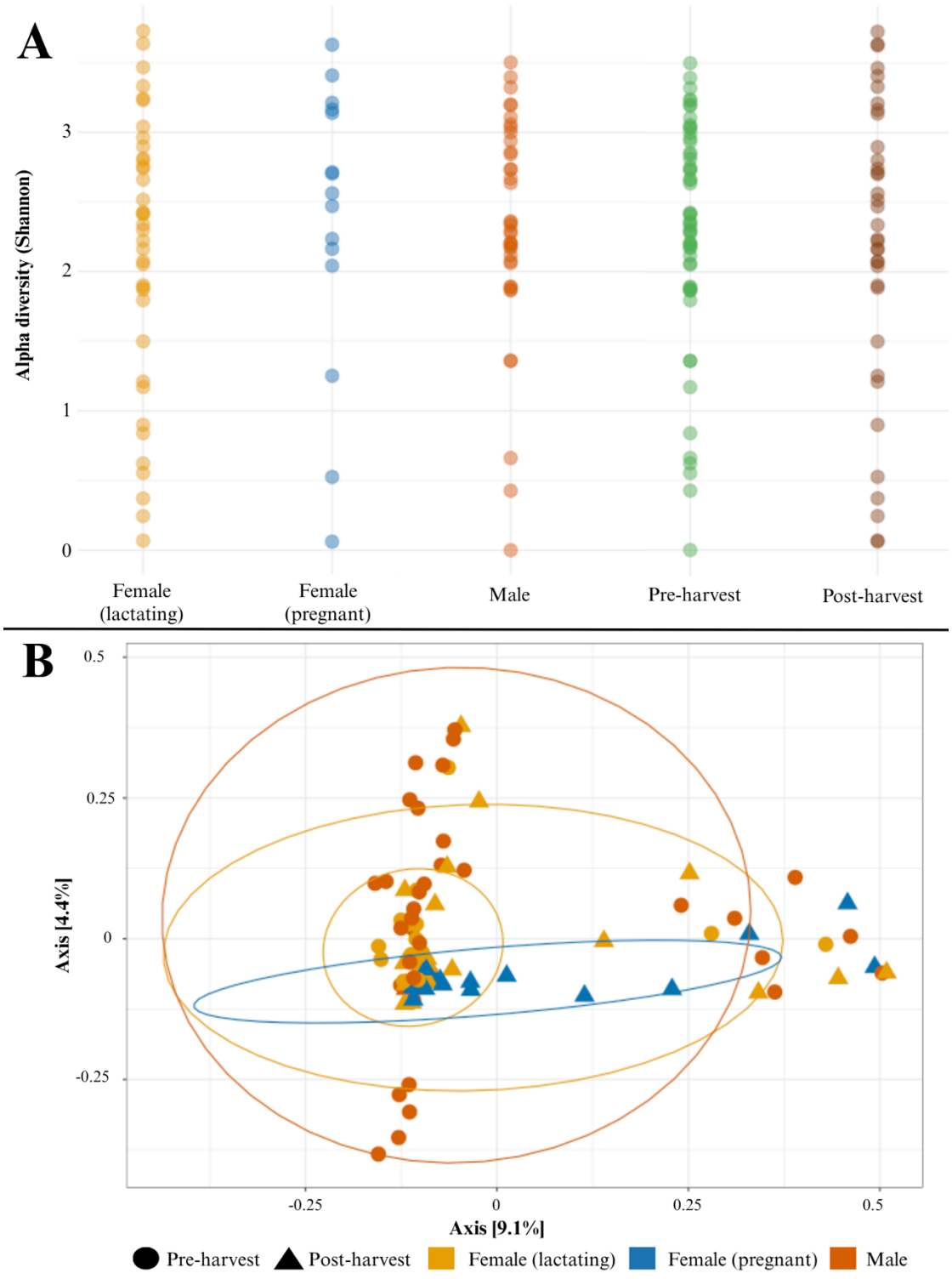


Figure 2.3. Diet diversity of New Zealand long-tailed bats (*Chalinolobus tuberculatus*) categorised by reproductive and harvest status: **A** Shannon's alpha diversity values of dietary taxa across reproductive categories (lactating females, pregnant females, and males) and between forestry harvest status (pre- and post-harvest). Each point represents a single faecal sample; **B** Principal Coordinates Analysis (PCoA) of Bray-Curtis dissimilarities depicting variation in diet composition (beta diversity). Points are coloured by reproductive status and shaped by harvest status as indicated by the legend. Ellipses indicate 95% confidence intervals for each reproductive group.

2.4.4. Taxonomic composition of prey across reproductive and harvest states

Community composition analysis revealed that the majority of individuals across all reproductive and harvest categories belonged to the class Insecta, comprising from 91.3% (males) to 95.6% (lactating females) of diets (92.0% for pregnant females. Similarly high proportions were observed when the data was defined as pre-harvest (89.9%) and post-harvest (96.4%). Arachnida was the second most abundant class, ranging from 3.3% post-harvest to 8.8% pre-harvest, and was highest in pregnant females (7.4%). Other classes, including Malacostraca and Collembola, were consistently detected at low levels (< 2%) (Table 2.4).

At the order level, Lepidoptera and Diptera were dominant across all groups. Males had the highest proportion of Lepidoptera (52.4%), while lactating females consumed both Lepidoptera (32.1%) and Diptera (20.8%) in relatively high amounts (Table 2.4). Pregnant females had the highest proportion of Diptera (61.1%) and moderate levels of Lepidoptera (9.49%) and Coleoptera (9.79%). Pre- and post-harvest comparisons revealed a slight decrease in Diptera (31.6% to 27.3%) and an increase in Lepidoptera (28.1% to 32.3%). Coleoptera also increased post-harvest (14.2% to 17.1%) (Table 2.4).

Less common arthropod orders, including Mesostigmata, Trichoptera, and Sarcoptiformes, were more frequently detected in reproductive females, particularly lactating individuals. For example, Mesostigmata accounted for 2.46% of lactating female diets and 3.73% of pre-harvest samples (Table 2.4).

Table 2.4. Relative abundance of prey taxa identified in *Chalinolobus tuberculatus* diet samples across reproductive and harvest statuses. Values represent the percentage contribution of each taxonomic class and order to total prey composition.

Taxonomic Level	Female (lactating)	Female (pregnant)	Male	Pre-harvest	Post-harvest
Class					
Insecta	95.6	92.0	91.3	89.9	96.4
Arachnida	4.0	7.4	6.9	8.8	3.3
Malacostraca	0.1	0.4	1.7	1.0	0.2
Collembola	0.2	0.2	< 0.1%	0.4	0.1
Hexanauplia	< 0.1%	–	< 0.1%	< 0.1%	–
Protura	< 0.1%	–	< 0.1%	< 0.1%	< 0.1%
Symphyla	–	–	< 0.1%	< 0.1%	–
Taxonomic Level	**Female (lactating)**	**Female (pregnant)**	**Male**	**Pre-harvest**	**Post-harvest**
Order					
Amphipoda	–	< 0.1%	< 0.1%	< 0.1%	–
Araneae	0.28	3.41	0.19	0.45	1.31
Calanoida	< 0.1%	–	< 0.1%	< 0.1%	–
Coleoptera	19.1	9.79	14.1	14.2	17.1
Cumacea	< 0.1%	0.23	1.95	1.01	0.11
Decapoda	0.1	0.19	–	< 0.1%	0.14
Diptera	20.8	61.1	14.9	31.6	27.3
Entomobryomorpha	0.23	0.13	–	0.36	< 0.1%
Ephemeroptera	< 0.1%	0.12	< 0.1%	< 0.1%	< 0.1%
Hemiptera	19.8	7.65	6.75	9.34	17.6
Hymenoptera	1.34	< 0.1%	1.53	2.67	0.13
Isopoda	–	–	< 0.1%	< 0.1%	–
Ixodida	< 0.1%	–	–	< 0.1%	–
Lepidoptera	32.1	9.49	52.4	28.1	32.3
Mesostigmata	2.46	< 0.1%	0.21	3.73	0.13
Neuroptera	< 0.1%	–	1.25	0.64	< 0.1%
Orthoptera	< 0.1%	0.48	2.02	1.07	0.18
Poduromorpha	–	–	< 0.1%	< 0.1%	–
Psocoptera	0.1	0.91	0.1	0.11	0.39
Sarcoptiformes	0.36	2.82	1.51	1.03	1.21
Scorpiones	< 0.1%	–	–	< 0.1%	–
Siphonaptera	1.55	–	–	2.4	–
Thysanoptera	< 0.1%	< 0.1%	< 0.1%	< 0.1%	< 0.1%
Trichoptera	0.57	2.65	1.06	0.82	1.33
Trombidiformes	0.98	0.92	1.72	2.19	0.45

2.4.5. Specialist prey taxa exclusive to reproductive and harvest status

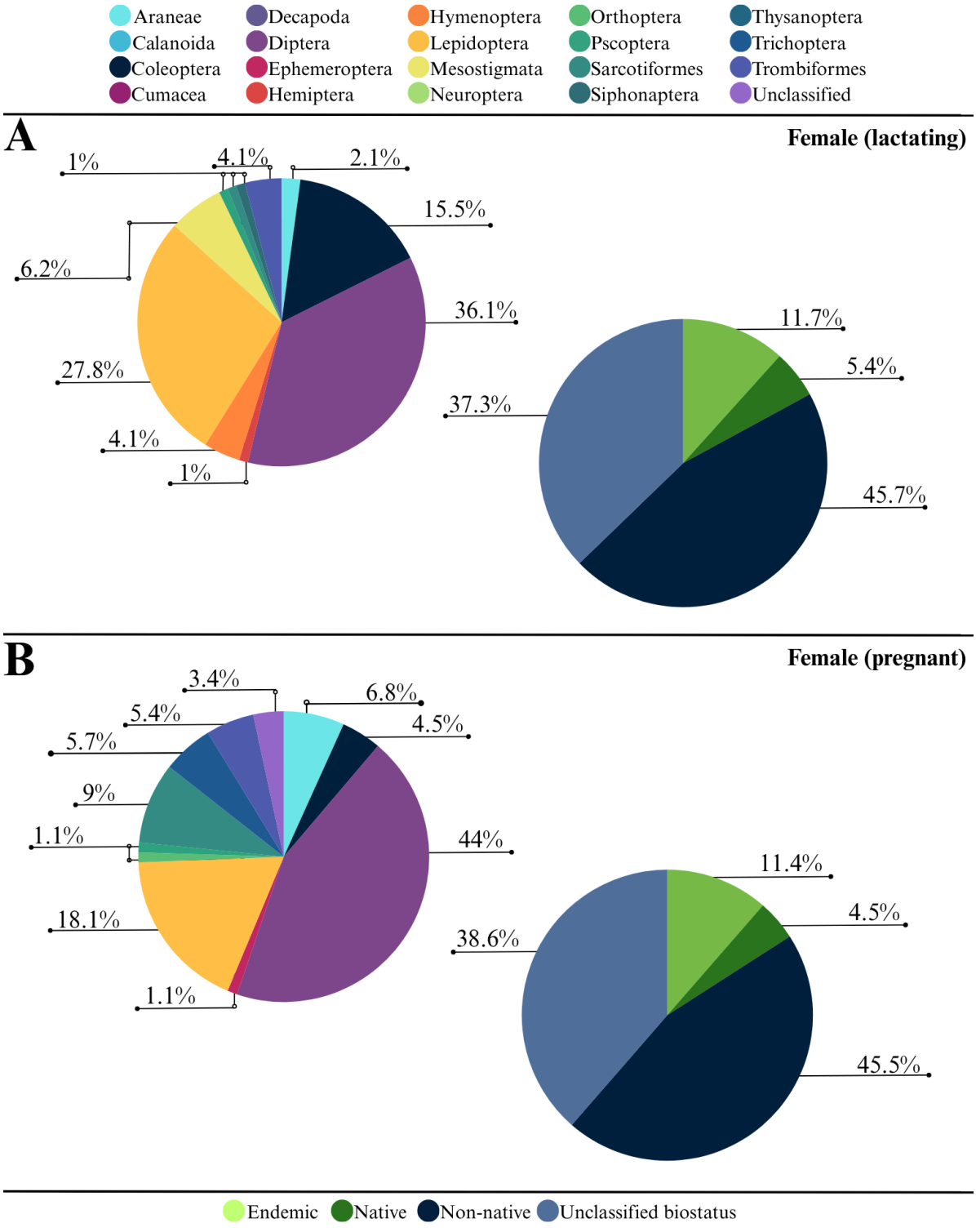
Reproductive group comparisons

A total of 73 and 64 unique arthropod species were identified in the diets of lactating (n = 38) and pregnant (n = 15) females, respectively (Fig. 2.4A,B, Supplementary Fig. S2.3, Supplementary Table S2.4). Diets in both groups were dominated by taxa from the class Insecta, detected in 86.6% of samples from lactating females and 79.5% from pregnant females. Arachnida was the second most frequently detected class, present in 13.4% and 20.5% of samples, respectively (Fig. 2.4A,B, Supplementary Fig. S2.3, Supplementary Table S2.4). At the order level, Diptera were more commonly detected in pregnant females (45.9%) compared to lactating females (36.1%). In contrast, lactating females exhibited broader dietary composition, with higher proportions of Lepidoptera (27.8% vs. 18.8%) and Coleoptera (15.5% vs. 4.7%). Mesostigmata (6.2%) and Hymenoptera (4.1%) were detected exclusively in lactating females, whereas Sarcoptiformes (9.4%) and Araneae (7.1%) were more frequently detected in pregnant females compared to lactating females (1.0% and 2.1%, respectively). Non-native species constituted approximately 45% of the unique taxa in both reproductive categories. Endemic and native species were detected at low levels, and approximately 38% of taxa in each group could not be confidently assigned a biological status due to limitations in taxonomic resolution, with many identified only to genus level or higher (Fig. 2.4A,B, Supplementary Fig. S2.3, Supplementary Table S2.4).

Pre- and post-harvest comparisons

Dietary composition also differed between pre- and post-harvest samples, with 105 unique arthropod species identified pre-harvest and 83 post-harvest (Fig. 2.4C,D, Supplementary Fig.

S2.3, Supplementary Table S2.5). Insecta were the most frequently detected class in both groups, present in 86.7% of pre-harvest samples and 81.6% of post-harvest samples. Arachnida were also well represented, occurring in 10.4% of pre-harvest samples and 17.5% of post-harvest samples. Diptera were similarly prevalent in both groups (35.8% pre-harvest, 39.3% post-harvest). Lepidoptera were more frequently detected in pre-harvest samples (30.6%) than in post-harvest samples (23.2%), while Coleoptera were more common post-harvest (12.5%) (Fig. 2.4C,D, Supplementary Fig. S2.3, Supplementary Table S2.5). Taxa from the class Hexanauplia were detected exclusively in pre-harvest samples, although at low frequency (0.7%). Non-native taxa accounted for 43.0% of species in pre-harvest samples and 50.9% in post-harvest samples. Native and endemic species were detected at low levels in both groups, with a slightly greater proportion of native taxa recorded post-harvest (7.4%) than pre-harvest (3.5%). As with the reproductive group comparisons, approximately 38% of taxa in each harvest category could not be assigned a biological status due to insufficient taxonomic resolution (Fig. 2.4C,D, Supplementary Fig. S2.3, Supplementary Table S2.5).


Araneae
Calanoida
Coleoptera
Cumacea
Decapoda
Diptera
Ephemeroptera
Hemiptera
Hymenoptera
Lepidoptera
Mesostigmata
Neuroptera
Orthoptera
Pscoptera
Sarcotiformes
Siphonaptera
Thysanoptera
Trichoptera
Trombiformes
Unclassified
A
Female (lactating)
1%
4.1%
2.1%
15.5%
6.2%
36.1%
27.8%
4.1%
1%
11.7%
5.4%
37.3%
45.7%
B
Female (pregnant)
3.4%
6.8%
5.4%
4.5%
5.7%
9%
1.1%
44%
18.1%
1.1%
11.4%
4.5%
38.6%
45.5%
Endemic
Native
Non-native
Unclassified biostatus

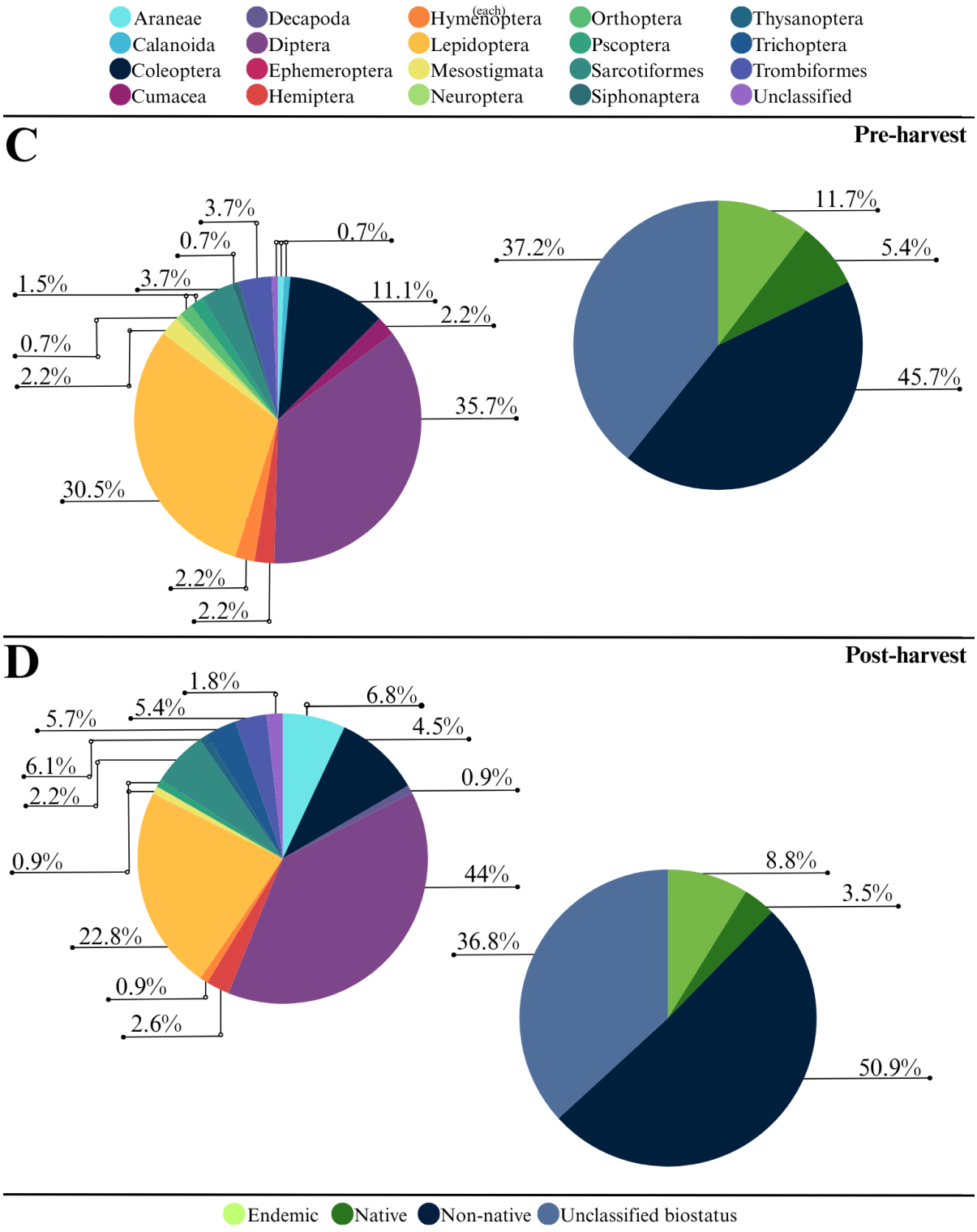


Figure 2.4. Specialist dietary composition of New Zealand long-tailed bats (*Chalinolobus tuberculatus*) across: **A** and **B** reproductive status; and **C** and **D** forestry harvest status, based on DNA metabarcoding of faecal samples. Each pie chart shows the proportional representation of the taxonomy (order) of the prey species identified, alongside the biological status (endemic, native, non-native, unknown) of detected species. These samples represent specialist diets unique to lactating versus pregnant females, and unique to pre- and post-harvest individuals.

2.4.6. Effects of proximity to roads and water sources on dietary diversity

Spearman's rank correlations revealed no significant relationship between individual dietary alpha diversity and distance to roads ($\rho = -0.053$, $p = 0.652$) (Fig. 2.5A). However, a moderate positive correlation was observed between alpha diversity and distance to natural water bodies ($\rho = 0.333$, $p = 0.004$), with individuals located further from natural water sources exhibiting slightly higher Shannon diversity (Fig. 2.5B). Similarly, distance to artificial waterpoints showed a weak positive trend with dietary diversity ($\rho = 0.214$, $p = 0.067$) (Fig. 2.C).

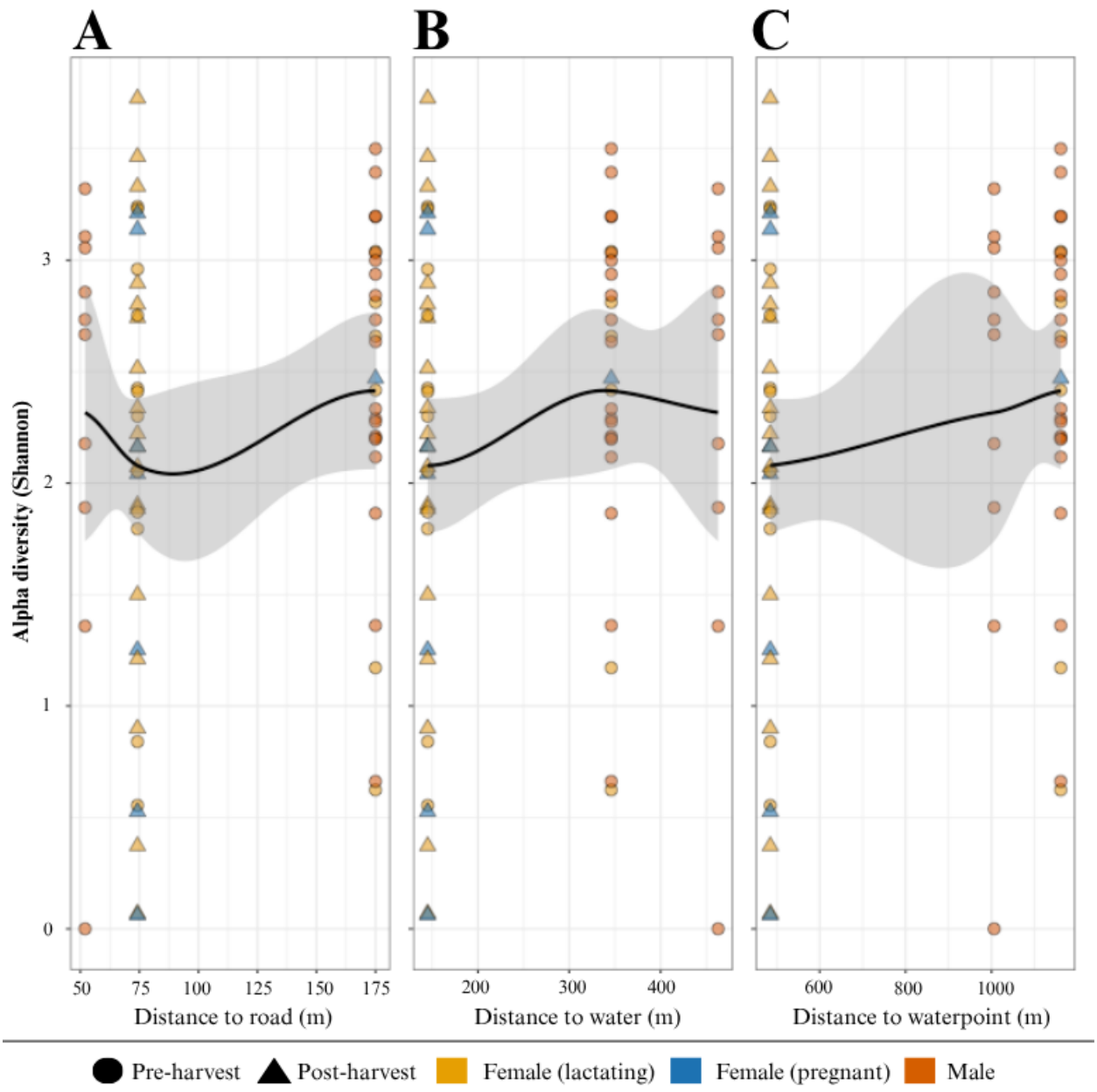


Figure 2.5. Relationships between dietary diversity (Shannon index) and distances to three landscape features: **A** distance to road (m); **B** distance to natural water (m); and **C** distance to artificial waterpoints (m). Points represent individual faecal samples and are coloured by reproductive status, shaped by harvest status; shaded areas indicate 95% confidence intervals of the smoothed (LOESS) fit.

2.5. Discussion

This study represents the most comprehensive analysis of *C. tuberculatus* diet to date, employing high-resolution DNA metabarcoding to integrate dietary data with reproductive status and forestry harvest context, providing new insights into how both physiological condition and environmental disturbance influence prey selection and foraging strategy in this generalist insectivorous bat.

We identified over 3,000 unique arthropod taxa in our bat faecal samples, more than 1,000 of which were identified to species level. This species richness and its broad taxonomic range aligns with the species' known behaviour as a nocturnal generalist aerial hawker, highlighting its capacity to exploit a wide variety of prey across dynamic and modified plantation forest environments (O'Donnell, 2000, 2001, 2002). Our results are consistent with previous work, which found high arthropod diversity from faecal samples collected in Pureora (native) and Kinleith (plantation) forests using visual methods (identifying 2,247 arthropod fragments, with major groups including Diptera (40%), Lepidoptera (24%), and Coleoptera (18%)), though species-level identification was not achieved (Gurau, 2014). Subsequent metabarcoding of faecal samples from six individuals in Whirinaki Forest Park (native) identified 24 dietary taxa with a predominance (62%) of aquatic insects (particularly Ephemeroptera and Trichoptera), small-bodied Lepidoptera, and other terrestrial insects (Ling et al. 2023). Fourteen of those taxa overlapped with species detected in our study, highlighting the potential for spatial variation among sites. Future work could revisit Whirinaki Forest Park and Pureora to see if higher sample numbers and eDNA methods would recover additional unique arthropod taxa in similar richness to that detected here and/or showcase the role of spatial variation and forest type (native vs exotic plantation) in determining bat diet breadth.

Nearly 40% of the *C. tuberculatus* diet in our study comprised non-native taxa, regardless of reproductive stage or forestry harvest condition. This high proportion of introduced species highlights the potential ecological role of *C. tuberculatus* in suppressing populations of invasive pest insects within plantation forest ecosystems. Several of the identified non-native taxa are known pests in plantation forestry, whose feeding activities cause direct economic losses and degrade ecosystem health. For example, we detected the burnt pine longhorn beetle (*Arhopalus ferus*), whose wood-boring behaviour compromises timber structural integrity and reduces commercial value (Wang & Leschen, 2003; Pawson, 2021). Detected defoliators, such as the brownheaded leafroller (*Ctenopseustis obliquana*) and the blacklegged leafroller (*Planotortrix notophaea*) inflict damage on plantation trees by consuming leaves and shoots, thereby reducing photosynthetic capacity and tree growth rates (Stevens et al., 2002; Lo et al., 2018; Suckling et al., 1998; Newcomb et al., 2016). The detected borer beetle (*Pachyodes peregrinus*) also poses a threat by tunnelling within stems and branches, further impacting timber quality (Zondag, 1982; Brockerhoff et al., 2003). Additionally, we detected the endemic common grass moth (*Orocrambus flexosellus*), which is known to cause defoliation and damage in plantation pine forests (Patrick, 2004; Cowley, 1988). Notably, we also detected horticultural and agricultural pests in the bats' diets, including the blueberry bud mite (*Acalitus vaccinii*, which damages buds and reduces fruit yields; Baker & Neuzig, 1970; Craemer, 2018), the silverleaf whitefly (*Bemisia tabaci*, an efficient vector of plant viruses and a pest resistant to multiple insecticides; Barro et al., 2011; Scott et al., 2007), and the diamondback moth (*Plutella xylostella*, a globally important crucifer pest known for rapid pesticide resistance development; Cameron et al., 1997; Mason, 2022), underscoring the bat's broad foraging scope and the mutual value of agricultural and horticultural areas to bats. Likewise, these findings highlight the value of the bats to managers as

pest control agents, and suggests that bolstering bat population sizes could help reduce other current pest control methods, including chemical pesticides

Dietary composition reflects consumed prey but also provides inferential information about foraging behaviour among sexes and/or reproductive states (Clare et al., 2011; Schoener, 1971), with reproductive status in particular expected to be a key driver of foraging behaviour in insectivorous bats due to the elevated energetic and nutritional demands associated with pregnancy and lactation (Barclay, 1991; Kunz et al., 1995; Kurta et al., 1989). For instance, Barclay (1989) demonstrated that female hoary bats (*Lasiurus cinereus*) significantly altered their foraging behaviour throughout reproduction, increasing foraging time and adjusting habitat use during lactation to meet the heightened energetic demands of raising young. Consistent with these results, we found that pregnant and lactating females exhibited significantly higher dietary diversity than males. Moreover, lactating females consumed the broadest range of prey, with higher proportions of key Lepidoptera and Coleoptera groups, and consumed some unique prey orders (e.g., Mesostigmata and Hymenoptera). These patterns suggest that pregnant females, with their comparatively lower diet diversity and reduced manoeuvrability due to increased body mass (which nearly doubles during pregnancy in *C. tuberculatus*; O'Donnell, 2001b), may forage over shorter distances and/or selectively target more easily captured or sedentary prey like non-flying insects and spiders to conserve energy while fulfilling nutritional needs (Maucieri et al., 2021; Mclean & Speakman, 1999; O'Donnell & Borkin, 2021). This reduced flight efficiency may limit their ability to adapt to spatial shifts in prey availability following disturbance, further constraining their dietary options. Thus, trade-offs between prey capture effort and energy intake may constrain dietary diversity during pregnancy, a pattern also observed in other temperate and tropical bat species (Encarnação & Dietz, 2006; Haarsma et al., 2023; Henry et al., 2002).

Forest management practices were associated with subtle but significant shifts in *C. tuberculatus* diet composition. Post-harvest landscapes showed increased consumption of Lepidoptera and Coleoptera and a relative decline in Diptera. These dietary changes likely reflect structural and microclimatic alterations caused by harvesting, such as reduced canopy cover, shifts in moisture and temperature regimes, and regeneration of understory vegetation that favours certain insect groups (Beese et al., 2019; Gaigher et al., 2024; Hébert, 2023). Additionally, edge habitats and early successional growth following harvest can boost the abundance of opportunistic prey taxa (Law et al., 2016; Perry et al., 2007). Such environmental changes may require bats to adjust their foraging strategies to exploit shifting prey communities (Wright et al., 2023). Similar habitat-driven dietary partitioning has been observed in other insectivorous bat systems. For example, Andriollo et al. (2021) used DNA metabarcoding to study three sympatric *Plecotus* species and found that dietary differences were largely shaped by variation in foraging habitat preferences and seasonal prey dynamics, despite strong morphological similarity among species.

Finally, environmental factors can drive diet dynamics in plantation forest landscapes, which are typically highly fragmented, forming mosaics of forest patches, clearings, roads, and water bodies. This fragmentation alters arthropod community composition by modifying microclimates, vegetation structure, and resource availability, ultimately influencing prey availability and foraging opportunities (Law et al., 2016; Perry et al., 2018; Pinksen et al., 2020). Although Borkin and Parsons (2011a) did not assess diet directly, their radio-tracking study showed that bats commonly forage along riparian corridors and forest edges, which are typically associated with higher insect abundance. Similarly, we found a moderate positive correlation between dietary diversity and distance from water sources, suggesting that individuals foraging farther from water consume a broader range of prey. This pattern may reflect compensatory

foraging, where bats in less productive habitats diversify their diets to meet energetic demands (Barclay, 1991; Clare et al., 2011). High insect availability near waterways may also explain why female *C. tuberculatus* preferentially roost close to water (Borkin & Parsons, 2009; Borkin & Parsons, 2011b).

Although our study suggests that *C. tuberculatus* may be capable of adapting its diet in response to habitat changes, foraging behaviour adjustments may entail increased energetic costs or greater exposure to predation and disturbance (Kunz et al., 1995; O'Donnell & Borkin, 2021). Thus, future studies that combine arthropod surveys, microhabitat assessments, and fine-scale bat tracking will provide deeper insights into how various factors mediate foraging behaviour and dietary responses in long-tailed bats to best optimise management decisions. Additional caveats in our study relate to DNA metabarcoding. While this method undoubtedly offers improved taxonomic resolution for dietary studies (Evans et al., 2016; Liu et al., 2020), primer bias (Deagle et al., 2019), differential digestibility of prey DNA and variation in gut passage times (Panni & Pizzolotto, 2018), and gaps and inconsistencies in reference databases (Porter & Hajibabaei, 2018; Andersen et al., 2019; Keck et al., 2023) can all cause conservative estimates of dietary diversity. Moreover, we focused exclusively on arthropods in this study, which may have excluded some important non-arthropod components of the bat diet, while in contrast some of the taxa we detected are likely to be non-diet environmental contaminants or examples of secondary predation.

Despite the noted limitations (many of which are active areas of research), metabarcoding remains a powerful and sensitive tool for elucidating complex dietary patterns and trophic relationships. Its application in this study revealed valuable insights into the diet of *C. tuberculatus,* suggesting that this treasured species may adjust its diet in response to both environmental changes

and physiological state and highlighting its potential role in pest suppression and vulnerability to large-scale habitat modification.

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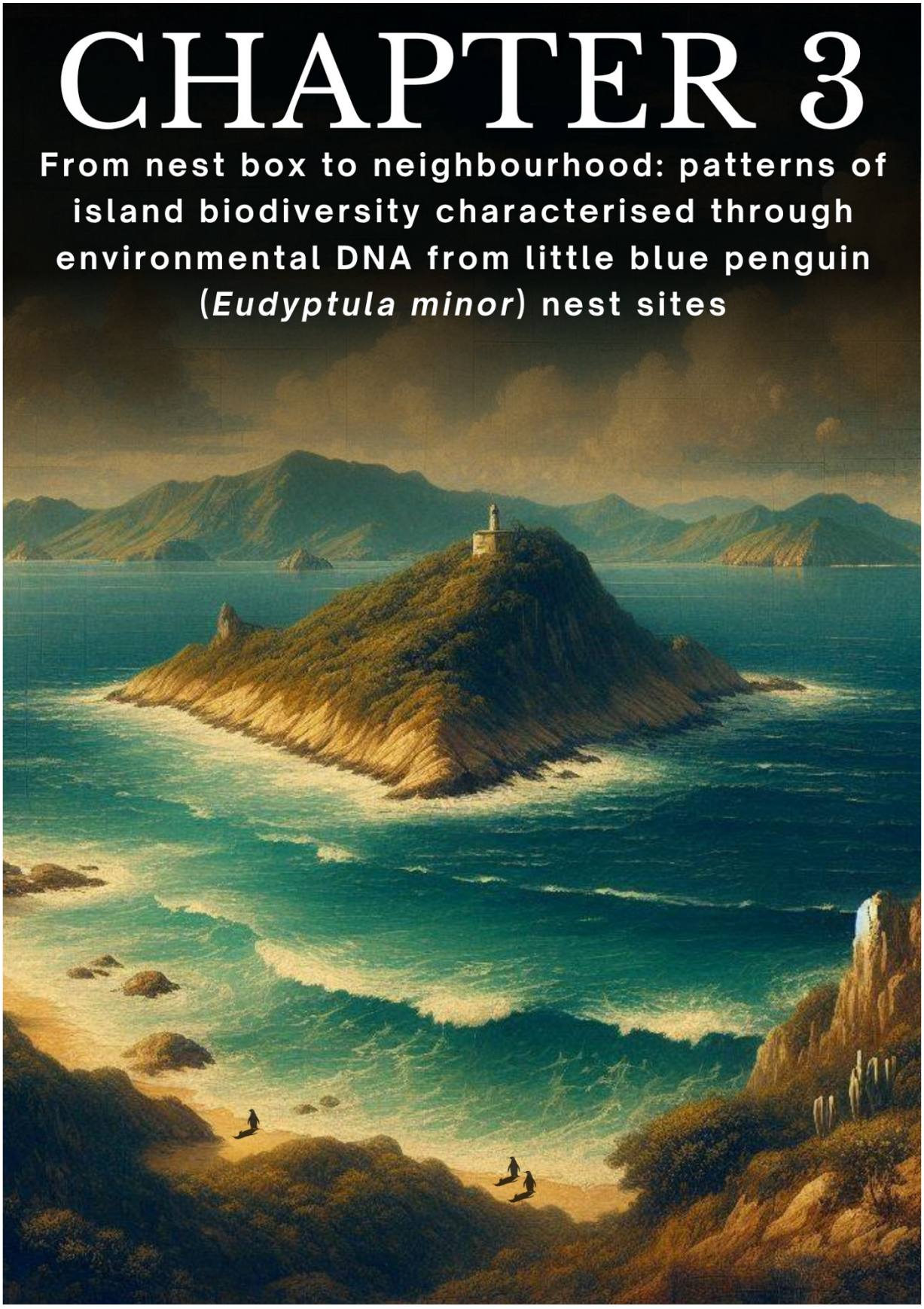
CHAPTER 3

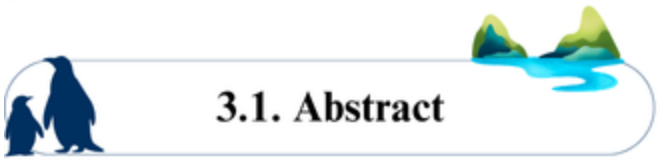
From nest box to neighbourhood: patterns of island biodiversity characterised through environmental DNA from little blue penguin (*Eudyptula minor*) nest sites



To be submitted to *Environmental DNA*: Stanners, A. & McGaughran, A. From nest box to neighbourhood: patterns of island biodiversity characterised through eDNA from little blue penguin (*Eudyptula minor*) nest boxes.

Contributions: AS and the Wellington Harbour Little Penguin volunteer team collected the faecal samples. AS performed the DNA extractions and PCR, and the data analysis with assistance from AM. AS wrote the first draft of the manuscript and AM provided feedback, resulting in the final version.

Data accessibility to be added.



3.1. Abstract

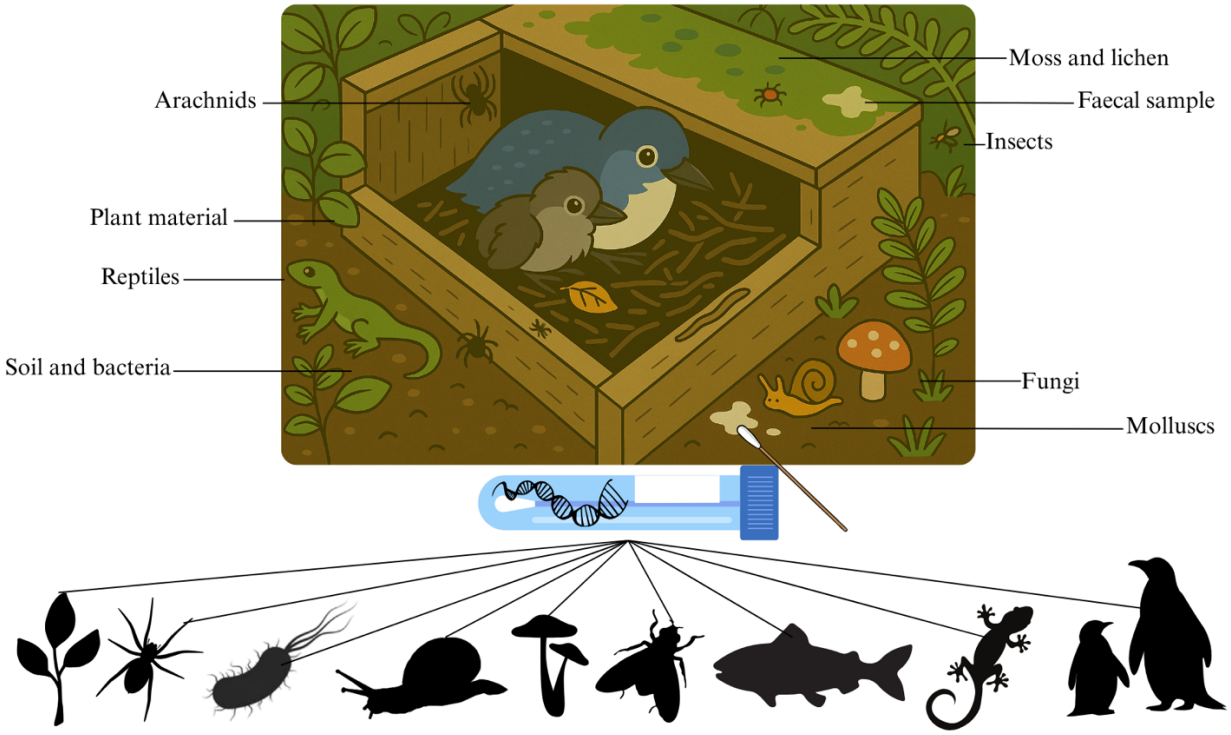
Environmental DNA (eDNA) from seabird faecal samples can provide insights into both diet and the broader biodiversity of the ecosystems the birds inhabit. Seabirds like the little blue penguin (*Eudyptula minor; kororā*) are sensitive to changes in prey availability and habitat condition, yet their diet and foraging plasticity remain poorly resolved in some regions.

In this study, we used DNA metabarcoding of 96 faecal samples collected from 74 unique nests across eight locations in an *E. minor* colony on Matiu/Somes Island (Wellington Harbour, Aotearoa New Zealand) to investigate both diet and the incidental detection of non-target ('bycatch') DNA, and how these varied with nest location (east vs. west), and season (spring vs. summer).

We detected six marine fish species known to be components of the *E. minor* diet. In addition, the faecal samples contained a diverse range of non-diet taxa, including soil invertebrates, plants, fungi, arachnids, and other local species that reflected the surrounding microhabitat. Within this bycatch data, community composition varied significantly by season, with summer samples exhibiting higher alpha diversity and increased representation of taxa, such as Bacillota, Rotifera, and Basidiomycota, potentially reflecting elevated biological activity and eDNA turnover in warmer months. While spring samples yielded more unique taxa overall, terrestrial-affiliated species dominated in both seasons. Spatial differences were more subtle, but eastern nest sites showed slightly higher taxonomic richness and a greater number of specialist taxa—particularly

nematodes and discoseans—potentially suggesting greater microhabitat heterogeneity and reduced anthropogenic disturbance compared to the western side of the island.

Collectively, our results demonstrate that, even in the absence of strong dietary signals, eDNA samples from seabird colonies can act as a valuable lens into local biodiversity and ecological processes. Thus, our findings underscore the potential of nest-associated eDNA for broad environmental monitoring, habitat assessment, and the identification of ecosystem change in coastal environments.



Graphical abstract. Environmental DNA (eDNA) sources within and around a seabird nest: DNA traces from soil, plant material, feathers, faeces, and other nest components enable detection of a wide range of taxa, including plants, fungi, insects, arachnids, molluscs, reptiles, and other vertebrates.

3.2. Introduction

Biodiversity is declining at an unprecedented rate globally, with coastal ecosystems among the most ecologically rich yet inherently vulnerable (Ayyam et al., 2019; Economidou, 1982; Monaco & Prouzet, 2014). They function as critical interfaces between marine and terrestrial systems and support a wide range of endemic and specialised species (Finkl & Makowski, 2021). However, the pressures of human development, climate change, pollution, and biological invasions have collectively degraded these ecosystems, leading to habitat loss, altered species interactions, and reduced ecological resilience (Adla et al., 2022; He & Silliman, 2019; Newton et al., 2020; Hess et al., 2008; Sahavacharin et al., 2022). Despite their importance, our understanding of how biodiversity in island coastal ecosystems responds to environmental change across fine spatial and temporal scales remains limited. This is particularly true for microhabitats associated with seabird colonies, which support distinct and dynamic biological communities (Durrett et al., 2014; Grant et al., 2022; Signa et al., 2021).

At local scales, biodiversity is shaped by a combination of abiotic and biotic drivers that operate across space and time. Spatially, habitat structure, microclimate, resource availability, disturbance history, and species interactions all shape local community structure (McGill et al., 2006; Siefert et al., 2013; Stein et al., 2014). Temporally, variation may arise from weather extremes, as well as from seasonal cycles, migration, and ecological succession, with each able to impact species richness and/or turnover (Holyoak et al., 2020; Vellend et al., 2007; Wolkovich et al., 2014). Together, spatial and temporal gradients—even within relatively small units, such as individual islands or habitat patches—play a fundamental role in structuring community

composition. Climate change compounds these effects by altering habitat quality, stability, and metapopulation dynamics, making specialised species within coastal islands particularly vulnerable to fragmentation and environmental shifts (Holyoak & Heath, 2016).

Among the visible ecological consequences of coastal disruption are declines in species that rely on these environments for critical life stages (Costello et al., 2015; Harley et al., 2006; Marshall & Morgan, 2011). Seabirds, many of which breed exclusively in coastal or island habitats, have experienced some of the steepest population declines of any avian group (Burger, 2006; Furness, 2012; Paleczny et al., 2015; Phillips et al., 2023). Their life histories are characterised by high site fidelity, long generation times, and a reliance on both marine and terrestrial resources, making them particularly sensitive to environmental changes (Naves et al., 2006; Jiguet et al., 2007; Schreiber & Burger, 2001). Habitat modification, invasive predators, and human disturbance have all been linked to reduced breeding success, while changes in ocean productivity and environmental variability have disrupted foraging behaviour and reduced habitat quality (Harley et al., 2006; Sheaves, 2009; Spalding et al., 2014; Weiser et al., 2018). Small or isolated populations may be especially vulnerable, as even moderate reductions in reproductive output can lead to long-term population instability (Baron et al., 2013; Cutting et al., 2021).

The little blue penguin (*Eudyptula minor; kororā*) is the smallest penguin species globally and exemplifies the vulnerability of coastal breeders to environmental pressures. Found along temperate coastlines in Aotearoa New Zealand and southern Australia, *E. minor* nests in burrows close to the shoreline, often in close proximity to human activity. As such, it is exposed to habitat degradation, domestic animal attacks, road mortality, and recreational disturbance (Cannell et al., 2016; Poupart et al., 2017; Whitehead et al., 2019). *E. minor* shows high site fidelity and limited

dispersal, and its reproductive success and colony persistence are tied to both the availability of suitable nesting habitat and to local environmental conditions (Ropert-Coudert et al., 2009).

Monitoring nocturnal and amphibious seabirds like *E. minor* using traditional field surveys (and often in remote island systems) poses particular challenges. For example, logistical constraints and environmental complexity can hinder accurate, high-resolution assessments (Barajas et al., 2020; Buxton & Jones, 2012). Traditional surveys also often rely on visual observations, trapping, or acoustic detection, which can be labour-intensive, biased toward conspicuous species, or insensitive to cryptic or nocturnal species (Longino & Colwell, 1997; Ward-Paige et al., 2010). These limitations constrain our ability to monitor community-level dynamics or detect early ecological shifts (Yoccoz et al., 2001). DNA-based approaches offer a powerful alternative biomonitoring method, enabling high-resolution, multi-taxa assessments from minimally invasive environmental samples (Beng & Corlett, 2020; Deiner et al., 2017; Taberlet et al., 2012). eDNA and metabarcoding have proven especially effective in detecting rare, cryptic, or elusive taxa across terrestrial, freshwater, and marine environments, with increasing applicability in remote and island systems (Ushio et al., 2022; Bálint et al., 2018).

DNA metabarcoding approaches include tools that enable assessment of biodiversity from environmental sources, including faeces, soil, and sediment. Recent work has shown that faecal samples can detect both diet DNA and incidental DNA from the surrounding environment (Valentini et al., 2016; Wang et al., 2022). For example, metabarcoding studies targeting diet have incidentally revealed parasites, symbionts, and microbial communities, while soil eDNA surveys routinely detect non-target taxa that reflect broader biodiversity patterns (Dickie et al., 2018; Capo et al., 2020). Thus, including multiple substrates in a single experiment may result in the capture

of distinct but complementary snapshots of the biotic environment (Shehzad et al., 2012; Swift et al., 2018).

In this study, we used DNA metabarcoding of environmental samples collected from *E. minor* nesting sites on Matiu/Somes Island (Wellington Harbour, Aotearoa New Zealand) during spring and summer months (October-December), to investigate local temporal and spatial biodiversity signals. As well as a focus on dietary species, we evaluated the potential of faecal eDNA sampling to provide dual insights into broader biodiversity dynamics via eDNA bycatch. We hypothesised that *samples* would contain a mix of marine and terrestrial DNA, reflecting the interaction of *E. minor* with both environments. We further hypothesised that taxonomic composition would vary across: (i) time, to reflect differences in seabird (associated with breeding behaviour) and general biodiversity activity; and (ii) space, in response to microhabitat and landscape-scale ecological differences between the easter and western sides of the island**.**

3.3. Methods

3.3.1. Study area and sample collection

Sampling for this study took place on Matiu/Somes Island in Wellington Harbour, Aotearoa New Zealand, where 37 nest boxes housing *E. minor* are distributed across the northern half of the island—spanning eight spatially distinct eastern and western zones, based on the most likely direction the birds enter the harbour while foraging (Fig. 3.2; Table 3.1). Nest boxes were monitored from early September to late December 2024 and faecal samples were collected on three occasions: 30 October, 14 November, 7 December (Table 3.1; Supplementary Table S3.1).

Faecal material was collected using sterile Isohelix© swabs, which were used to sample visible deposits either within the nest box or tunnel, or at the entrance area. Swabs were labelled with the corresponding nest box ID and date, sealed in sterile containers, and stored at 4 °C before transfer to the University of Waikato for DNA extraction. Although the primary target of each swab was fresh faecal material, many samples also contained trace amounts of surrounding soil or substrate, collected incidentally as part of the swabbing process.

Table 3.1. Summary of eDNA samples collected by location from September to December 2024. Colours correspond to map markers in Fig. 3.2.

Site ID	Side of island	Map ID	No. samples
Accommodation (AC)	West	Orange	12
Cable bay (CB)	West	Green	13
Caretaker's cottage (CC)	East	Yellow	10
Gun emplacement (GE)	East	Pink	2
Nursery (NA)	East	Red	15
North point (NP)	East	Mid blue	25
Wharf (WA)	East	Purple	17
Workshop paddock (WP)	East	Dark blue	18
		Total samples	**114**

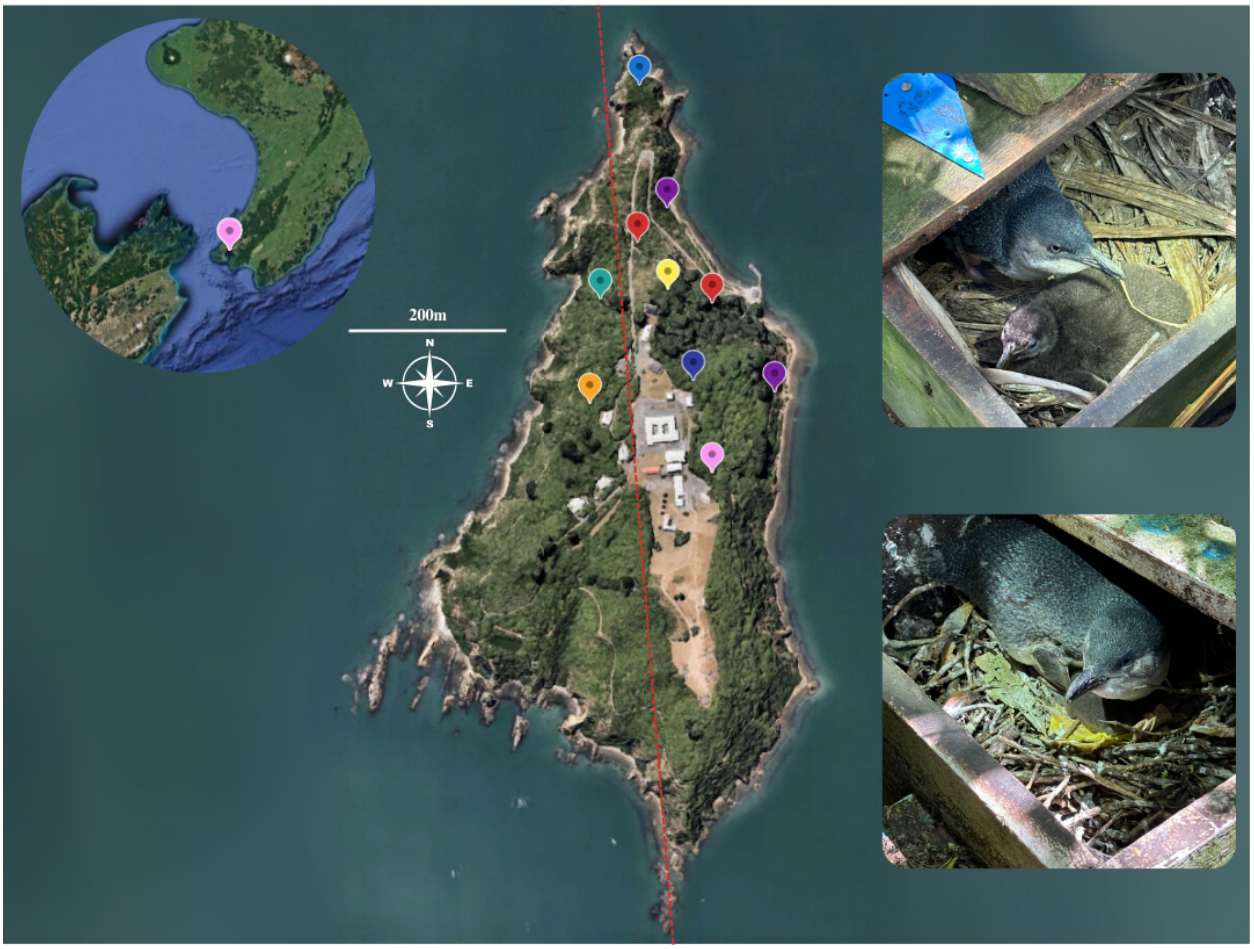


Figure 3.1. Location of Matiu/Somes Island. The satellite image shows nest box sites (extracted from Fig. S3.1) for little blue penguins (*Eudyptula minor*), divided into eastern and western sectors (red dotted line). Coloured markers indicate different monitoring locations, as listed in Table 3.1. The inset map on the left displays the island's geographic position within New Zealand, while the photos on the right show *E. minor* adults and/or chicks inside a nest box on Matiu/Somes Island (Stanners, 2024).

All sample collection for this study was carried out by or in conjunction with an established volunteer conservation group (Wellington Harbour Little Penguins), with express permission granted by the island's Kaitiaki Board (which oversees the protection and management of the island's natural and cultural resources) in accordance with their tikanga (customs/protocols) and decision-making processes. No live handling or disturbance of penguins occurred, and all procedures were aligned with ethical standards for minimally invasive wildlife research.

3.3.2. DNA extraction and metabarcoding

Total genomic DNA was extracted from eDNA swabs using a modified cetyltrimethylammonium bromide (CTAB) protocol, optimised for high-yield DNA recovery from low-quality environmental samples while minimising contamination. Chilled swabs were warmed at room temperature prior to extraction. Each sample was incubated in 720 µL of CTAB extraction buffer, which comprised 270 µL sodium dodecyl sulphate (SDS), 270 µL phosphate-buffered saline (PBS), and 180 µL CTAB. Samples were vortexed to ensure thorough mixing and incubated at 65 °C for 30 min with gentle agitation (150 rpm on an orbital mixer or heat block).

Following incubation, 350 µL of chloroform:isoamyl alcohol (24:1) was added to each sample. Tubes were vortexed for 20 s and centrifuged at 12,000 rpm for 5 min to separate the phases. The upper aqueous layer was carefully transferred to new sterile microcentrifuge tubes, and a second chloroform:isoamyl alcohol extraction (500 µL) was performed to further purify the DNA. After vortexing and incubation on a hula mixer for 20 min, samples were centrifuged again at 12,000 rpm for 5 min. The resulting supernatant was transferred to fresh tubes, and volumes were recorded.

To precipitate the DNA, 0.33× volume of 7.5 M ammonium acetate was added, followed by centrifugation. The supernatant was then transferred to a new tube, and 0.54× volume of isopropanol was added. Samples were gently inverted 20 times and stored overnight at −20 °C. The following day, DNA was pelleted by centrifugation at 13,200 rpm for 20 min. Pellets were washed with 1 mL of ice-cold 70% ethanol and centrifuged again. Residual ethanol was removed using progressively finer pipette tips, and DNA pellets were dried in a Speed-Vac (medium heat, 5–15 min). Dried pellets were resuspended in 20 µL of ultrapure water. DNA concentration and

purity were quantified using a Qubit fluorometer or DeNovix spectrophotometer. Samples were stored at −20 °C until PCR amplification. Alongside sample extractions, an extraction control, consisting of a sterile swab, was performed.

DNA metabarcoding targeted the mitochondrial cytochrome c oxidase subunit I (COI) gene region, with PCR amplification conducted using the mlCOIintF (GGWACWGGWTGAACWGTWTAYCCYCC; Leray et al., 2013) and jgHCO2198 (TAAACTTCAGGGTGACCAAAAAATCA; Geller et al., 2013) primers. Each 27 µL PCR reaction included 10 µL of MyFi© 5× reaction buffer, 1 µL each of 10 µM forward and reverse primers, 1 µL of 20 mg/mL bovine serum albumin (BSA), 1 µL of MyFi© DNA polymerase (2 U/µL), 15 µL of nuclease-free water, and 2 µL of extracted DNA. Thermal cycling was carried out with an initial denaturation at 95 °C for 4 min, followed by 40 cycles of denaturation at 95 °C for 20 s, annealing at 46 °C for 45 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 5 min. Negative controls, in which PCR-grade water replaced the DNA template, were included in all amplification runs to monitor for potential contamination. Post-amplification, DNA quantity and quality were assessed using a Qubit fluorometer and agarose gel electrophoresis. Sequencing of the amplified COI gene region, as well as an extraction and PCR control, was conducted by SeQuench Ltd (Nelson, New Zealand) using an Illumina MiSeq platform with a v2 500-cycle kit (2 × 251 bp paired-end reads) and Nextera XT v2 Index Kit Set C dual indices. Prior to sequencing, amplified products were purified and size-selected using solid-phase reversible immobilisation (SPRI) beads according to the manufacturer's protocol to retain fragments of approximately 300–400 bp.

3.3.3. Data analysis

Individual FASTQ read files were processed using R v4.2.1 (R Core Team, 2024) and the DADA2 pipeline v1.34.0 (Callahan et al., 2016). Initially, reads were truncated to remove primer sequences, followed by trimming based on quality score profiles, with up to 30 bp removed from each read end depending on the sequencing quality (Q score). Reads containing ambiguous bases (maxN = 0) or more than two expected errors (maxEE = c(2,2)) were excluded. Error rates for the COI amplicon were estimated, after which the reads were dereplicated and denoised. Chimeras were removed from merged reads *de novo*, resulting in a high-resolution table of amplicon sequence variants (ASVs).

Taxonomic classification of the ASVs was performed using a custom reference database built with CRABS v1.7.6 (Jeunen et al., 2022), according to the methods described at https://github.com/gjeunen/reference_database_creator. The reference database was constructed using sequences obtained from the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/) on 17 March 2025, using the following search terms: COI[All Fields] OR COXI[All Fields] OR CO1[All Fields] OR COX1[All Fields] OR cytochrome oxidase subunit i[All Fields] OR cytochrome oxidase subunit 1[All Fields] OR cytochrome oxidase subunit I[All Fields] AND (mitochondrion[filter] AND ("200"[SLEN] : "50000"[SLEN])). Sequences were retrieved, dereplicated, and filtered using CRABS default settings. The COI amplicon region was extracted, primer regions were removed via pairwise global alignment, and sequences were dereplicated and filtered (Table S3.2). The final reference database was exported in a format compatible with DADA2.

Following the initial taxonomic assignment in DADA2, some ASVs were further identified to the species level using BLAST searches against the NCBI nucleotide database, during which only ASVs with strong matches (e-value > 1e–50) were retained. Per-sample ASV counts, taxonomic assignments, and associated metadata were imported into R v4.4.2 for further analysis using the phyloseq package v1.50.0 (McMurdie & Holmes, 2013). Raw read counts per sample ranged from 0 to 210,278 (Table S3.2).

Samples were initially filtered to remove any individuals with a raw read count of < 5, and the proportion of 'host' (*E. minor*), 'diet', and 'environmental' ASVs was calculated. ASVs assigned to the host genera were removed, as were any reads assigned to *Homo sapien* or lacking phylum-level classification. Next, we subset the data into 'diet' (defined as the class Actinopteri*,* ray-finned fishes) and 'environmental' datasets (i.e., all other non-filtered taxa). These filtering steps enabled a more focused exploration of the environmental and dietary components. However, we note that the "environmental" dataset may still include indirect diet taxa (e.g., microbial symbionts or gut microbiota of prey), as well as non-nest taxa, such as *E. minor* microbiome taxa or other incidental DNA traces. The overall filtering process resulted in a final diet dataset comprising 7 unique ASVs (6 species) and a final environmental dataset comprising 3,193 unique ASVs (636 distinct species) across the 96 analysed samples.

The dataset was normalised using the DESeq2 geometric mean-based "poscounts" method, which is better suited for zero-inflated count data than traditional rarefaction approaches and retains all available reads. Metadata variables location and season were explicitly defined as factors and incorporated into the normalisation model formula. Following DESeq2 normalisation and standardisation of library sizes across samples, the dataset comprised 2,920 taxa and 87

samples. During preliminary analyses, ordination of the normalised dataset was performed using PCoA with the ordinate function in phyloseq. This revealed that control samples (PCR and extraction blanks, comprising 0.72% of total reads) had negligible influence on observed community composition. These control samples were therefore excluded from all subsequent analyses.

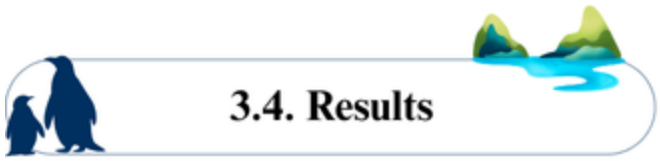
Relative abundance of the top 20 taxa within the environmental dataset was plotted for the full (non-normalised) dataset. Subsequent analyses on this dataset included alpha diversity (Shannon's diversity index) and taxonomic richness (ASV count) across seasons (spring, summer) and nest locations (Accommodation, Cable Bay, Caretaker's Cottage, Gun Emplacement, Nursery, North Point, Wharf, Workshop Paddock).

Bray-Curtis dissimilarities were computed to assess multivariate patterns in community composition. Ordinations were performed in phyloseq as described above*, g*roup differences were tested using PERMANOVA (adonis2, 999 permutations, $\alpha = 0.05$) via *vegan* v2.6-8 (Oksanen et al., 2018), and beta dispersion was assessed using the betadisper function to test for homogeneity of variance among groups.

To evaluate differences in taxon recovery across seasonal groups, clamtest analysis was performed on the normalised dataset using the clamtest function from vegan. This method identified specialist taxa occurring exclusively within each group, based on pairs of categorical factors, including season (spring vs. summer) and island region (east vs. west). Identified specialists were subsequently analysed for taxonomic identity, habitat classification (aquatic, terrestrial, both), and phylum-level distribution using online searches to assign habitat status. iNaturalist was predominantly used for this, alongside Google searches based on genus or species

names, and taxa that could not be confidently categorised, or that inhabited aquatic and terrestrial ecosystems were labelled as “both”.

All visualisations were generated using base R functions or the ggplot2 package.



3.4. Results

3.4.1. Overall dataset composition

E. minor was among the most frequently detected species in the overall dataset (69% of reads), consistent with the sampling context. The majority of non-host sequences that were of likely environmental (or prey/host microbiome; see Section 3.3.3) origin, comprised 30.9% of all ASVs. Six species were able to be specifically associated with *E. minor* diet based on published records (Flemming et al., 2013; Fraser et al., 2004; Shaw, 2008): *Hyporhamphus ihi* (garfish), *Engraulis japonicus* (Japanese anchovy), *Fosterygion flavonigrum* (yellow-black triplefin), *Stolephorus* sp. (anchovy species), *Pseudophycis bachus* (red codling), and *Thyrsites atun* (snoek or barracouta) (Fig. S3.2).

3.4.2. Habitat-specific taxonomic composition of the environmental dataset

Within the environmental data subset, Bacteria dominated at the Kingdom level, comprising 54.4% of total assignments (Fig. 3.2A). This was followed by Fungi (19.7%), Animalia (18.5%), Protista (5.1%), Virus (1.9%), and Plantae (0.3%). When habitat classification was restricted to the subset of environmental ASVs resolved to species level, the distribution was skewed toward terrestrial taxa (64.1%), with fewer species classified as aquatic (16.3%) or associated with both environments (19.6%) (Fig. 3.2B).

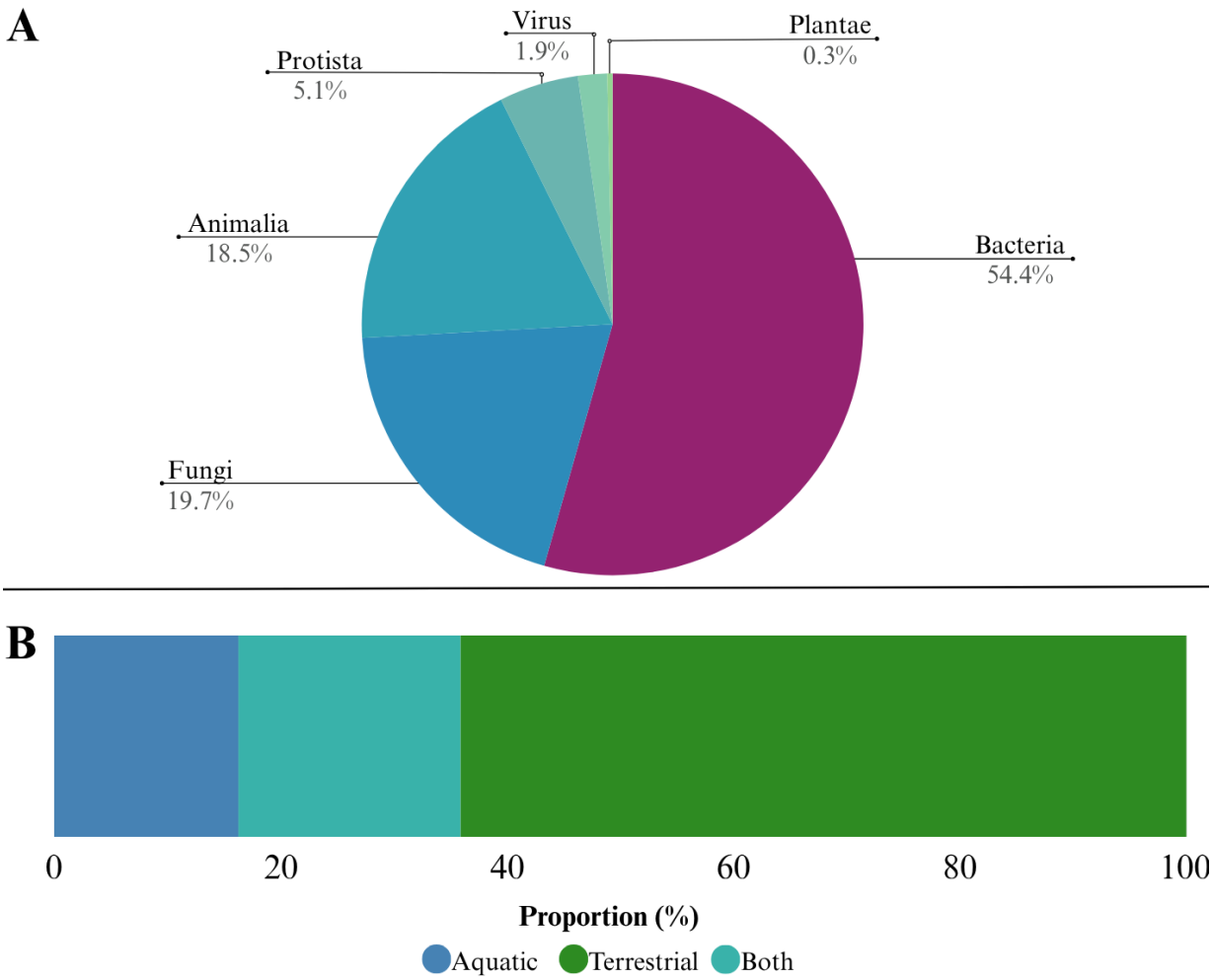


Figure 3.2. Proportion of ASVs assigned to ecological and taxonomic categories: **A** Taxonomic distribution of *E. minor*-associated ASVs at the kingdom level; **B** Horizontal stacked bar plot showing habitat classification proportion by species.

The 20 most abundant species in the environmental dataset represented a taxonomically diverse assemblage spanning Bacteria, Protista, and Animalia. Bacterial taxa accounted for approximately 37.4% of total reads, and were dominated by members of the phylum Bacteroidota, including *Aggregatimonas sangjinii* (16.83%), *Flavobacterium* sp. (3.98% and 0.68%), *Maribacter* sp. (2.85%), and *Winogradskyella schleiferi* (0.97%), as well as taxa from Pseudomonadota such as *Bradyrhizobium* sp. (7.12%). Nematodes contributed approximately 23.4% of environmental dataset reads, primarily from *Oscheius* sp. (13.02%) and *Rhabditis* sp.

(10.38%), both of which are typically associated with terrestrial substrates (Bongers, 1990; Sudhaus, 2011). Protistan parasites from the genus *Eimeria* (phylum Apicomplexa) were also highly represented, accounting for 3.62% of total reads. Additional abundant taxa in the environmental dataset included the cestode *Versteria mustelae* (2.21%), the trematode *Schistosomatidae* sp. (0.89%), the flea *Parapsyllus humboldti* (1.16%), the mite *Tyrophagus curvipenis* (1.84%), the springtail *Entomobrya* sp. (1.20%), and a member of *Eupodidae* (5.67%)—all of which are arthropods or ectoparasites (Brant & Loker, 2009; Espinaze Pardo et al., 2019; Murillo et al., 2018; Shanebeck et al., 2024; Walter & Proctor, 2013). Fungal taxa, such as *Rigidoporus microporus* (1.73%) and *Apiospora arundinis* (1.00%), were also detected, alongside additional eukaryotes, including the moth *Monopis crocicapitella* (0.78%), *Bdelloidea* (0.62%), and the avian herpesvirus *Iltovirus gallidalpha*-1 (1.36%) (Ai et al., 2024; Davison et al., 2009; Kutikova, 2003; Saidi et al., 2023).

The total environmental dataset species composition reflected both terrestrial and aquatic habitats (Table 3.2). In the aquatic category (16.3% of the total environmental dataset), Bacteroidota accounted for 95.6% of total abundance, followed by Bacillariophyta (1.2%). All remaining phyla, including Annelida, Chordata, Mollusca, and Oomycota, each contributed less than 0.5% of the total aquatic abundance (Table 3.2). In terrestrial samples (64.1% of the total dataset), Nematoda comprised the largest proportion of phyla at 40.0%, followed by Arthropoda (22.4%), Ascomycota (15.5%), Pseudomonadota (11.8%), and Basidiomycota (4.0%). Other phyla such as Mucoromycota, Oomycota, Annelida, and Evosea were present at lower relative abundances (Table 3.2). The ‘both’ habitat category (19.6% of total) showed a more even distribution of phyla. Bacteroidota was again dominant at 42.3%, followed by Apicomplexa (25.0%), Peploviricota (9.4%), Platyhelminthes (6.3%), Arthropoda (4.4%), Nematoda (4.1%),

and Ascomycota (2.4%). Other phyla including Basidiomycota, Bacillota, and Oomycota were present in smaller proportions (Table 3.2).

Table 3.2. Total ASV read abundance per phylum across three habitat types: aquatic, terrestrial, and both. Values represent the summed read counts for each phylum within samples assigned to each habitat category, with proportions in parentheses. Dashed entries (–) indicate no detectable ASVs for that phylum in the corresponding habitat. Row and column totals are provided to highlight overall taxonomic and habitat-level abundance patterns.

Phylum	Aquatic	Both	Terrestrial	Total
Actinomycetota	-	-	3 (> 0.01%)	3 (> 0.01%)
Annelida	979 (0.48%)	-	2,360 (0.44%)	3,339 (0.38%)
Apicomplexa	23 (0.01%)	31,629 (24.17%)	-	31,652 (3.64%)
Arthropoda	21 (0.01%)	5,514 (4.21%)	118,471 (22.21%)	123,996 (14.26%)
Ascomycota	3 (0.00%)	3,061 (2.34%)	82,182 (15.41%)	85,246 (9.80%)
Bacillariophyta	2,534 (1.23%)	-	-	2,534 (0.29%)
Bacillota	-	1,581 (1.21%)	248 (0.05%)	1,829 (0.21%)
Bacteroidota	196,883 (95.89%)	53,404 (40.79%)	2,085 (0.39%)	252,372 (29.02%)
Basidiomycota	-	1,800 (1.37%)	21,345 (4.00%)	23,145 (2.66%)
Chlorophyta	-	67 (0.05%)	75 (0.01%)	142 (0.02%)
Chordata	919 (0.45%)	-	140 (0.03%)	1,059 (0.12%)
Ciliophora	116 (0.06%)	3 (> 0.01%)	-	119 (0.01%)
Cnidaria	616 (0.30%)	-	-	616 (0.07%)
Discosea	423 (0.21%)	3,174 (2.42%)	-	3,597 (0.41%)
Evosea	-	-	568 (0.11%)	568 (0.07%)
Gastrotricha	165 (0.08%)	-	-	165 (0.02%)
Mucoromycota	-	-	4,544 (0.85%)	4,544 (0.52%)
Mollusca	906 (0.44%)	-	-	906 (0.10%)
Nematoda	-	5,142 (3.93%)	211,636 (39.67%)	216,778 (24.93%)
Oomycota	885 (0.43%)	361 (0.28%)	3,511 (0.66%)	4,757 (0.55%)
Peploviricota	-	11,900 (9.09%)	-	11,900 (1.37%)
Platyhelminthes	509	7,967	19,347	27,823

	(0.25%)	(6.08%)	(3.63%)	(3.20%)
Porifera	288 (0.14%)	-	-	288 (0.03%)
Pseudomonadota	83 (0.04%)	179 (0.14%)	62,610 (11.74%)	62,872 (7.23%)
Rhodophyta	562 (0.27%)	-	-	562 (0.06%)
Streptophyta	-	-	51 (0.01%)	51 (0.01%)
Tubulinea	102 (0.05%)	345 (0.26%)	-	447 (0.05%)
Uroviricota	-	257 (0.20%)	-	257 (0.03%)
Total	**205,265**	**130,914**	**533,343**	**869,522**

3.4.3. Temporal and spatial variation in biodiversity

We observed seasonal differences in alpha diversity in the environmental dataset, with summer samples exhibiting higher Shannon diversity values compared to spring (Fig. 3.3A). When examining spatial patterns across nesting areas, alpha diversity varied among sites on both the eastern and western sides of the island in a manner consistent with the reduced sample sizes at Caretaker's Cottage and Gun Emplacement (Fig. 3.3B,C).

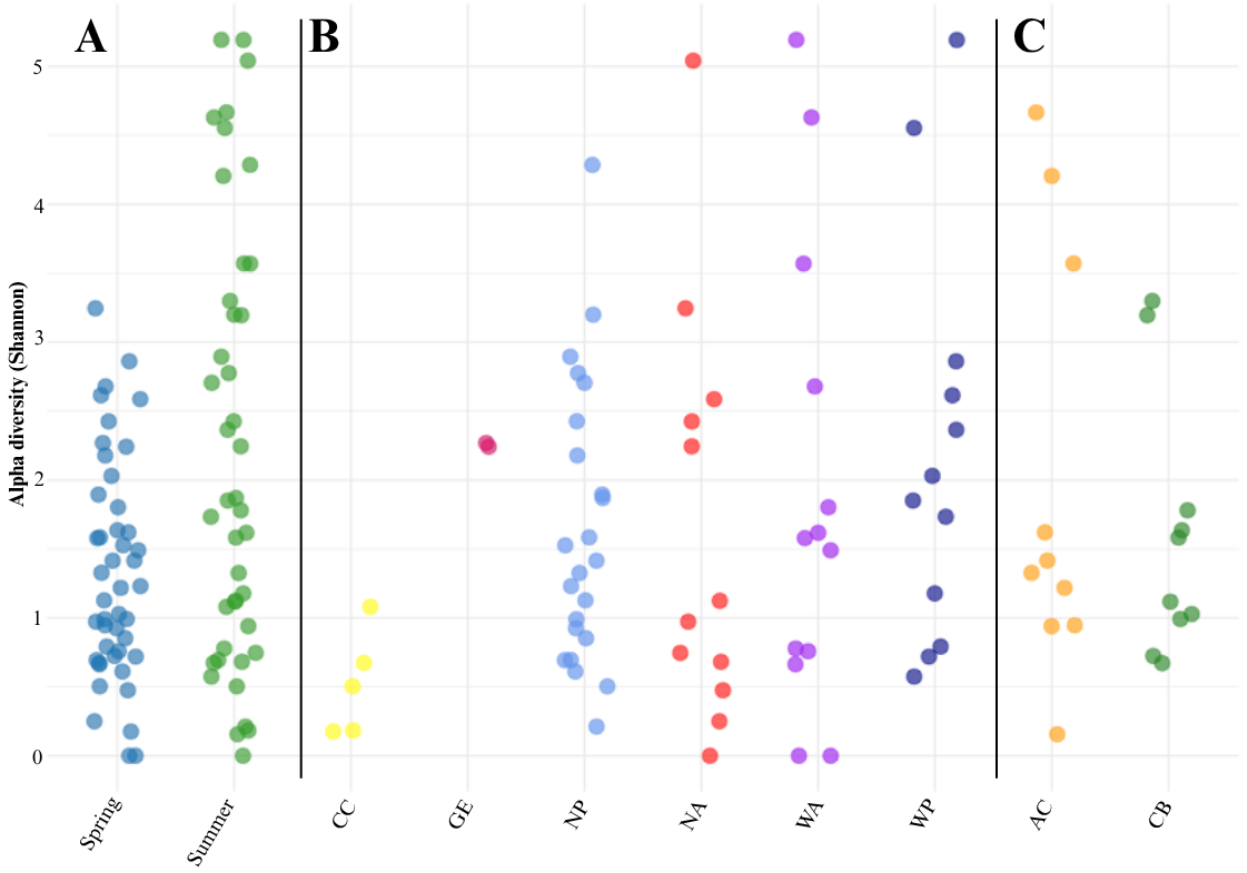


Figure 3.3. Alpha diversity (Shannon index) of taxa across: **A** seasons; **B** eastern nest locations; and **C** western nest locations. Each point represents an individual sample. Colours correspond to groupings within each panel and are consistent with those presented in Figure 3.1. In panel B, CC = Caretaker's Cottage, GE = Gun Emplacement, NP = North Point, NA = Nursery Area, WA = Wharf Area, and WP = Workshop Paddock. In panel C, AC = Accommodation and CB = Cable Bay.

nMDS ordination revealed no clear separation in community composition (beta diversity) between seasons, with spring and summer samples exhibiting some overlap (PERMANOVA: $F_{1,85} = 1.292$, $p = 0.061$; BETADISPER: $F_{1,85} = 1.514$, $p = 0.222$) (Fig. 3.4A). Similarly, no significant differences in beta diversity were detected between eastern and western nest orientations (PERMANOVA: $F_{1,85} = 1.100$, $p = 0.263$; BETADISPER: $F_{1,85} = 0.084$, $p = 0.746$) (Fig. 3.4B). Differences among individual nest locations were not statistically significant (PERMANOVA: $F_{7,79} = 1.086$, $p = 0.133$; BETADISPER: $F_{7,79} = 22.974$, $p = 0.001$) (Fig. 3.4C), though the Gun Emplacement site (n = 2) exhibited notably lower within-group dispersion.

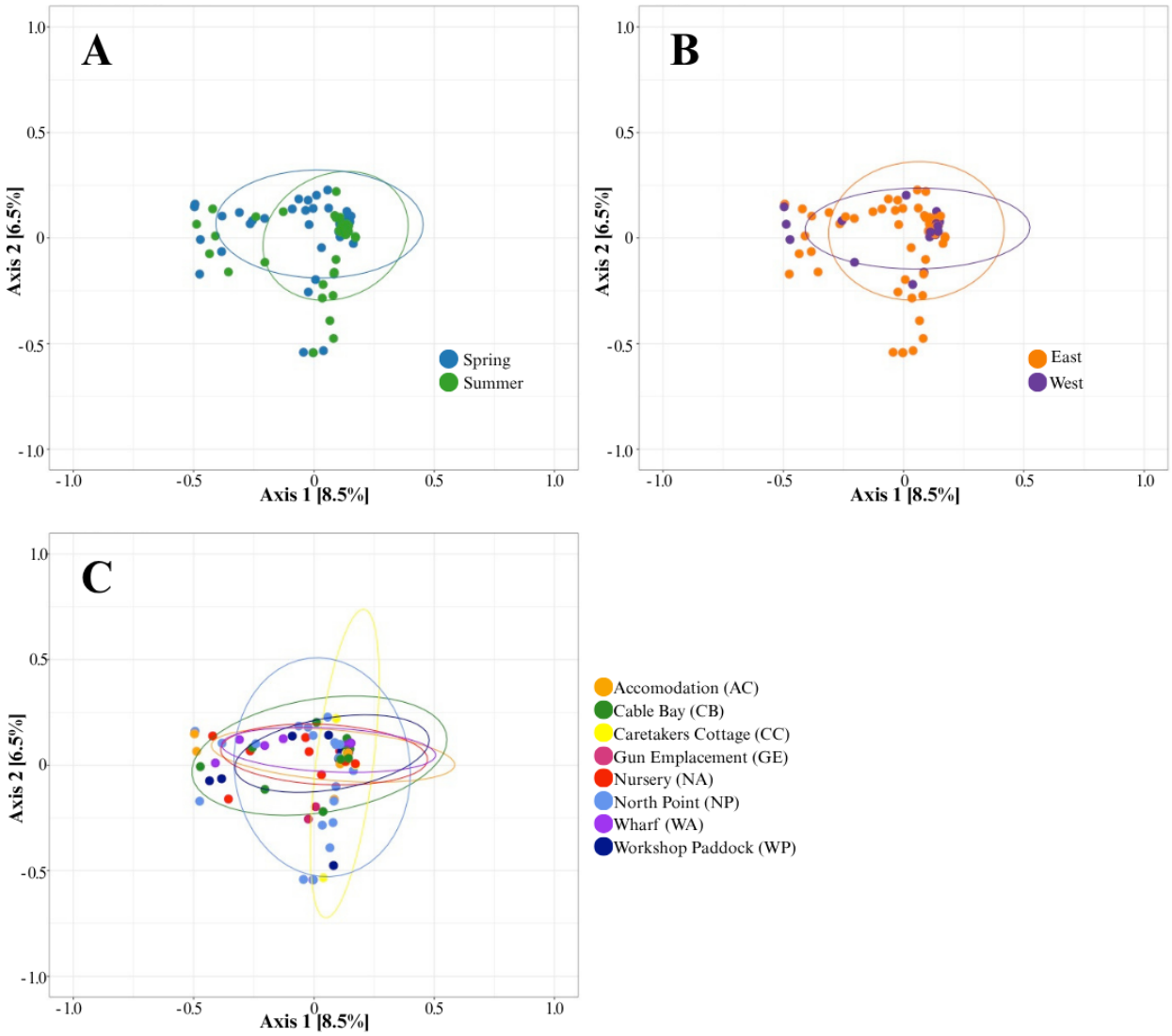


Figure 3.4. Non-metric Multidimensional Scaling (nMDS) ordination of Bray-Curtis dissimilarities, depicting variation in community composition (beta diversity) among: **A** seasons; **B** nest island orientations; and **C** nest locations according to the provided keys. Colours in panel C are coordinated with those shown in Figures 3.1-3.2 and Table 3.1. Ellipses represent 95% confidence intervals for each group.

3.4.4. Specialist taxa exclusive to season and nest location

Seasonal comparisons

The clamtest analysis identified 393 spring-exclusive and 273 summer-exclusive specialist ASVs, comprising 263 and 157 unique species, respectively, in the environmental dataset (Fig. 3.5A,B;

Table S3.3). Across both seasons, the most frequently represented species belonged to the phyla Ascomycota (spring: n = 60 species; summer: n = 31), Bacteroidota (n = 54; 15), Arthropoda (n = 60; 49), and Basidiomycota (n = 10; 9). Other commonly represented phyla included Discosea (spring: n = 11), Nematoda (n = 11; 4), and Oomycota (n = 15; 3). Several groups exhibited clear seasonal biases: Bacillota (n = 6) and Rotifera (n = 6) were more abundant in summer, whereas Discosea (n = 11) and Evosea (n = 4) were more prevalent in spring. Chordata were also detected more frequently in spring (n = 5) than in summer (n = 4).

Analysis of these seasonal patterns based on habitat classification revealed that terrestrial-affiliated species dominated in both seasons, comprising 165 species (63.2%) in spring (Fig. 3.5A) and 94 species (62.7%) in summer (Fig. 3.5B). Species classified as occurring in both aquatic and terrestrial environments accounted for 58 species (22.2%) in spring and 28 species (18.7%) in summer. Aquatic-affiliated specialists represented a smaller proportion, with 38 species (14.6%) in spring and 28 species (18.7%) in summer.

Spatial comparisons

Spatial clamtest comparisons showed that 152 species were identified in samples from the eastern side of the island and 122 species from the west (Fig. 3.5C,D; Table S3.3). The most abundant species again corresponded to consistent phyla across regions, including Ascomycota (east: n = 38; west: n = 27), Arthropoda (n = 34; 31), Bacteroidota (n = 24; 15), and Basidiomycota (n = 8; 10). Nematoda were more frequently detected in eastern samples (n = 9) than in western ones (n = 1), while Mucoromycota (n = 3; 0) and Pseudomonadota (n = 1; 7) were more common in the west. Chordata were more evenly distributed (east: n = 3; west: n = 4).

As with seasonal trends, habitat-based analysis of spatial data revealed a dominance of terrestrial-affiliated species, comprising 102 species (68%) in the east (Fig. 3.5C) and 74 species (63.2%) in the west (Fig. 3.5D). Species classified as occurring in both habitats made up 29 species (19.3%) in the east and 16 species (13.7%) in the west. Aquatic-affiliated specialists were more prevalent in western samples (27 species; 23.1%) than in the east (19 species; 12.7%).

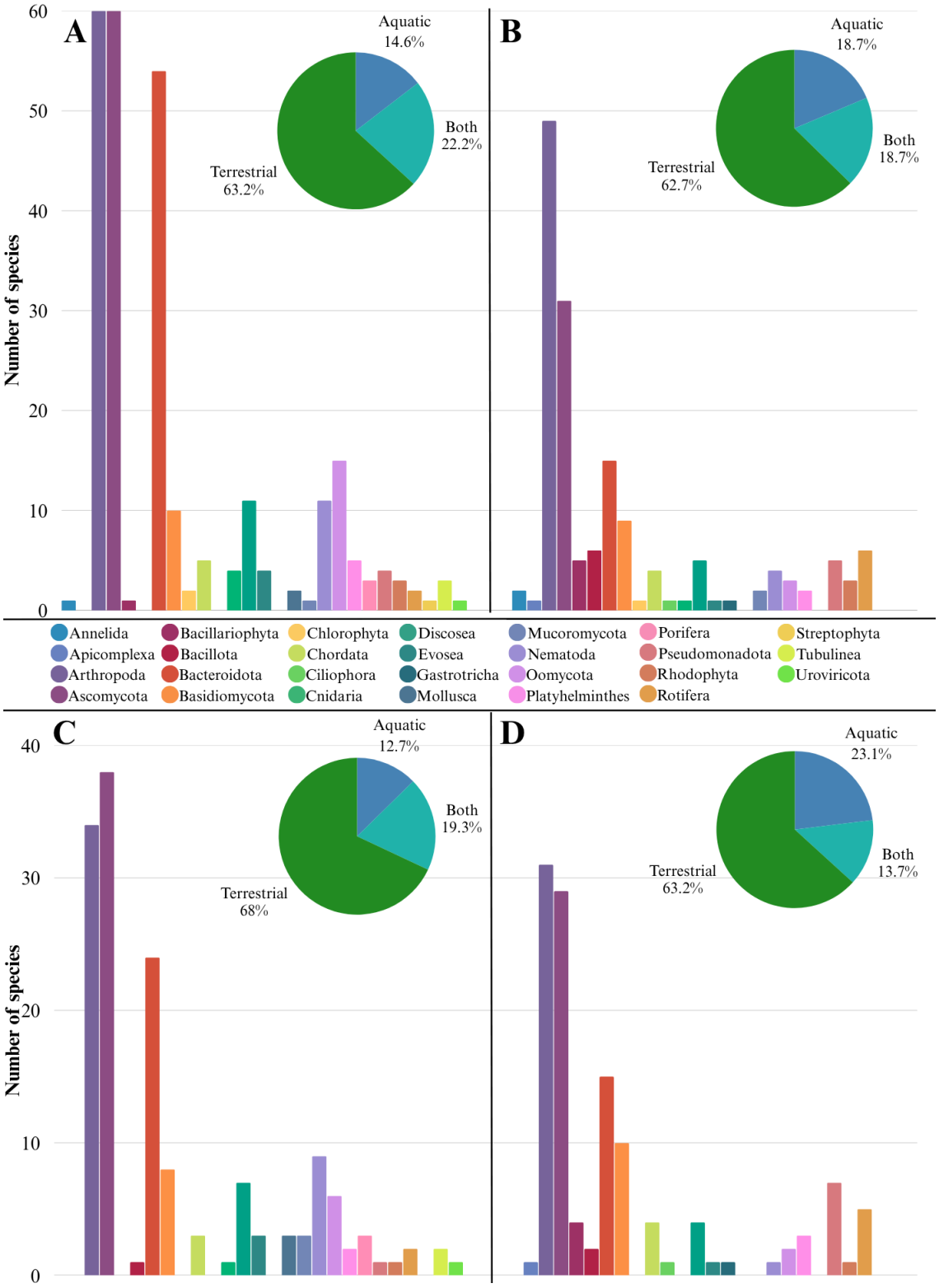


Figure 3.5. Specialist taxonomic composition of species across seasonal and spatial comparisons based on DNA metabarcoding of environmental DNA (eDNA) samples. Panels show the number of specialist species detected in: **A** spring; **B** summer; **C** eastern nest sites; and **D** western nest sites. Bar plots display the number of species assigned to each phylum, with colours representing phylum identity. Corresponding pie charts illustrate the proportion of species classified by habitat preference: terrestrial, aquatic, or both.

3.5. Discussion

We used eDNA metabarcoding analysis of nesting *E. minor faecal material* to show that biodiversity signals at Matiu/Somes Island varied temporally, while highlighting the value of nest-associated eDNA for both diet and 'bycatch' observations.

Because our samples were collected from guano-rich substrates within active nests, host DNA unsurprisingly dominated the sequence pool (69%). Although we attempted to reduce host-derived sequences using host-specific peptide nucleic acid (PNA) blocking primers, these efforts were ultimately unsuccessful. The relatively low detection of diet taxa in our study (six species) suggests that dietary DNA can persist within nest substrates but is likely degraded—potentially due to passage through two digestive tracts as adults regurgitate prey to feed chicks (Deagle et al., 2010; McInnes, Alderman, Deagle, et al., 2017)—or present in low overall concentrations (Pompanon et al., 2012). Consistent with this, McInnes et al. (2017) found that DNA from dietary sources could be detected in regurgitated samples collected from nests of shy albatross (*Thalassarche cauta*), but the detectability of key prey taxa was reduced, likely due to degradation and contamination from environmental and microbial sources within the nesting material. Similarly, Nimz et al. (2021) demonstrated that, even in highly digested regurgitated material from Christmas shearwaters (*Puffinus nativitatis*), dietary DNA could be recovered using metabarcoding techniques, but the prey signal was often fragmented and incomplete. In our study, differences in sample age, quality, and guano composition—such as varying freshness or the extent of degradation—may have contributed to low diet DNA concentrations and detectability. In particular, we were unable to differentiate between adult and chick faecal material, and differences

in digestive physiology and defecation behaviour between adults and chicks may have influenced the amount and quality of DNA present (Alberdi et al., 2020; Barrett et al., 2007; de Sousa et al., 2019; Videvall et al., 2018). Of additional note, among the six fish taxa we detected that were consistent with known *E. minor* dietary profiles, the identification of *E. japonicus* (Japanese anchovy) is unlikely to reflect a true diet species given its absence from the Southern Hemisphere. Instead, this detection likely represents *Engraulis australis* (Australian anchovy), a species known from New Zealand waters, that (given COI sequences for *E. australis* are available on GenBank) may have been misassigned due to haplotype variation.

Despite low detection numbers, our findings were broadly consistent with previous studies on *E. minor* diet composition in New Zealand. For example, the NZ Penguin Initiative (2025) used DNA metabarcoding to assess seabird prey across multiple coastal sites for *E. minor* and identified pelagic schooling fishes, such as anchovies (*Engraulis* sp., *Stolephorus* sp.) and triplefins (*Forsterygion flavonigrum*) as frequent dietary components. Similarly, we detected both anchovies and triplefins in samples from Matiu/Somes Island, reinforcing the importance of these taxa as widespread and accessible prey for *E. minor* across New Zealand's coastal waters. Conversely, we detected two fish taxa not reported by NZ Penguin Initiative (2025): *Hyporhamphus ihi* (garfish) and *Pseudophycis bachus* (red cod), both native to New Zealand and likely representing locally available or seasonally abundant prey specific to the Matiu/Somes Island foraging grounds. However, several fish groups reported by NZ Penguin Initiative (2025)—including pilchards (*Sardinops* sp.), needlefishes (*Belonidae* sp.), mackerels (*Scombridae* sp.), sauries (*Scomberesocidae* sp.), and warhou (*Seriolella* sp.)—were absent from our dataset. This may reflect local variation in prey communities, seasonal fluctuations in prey availability, or differences in *E. minor* foraging behaviour across sites, but the limited scale of diet species detection in our

study limits broader inference about regional or seasonal prey variation as yet. Nevertheless, our results contribute to a growing body of seabird eDNA diet data (de Leeuw et al., 2024; Evans et al., 2025; Young et al., 2020), and we look forward to further insights into seabird foraging ecology and the dynamics of coastal marine ecosystems as a result.

The environmental component of our dataset encompassed a broad taxonomic spectrum, including microbial, invertebrate, and vertebrate taxa, with Bacteria—particularly members of Bacteroidota and Pseudomonadota—dominating the species classifications. While we refer to these sequences as "environmental," we note that some may originate from the microbiomes of *E. minor* (e.g., cloacal, gut, or skin-associated bacteria) or from consumed prey (e.g., gut microbiota), rather than from truly exogenous environmental sources (Pearce et al., 2017). This potential overlap complicates interpretation, particularly in guano-rich nesting substrates where dietary, host, and environmental DNA are co-deposited. Nevertheless, this pattern reflects the ecological complexity of nesting environments and the contribution of both terrestrial and marine inputs to seabird-dominated land areas. In our study, habitat classifications revealed a predominance (64.1%) of terrestrial-affiliated taxa, including soil-dwelling nematodes, decomposer fungi, and invertebrate ectoparasites. In contrast, aquatic-affiliated taxa in our environmental dataset consisted primarily of marine-associated Bacteroidota. These findings are consistent with previous studies highlighting the strong terrestrial signature of seabird nesting habitats and the localised influence of marine inputs (Benkwitt et al., 2022; Kolb et al., 2015; McInnes et al., 2017; Ramírez-Fernández et al., 2019).

We observed distinct seasonal variation in biodiversity in our environmental dataset, with higher alpha diversity (Shannon index) in summer samples and significant differences in the

composition of specialist taxa between spring and summer. While overall diversity was greater in summer, spring samples harboured a higher number of unique specialist taxa, many of which were associated with terrestrial environments. These patterns likely reflect a combination of abiotic and host-related factors, including seasonal shifts in microbial colonisation, activity of cold-tolerant or early-successional taxa, and decomposition dynamics (Cereghetti et al., 2025; Wu et al., 2011; Zhao et al., 2022). Warmer summer conditions are known to enhance microbial and invertebrate activity, accelerate decomposition, and promote microbial succession (Bradford et al., 2008; Shade et al., 2012). Moreover, summer coincides with peak chick-rearing and fledging in *E. minor*, when guano accumulation increases and adult birds frequently return to nests, likely contributing to elevated DNA shedding and turnover in nest environments (Ancel et al., 2013; Chiaradia & Kerry, 1999; Sutton & Arnould, 2022). These factors collectively influence the composition and detectability of taxa within the environmental DNA pool.

Higher summer diversity may also reflect seasonal changes in the surrounding biotic community. Pulses of insect emergence, microbial blooms, and plant senescence during warmer months can introduce transient DNA signals to the nesting environment via direct contact (e.g., insects entering burrows), airbourne dispersal, or trophic interactions (Bálint et al., 2018; Bohmann et al., 2014). These seasonal inputs expand the diversity of eDNA available for detection, particularly in open or semi-exposed nest sites. This pattern has been corroborated in arthropod metabarcoding studies, which show summer peaks in species richness driven by life cycle timing and increased environmental activity (Kirse et al., 2021). Similarly, broader eDNA studies have found that species detectability is higher during warmer periods due to increased biomass, organismal shedding rates, and microbial processing (Buxton et al., 2018; Çevik & Çevik, 2025; Troth et al., 2021).

Spatial variation across nest locations or regions was not a significant driver of biodiversity signals in our environmental dataset. This contrasted with our original hypothesis, and suggests that environmental exposure and anthropogenic gradients across the island landscape (the eastern side, facing open, less disturbed coastal waters and coastline, is more isolated from human activity and boat traffic, while the western side, oriented toward urban Wellington, is more sheltered but subject to higher levels of anthropogenic disturbance, including consistent vessel traffic and underwater noise exposure) are not strong enough to affect localised diversity. Alternatively, our methods may not have been sensitive enough to detect such differences. Previous work has shown that abiotic and biotic conditions can influence substrate composition, microbial assemblages, and community structure (e.g., lipid-degrading bacteria, meiofaunal taxa), while anthropogenic disturbance can restructure ecological communities, reduce microbial diversity, and shift taxa co-occurrence patterns (DiBattista et al., 2020; Hoang et al., 2024; Martin, 2022). Despite the non-significant overall spatial pattern, clamtest analysis identified aquatic-affiliated specialist taxa more frequently in western samples, potentially due to increased marine aerosol deposition or runoff exposure from boat wakes and harbour movement (Dueker et al., 2011; Nogales et al., 2011; Thomas et al., 2006). In contrast, eastern samples were enriched in nematodes, fungi, and other soil-associated taxa, possibly reflecting deeper or less disturbed soil profiles and more complex detrital environments (Bardgett & van der Putten, 2014; Ferris et al., 2001).

We identified some key trends in biodiversity variation and highlighted the value of bycatch data in overall biodiversity detection. Future research should aim to extend this by comparing sampling methods that are optimised toward recovering prey DNA with those focused specifically on soil or substrate composition. Additional work should aim to further assess spatial eDNA variation from such samples—ideally across broader scales, improve taxonomic resolution

and interpretability through methodological refinements (such as incorporating predator- and prey-specific primers and/or peptide nucleic acid host DNA blockers, increasing sample replication, and using freshly deposited faeces of known origin) and integrate eDNA metabarcoding with complementary methods like stable isotope analysis and invertebrate surveys to provide a more holistic understanding of seabird foraging ecology and surrounding ecosystem health.

3.6. References

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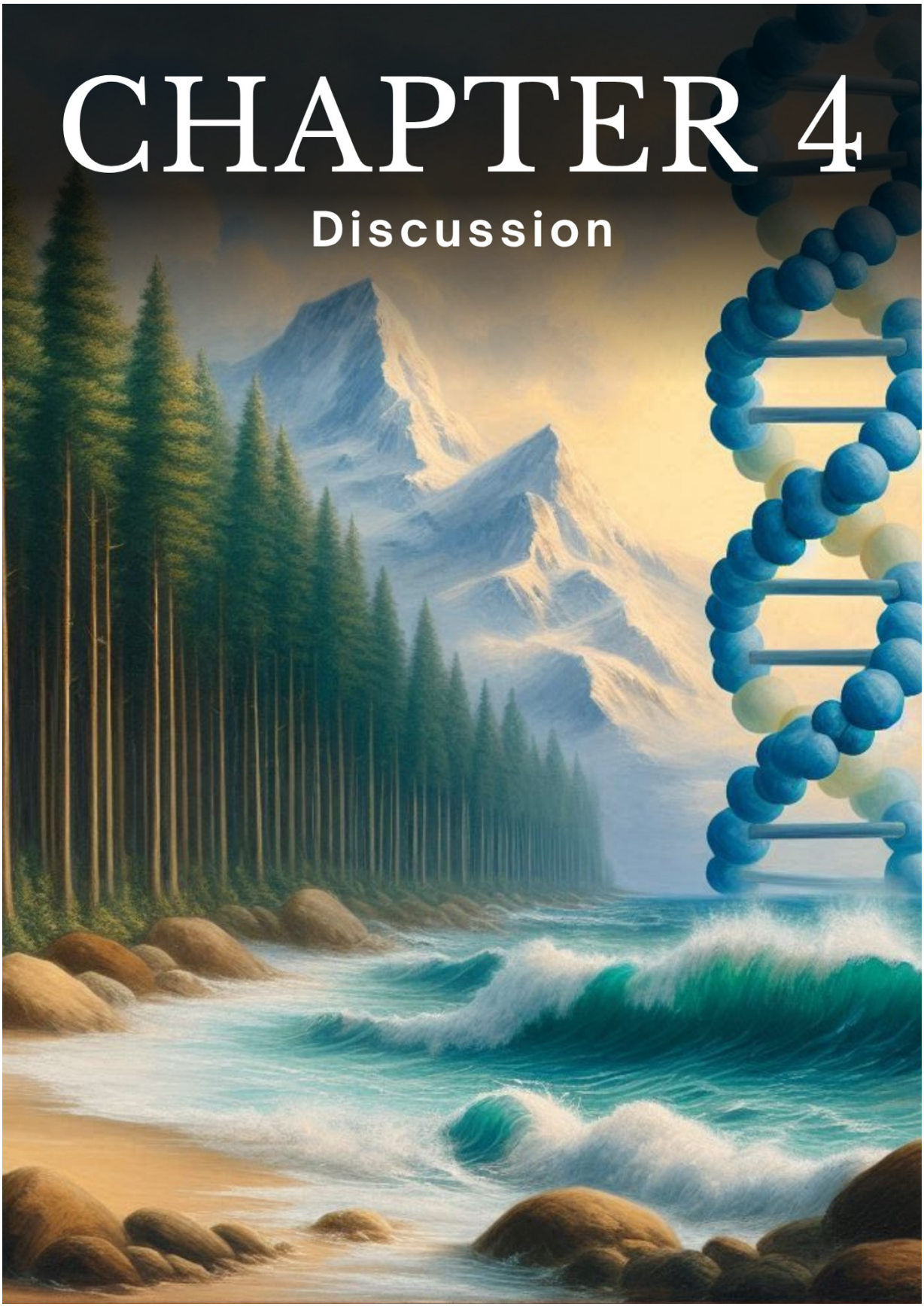
CHAPTER 4
Discussion

4.1. Thesis summary

This thesis investigates diet composition and ecosystem biodiversity using DNA metabarcoding of faecal samples from two keystone predators—the long-tailed bat *Chalinolobus tuberculatus* and the little blue penguin *Eudyptula minor*—in distinct ecological contexts. By combining dietary profiling with incidental biodiversity detection, these studies provide complementary perspectives on trophic ecology and environmental variation, illustrating how ecological context influences the type, scope, and resolution of information recovered through molecular approaches.

For *C. tuberculatus* (Chapter 2), faecal analysis revealed a diverse arthropod diet spanning multiple insect orders, including both native and non-native species. Dietary variation with reproductive status suggested that energetic demands influence prey choice, while differences between pre- and post-harvest periods indicated sensitivity to habitat modification. These findings point to a potential role of bats in regulating introduced insects and highlight the species' value as a bioindicator in plantation forests.

In *E. minor* (Chapter 3), metabarcoding produced limited direct dietary resolution but yielded a rich record of non-diet taxa, reflecting the surrounding biodiversity of the colony environment. Seasonal differences in community composition and subtle spatial patterns were linked to biological activity, microhabitat heterogeneity, and possible anthropogenic disturbance. These results demonstrate that, even when dietary data are sparse, predator-associated eDNA can provide meaningful ecological insights, with seabird colonies acting as sentinel sites for coastal biodiversity monitoring.

4.2. Ecological caveats

Many of the technical and ecological constraints relevant to eDNA metabarcoding in these studies are addressed in Chapters 2 and 3, but several broader considerations remain important when interpreting the findings.

eDNA persistence and detectability are influenced by environmental factors, such as temperature, pH, salinity, UV exposure, and microbial activity—all of which vary across habitats, seasons, and sampling times—potentially biasing detection probabilities and taxonomic resolution (Ke et al., 2025). Sample freshness is also critical, as DNA in faecal material degrades rapidly after deposition; delays in collection or suboptimal storage can also reduce sequence quality (Hawlitschek et al., 2018). Although careful timing, storage, and processing can mitigate these effects, they inevitably influence the scope and precision of results. For example, in Chapter 2, variation in guano composition—including freshness, age, and depositor (adult vs chick)—likely contributed to uneven taxonomic detection (Alberdi et al., 2020; Barrett et al., 2007; Videvall et al., 2018).

As with all metabarcoding studies, technical limitations such as primer bias, PCR inhibition (e.g., by uric acid), and variable amplification efficiency can reduce taxonomic recovery (Fiorello, 2020; Munch et al., 2019; Piñol et al., 2015). Taxonomic assignment may also be constrained by incomplete or inconsistent reference database coverage (Keck et al., 2023; Porter & Hajibabaei, 2018). For *E. minor* and other marine-feeding predators, using fish-specific primers and targeting freshly deposited, individual faecal samples of known origin could reduce environmental contamination and improve prey detection. For bats, the discrete, solid, and compact nature of the

faecal material likely facilitated more efficient DNA extraction and reduced environmental contamination, thereby improving sequence quality and increasing dietary sequence recovery. For all eDNA metabarcoding studies, the continued growth of reference databases and methodological advances (e.g., improved primer design, optimised extraction protocols, and bioinformatic pipelines) will further enhance taxonomic resolution and detection rates (Gold et al., 2022; Jerde et al., 2021).

Both of my case studies focused on a single keystone predator in each ecosystem. While such species can exert a disproportionate influence on ecological structure and function, concentrating solely on apex or near-apex predators narrows the scope of inference (Cottee-Jones & Whittaker, 2012; Ellison, 2019). For example, observed patterns may not represent species dynamics at other trophic levels, particularly among mid-level consumers, omnivores, or primary producers (Goedegebuure et al., 2017; Heath et al., 2014; Steiner, 2001). Ecosystems are shaped by complex multi-species relationships and shifts in one predator may not fully capture broader ecological responses or resilience mechanisms (Rosenbaum et al., 2024; Thorogood et al., 2023).

Ecological context is also important. For *C. tuberculatus*, prey availability in plantation forests is influenced by forestry management cycles, vegetation composition, and surrounding landscape connectivity (Froidevaux et al., 2021; Hendel et al., 2025). For *E. minor*, dietary opportunities vary with seasonal changes in ocean productivity, local prey migration, and the effect of weather conditions on foraging (Kuletz et al., 2024; Xavier et al., 2013). Spatial scale also influences results: data from a single foraging location—whether a forest block or a single island—may not represent the species' role across its full range (Bergsten et al., 2012; Lu & Jetz, 2023). Interactions with other predators may also influence biodiversity detections through their influence

on competition and predation, or on altered access to foraging grounds (Ferretti et al., 2023; Kanishka et al., 2025). Additionally, environmental conditions that were not assessed here—such as weather patterns, temperature variation, and extreme climatic events, as well as anthropogenic factors including forestry operations, heavy machinery, artificial lighting, vessel traffic, and noise disturbance—can alter predator behaviour and prey community structure, potentially confounding observed spatial or seasonal patterns (Doherty et al., 2022; Laws, 2017; Takemoto et al., 2014; Cross et al., 2021; Katabaro et al., 2022; Morin et al., 2021).

Taken together, these considerations underscore that metabarcoding captures only one dimension of each species' ecological role. Results should therefore be interpreted within a broader framework of interacting biotic and abiotic drivers, environmental variability, and human influence, placing species-specific findings in the context of whole-ecosystem processes and long-term biodiversity dynamics.

4.3. Future directions

Species-specific recommendations are outlined in Chapters 2 and 3, but several broader opportunities span both studies. For example, future work would benefit from pairing eDNA sampling with systematic environmental monitoring. Recording abiotic and biotic variables such as those outlined in Section 4.2, can provide essential context for interpreting dietary and biodiversity patterns. In particular, because key environmental factors influence eDNA persistence and detectability, collecting these data alongside biological samples can improve interpretive accuracy and comparability across sites and seasons (Ke et al., 2025). Furthermore, integrating eDNA with environmental and spatial datasets—such as GIS layers linking land use, climate, and topography—could reveal hidden patterns in community composition (Ahmed & McClenaghan, 2024). Such combined approaches could help disentangle the relative contributions of ecological processes, natural variability, and human disturbance to observed shifts, for example by modelling environmental gradients using frameworks like eDNAPlus (Diana et al., 2022). Remote sensing could further enhance interpretation of spatial structure and environmental drivers (Bauknecht et al., 2025), while eDNA's fine-scale temporal resolution enables tracking of seasonal turnover more effectively than many traditional methods (Seymour et al., 2021).

The approaches demonstrated here have strong potential to detect biodiversity change across species, habitats, and trophic levels. Predator-associated eDNA is a non-invasive method for assessing diet and biodiversity simultaneously, with applications to a wide range of terrestrial, freshwater, and marine predators (Nørgaard et al., 2021). Expanding sampling across multiple colonies, forests, seasons, and years would extend the generality of findings and help detect long-

term ecological trends. Comparative studies of related predators in different environments, or of species occupying similar niches, could also help to identify consistent drivers of dietary variation and biodiversity change (Harper et al., 2020; Riaz et al., 2023).

Methodological refinements could further improve both dietary resolution and biodiversity signal quality. For *E. minor*, freshly deposited faecal samples yield stronger prey DNA signals, as degradation can occur rapidly through microbial activity, UV exposure, and moisture (Hawlitschek et al., 2018; Ke et al., 2025). This would require sample collection timed to when the birds return to their nests at dawn and dusk, as fresh material is most likely to be available—a logistically demanding task that would benefit from well-coordinated field teams and flexible scheduling. For *C. tuberculatus* and other mobile species, targeted post-foraging or roost-based sampling could further reduce any contamination and improve sample quality.

Finally, future work should move beyond identifying prey taxa to understanding their ecological functions. Categorising prey species by traits such as their roles as pollinators, decomposers, or pests would link dietary composition to ecosystem services. In forestry contexts, quantifying pest suppression services could inform management, while in seabird colonies, biodiversity trends could serve as indicators of habitat condition. Building on the present study, which explored relationships with certain environmental features (e.g., distance to streams), further work could integrate detailed taxonomic composition and functional trait data with a broader set of landscape variables, including vegetation composition and habitat. Incorporating dietary and biodiversity data into trophic network models, including Molecular Ecological Network Analyses (Meyer et al., 2020), could reveal key species, structural vulnerabilities, and potential cascade effects under different scenarios.

4.4. Closing perspective

Collectively, my thesis demonstrates how predator-associated eDNA can provide novel insights into species interactions and biodiversity patterns within New Zealand's forests and coastal environments. Embedding such approaches within national monitoring programmes would not only support kaitiakitanga (guardianship/protection) but would also enable more responsive, evidence-based management of ecosystems facing the combined pressures of climate change, habitat modification, and introduced species. By integrating molecular ecology with environmental monitoring, functional ecology, and spatial modelling, predator-associated eDNA could become a cornerstone of applied ecological management in Aotearoa—guiding forestry practices that protect native bats, monitoring biodiversity change in sensitive seabird colonies, and strengthening our ability to anticipate and mitigate ecological change across terrestrial and marine systems.

4.5. References

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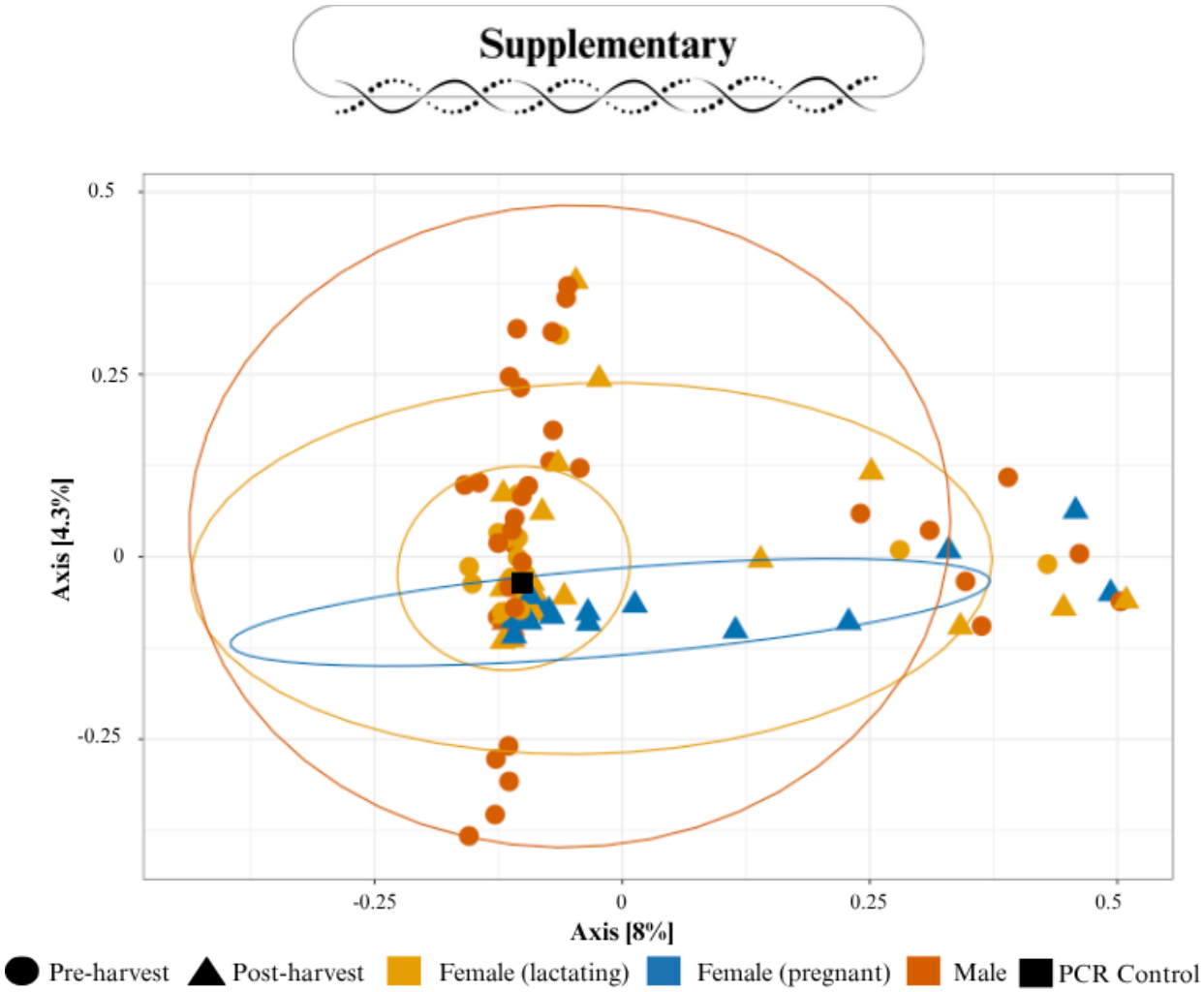

Figure S2.1. Ordination of COI amplicon data highlighting the placement of PCR and extraction controls relative to biological samples. Controls cluster tightly, indicating minimal contamination or bias, and are distinct from biological groupings categorised by harvest status and reproductive condition.

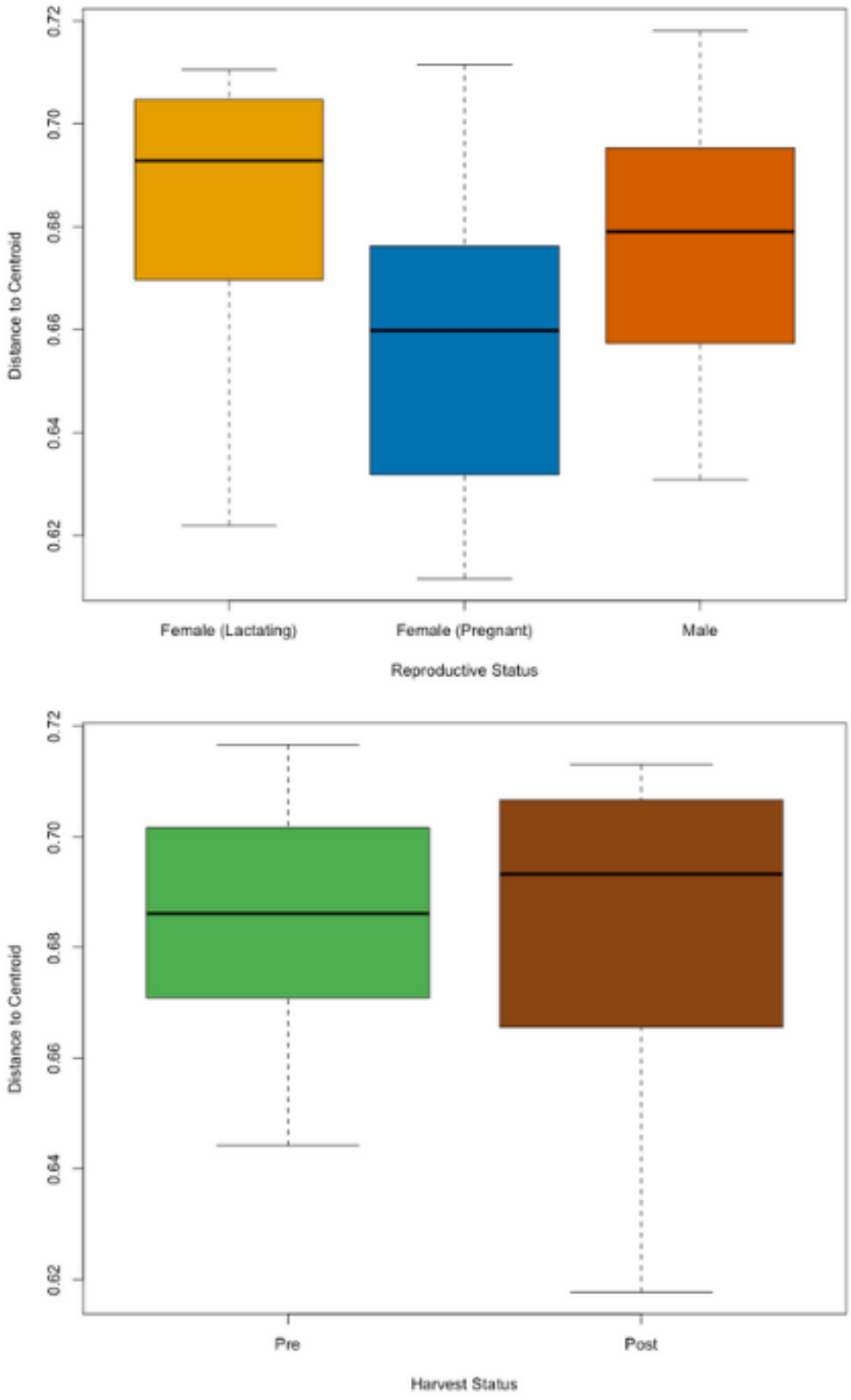

Figure S2.2. Multivariate homogeneity of group dispersions (betadisper analysis) based on COI amplicon data, shown by reproductive status (top) and harvest status (bottom). Boxplots represent distances of individual samples to their group centroid, with the median indicated by the bold horizontal line

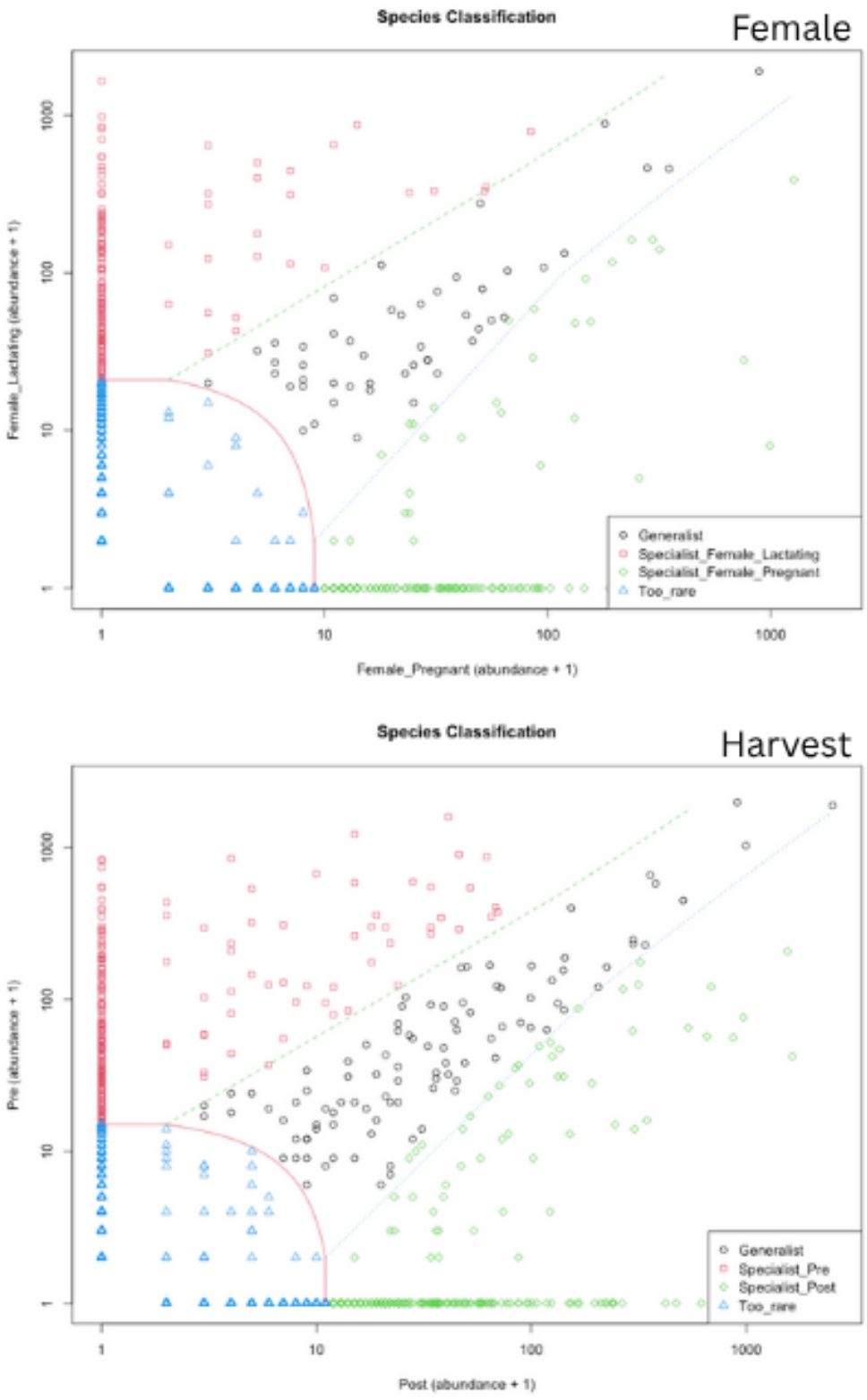

Figure S2.3. Clamtest analysis results, classifying ASVs by group association for (top) reproductive status (lactating vs. pregnant females) and (bottom) harvest status (pre- vs. post-harvest). ASVs are categorised as group-specific, generalist (shared between groups), or too rare to classify. Each point represents an individual ASV, plotted by relative abundance in each group

Table S2.1. Metadata for *Chalinolobus tuberculatus* faecal samples, including sample ID, collection date, reproductive and harvest status, roost type, and dominant sex where available.

Sample ID	Sample name	Collection date	Reproductive status	Roost	Dominant sex	Harvest status
UOW_24_01_001	Z2863	16/03/07	NA	NA	Male	Pre
UOW_24_01_002	Z2859	12/12/07	Lactating	NA	Female	Post
UOW_24_01_003	Carter2	02/11/08	NA	NA	Male	Pre
UOW_24_01_004	U9322	12/12/07	Lactating	NA	Female	Post
UOW_24_01_005	U9330	09/03/08	NA	NA	Male	Pre
UOW_24_01_006	U9330	09/03/08	NA	NA	Male	Pre
UOW_24_01_007	U9330	09/03/08	NA	NA	Male	Pre
UOW_24_01_008	U9330	09/03/08	NA	NA	Male	Pre
UOW_24_01_009	U9330	09/03/08	NA	NA	Male	Pre
UOW_24_01_010	U9303	04/02/09	NA	NA	Male	Post
UOW_24_01_011	Z2859	25/11/07	Pregnant	NA	Female	Post
UOW_24_01_012	Z2859	25/11/07	Pregnant	NA	Female	Post
UOW_24_01_013	Z2859	12/11/07	Pregnant	NA	Female	Post
UOW_24_01_014	Z2859	12/11/07	Pregnant	NA	Female	Post
UOW_24_01_015	Z2859	12/11/07	Pregnant	NA	Female	Post
UOW_24_01_016	Z2859	12/11/07	Pregnant	NA	Female	Post
UOW_24_01_017	Z2859	12/11/07	Pregnant	NA	Female	Post
UOW_24_01_018	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_019	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_020	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_021	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_022	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_023	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_024	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_025	KR3	25/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_026	BR1	16/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_027	BR1	16/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_028	BR1	16/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_029	BR1	16/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_030	BR1	16/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_031	BR1	16/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_032	KR3	23/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_033	KR3	23/02/08	NA	Kinleith Roost 3	Male	Pre
UOW_24_01_034	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_035	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_036	BR1	07/02/08	Lactating	B Roost 1	Female	Post

UOW_24_01_037	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_038	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_039	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_040	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_041	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_042	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_043	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_044	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_045	BR1	07/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_046	BR1	28/11/08	Pregnant	B Roost 1	Female	Post
UOW_24_01_047	BR1	08/02/08	Lactating	B Roost 1	Female	Post
UOW_24_01_048	BR1	08/02/09	Lactating	B Roost 1	Female	Post
UOW_24_01_049	BR1	08/02/10	Lactating	B Roost 1	Female	Post
UOW_24_01_050	BR1	08/02/11	Lactating	B Roost 1	Female	Post
UOW_24_01_051	BR1	08/02/12	Lactating	B Roost 1	Female	Post
UOW_24_01_052	BR1	08/02/13	Lactating	B Roost 1	Female	Post
UOW_24_01_053	BR3	17/12/07	Giving Birth	B Roost 3	Female	Post
UOW_24_01_054	BR3	28/02/07	Lactating	B Roost 3	Female	Pre
UOW_24_01_055	BR3	28/02/07	Lactating	B Roost 3	Female	Pre
UOW_24_01_056	BR3	28/02/07	Lactating	B Roost 3	Female	Pre
UOW_24_01_057	BR3	28/02/07	Lactating	B Roost 3	Female	Pre
UOW_24_01_058	BR3	28/02/07	Lactating	B Roost 3	Female	Pre
UOW_24_01_059	BR3	28/02/07	Lactating	B Roost 3	Female	Pre
UOW_24_01_060	BR1	26/11/07	Pregnant	B Roost 1	Female	Post
UOW_24_01_061	BR1	26/11/07	Pregnant	B Roost 1	Female	Post
UOW_24_01_062	BR1	26/11/07	Pregnant	B Roost 1	Female	Post
UOW_24_01_063	BR1	26/11/07	Pregnant	B Roost 1	Female	Post
UOW_24_01_064	BR1	26/11/07	Pregnant	B Roost 1	Female	Post
UOW_24_01_065	BR1	26/11/07	Pregnant	B Roost 1	Female	Post
UOW_24_01_066	BR1	12/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_067	BR1	12/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_068	BR1	12/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_069	BR1	12/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_070	BR1	12/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_071	BR1	12/02/07	Lactating	B Roost 1	Female	Pre
UOW_24_01_072	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_073	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_074	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_075	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre

UOW_24_01_076	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_077	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_078	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_079	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_080	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_081	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_082	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_083	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_084	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_085	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_086	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_087	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_088	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_089	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_090	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_091	KR1	22/02/08	NA	Kinleith Roost 1	Male	Pre
UOW_24_01_092	Extraction Control	NA	NA	NA	NA	NA
UOW_24_01_093	PCR Control	NA	NA	NA	NA	NA

Table S2.2. Per-amplicon sequencing statistics for each sample, including the number of raw, filtered, denoised (F, R, for forward and reverse reads, respectively), merged, and non-chimera reads.

Sample	Raw	Filtered	Denoised (F)	Denoised (R)	Merged	Non-Chimera
UOW-24-01-001	148238	120586	119865	119803	111302	109740
UOW-24-01-002	294832	237462	235107	234866	191589	172750
UOW-24-01-003	244393	197055	194307	194888	186211	185402
UOW-24-01-004	240920	195359	195031	194545	177477	175064
UOW-24-01-005	178733	144876	144246	144131	139372	139104
UOW-24-01-006	235578	191043	190159	190377	182486	182072
UOW-24-01-007	323819	262345	261431	261819	248192	246719
UOW-24-01-008	328063	266311	265323	265316	249865	248578
UOW-24-01-009	249748	202148	201441	201169	187771	185933
UOW-24-01-010	164378	129673	128579	128611	121037	120647
UOW-24-01-011	230399	186289	184471	184839	173759	165637
UOW-24-01-012	172285	139304	137859	137974	130698	128889
UOW-24-01-013	191844	154704	154004	154080	146573	146361
UOW-24-01-014	265421	215627	214228	214460	202367	200610
UOW-24-01-015	286189	231037	225489	225221	203982	184766
UOW-24-01-016	182368	146930	144456	144464	122149	118872
UOW-24-01-017	315465	253739	247784	249668	209743	204968
UOW-24-01-018	96203	78192	75963	76058	67523	66925
UOW-24-01-019	112684	91109	88846	88707	81522	81326
UOW-24-01-020	319104	258169	253746	252898	213950	197253
UOW-24-01-021	11460	9264	8611	8567	7917	7840
UOW-24-01-022	27106	22097	21809	21780	20299	20289
UOW-24-01-023	18769	15314	14377	14482	13358	13341
UOW-24-01-024	301829	244369	240596	240386	214731	203129
UOW-24-01-025	113099	91579	88000	88230	78529	77585
UOW-24-01-026	107980	87851	86069	85757	75902	75436
UOW-24-01-027	283555	229337	225918	225289	191924	179605
UOW-24-01-028	391	315	289	288	280	280
UOW-24-01-029	323491	263279	261943	261381	242318	204045
UOW-24-01-030	324267	263697	262390	262449	243559	214942
UOW-24-01-031	286853	232880	231612	231457	213812	210498
UOW-24-01-032	154732	125397	124316	124230	114933	114272
UOW-24-01-033	88175	71425	69366	69577	60411	60126
UOW-24-01-034	148913	120330	117613	117833	104946	103999
UOW-24-01-035	195334	157818	155343	155583	137954	133710
UOW-24-01-036	257616	209239	207962	208037	193891	189246
UOW-24-01-037	259894	210816	207292	207840	194051	192264

UOW-24-01-038	211119	172025	170653	170615	160179	157177
UOW-24-01-039	232359	188389	187227	186480	173044	171705
UOW-24-01-040	226077	182964	182612	182231	165326	164762
UOW-24-01-041	138007	112301	111234	111598	105935	105479
UOW-24-01-042	214256	173449	172965	172712	163356	162773
UOW-24-01-043	99595	80468	78706	78835	68367	67410
UOW-24-01-044	237739	193938	191796	192179	184635	182588
UOW-24-01-045	282847	228030	227653	226279	218695	218256
UOW-24-01-046	218335	177477	176531	176256	164774	159589
UOW-24-01-047	331995	269213	268095	267702	248203	242487
UOW-24-01-048	333119	265813	262330	262385	244562	237807
UOW-24-01-049	296509	241017	239795	239389	216141	214699
UOW-24-01-050	209648	170046	168247	167878	159213	158561
UOW-24-01-051	324377	263240	260747	261713	240497	237872
UOW-24-01-052	248667	201391	199918	199950	185374	170110
UOW-24-01-053	169040	135306	132994	132977	127239	125771
UOW-24-01-054	349727	283226	278372	278753	255182	252485
UOW-24-01-055	303911	246758	243626	243672	232538	230420
UOW-24-01-056	320468	259270	255799	255954	237138	234546
UOW-24-01-057	168588	136222	134203	133951	122046	111893
UOW-24-01-058	346612	281174	277328	277857	254404	233685
UOW-24-01-059	300660	243965	239630	240104	223191	212760
UOW-24-01-060	367349	297685	296053	296169	272930	266508
UOW-24-01-061	285434	231331	229797	229768	207754	203065
UOW-24-01-062	359798	291498	287043	287085	270638	265213
UOW-24-01-063	352850	287656	286267	286190	267954	261487
UOW-24-01-064	284271	231472	230745	230835	211473	208515
UOW-24-01-065	284570	230269	227046	227660	207623	205113
UOW-24-01-066	216375	175672	175260	175021	165841	165355
UOW-24-01-067	137240	110773	109058	109717	103320	96138
UOW-24-01-068	381049	310655	309154	309228	282214	279349
UOW-24-01-069	428372	347220	345014	344626	320720	295054
UOW-24-01-070	427554	346905	345962	344670	313425	309837
UOW-24-01-071	194892	159036	157987	158110	151756	151717
UOW-24-01-072	375855	304870	301345	301812	283565	280682
UOW-24-01-073	273123	215687	213337	213431	202315	199743
UOW-24-01-074	298189	242606	241661	241772	229140	200752
UOW-24-01-075	216517	174833	172937	173005	165089	157484
UOW-24-01-076	320521	260068	256283	256612	240635	233471

UOW-24-01-077	277563	225267	223738	223425	209627	197908
UOW-24-01-078	367096	297613	296137	296569	269904	264072
UOW-24-01-079	1551	1255	1187	1174	1093	1090
UOW-24-01-080	324747	263601	262327	262254	246838	233761
UOW-24-01-081	361944	295140	293672	293993	277911	266012
UOW-24-01-082	224645	181976	179146	179550	172315	171632
UOW-24-01-083	340849	276902	273322	273944	255311	244941
UOW-24-01-084	336746	271027	264687	265519	249023	244347
UOW-24-01-085	305386	248577	247359	247566	233312	206215
UOW-24-01-086	270220	219144	218286	218305	205549	203129
UOW-24-01-087	217191	176130	174308	174400	165826	164047
UOW-24-01-088	9775	7969	7659	7585	7029	7021
UOW-24-01-089	392891	316128	312757	313149	283865	270601
UOW-24-01-090	161352	130284	127884	127864	111522	110011
UOW-24-01-091	303998	246726	242467	242691	230518	220719
UOW-24-01-092	141040	114674	114015	114311	103485	103326
UOW-24-01-093	301383	244959	244048	243941	221522	206551

Table S2.3. Summary of total amplicon sequence variants (ASVs) and read counts recovered from COI amplicon sequencing controls. Values are reported separately for extraction and PCR controls.

Control type	No. of ASVs	No. of reads
Extraction control	43	103326
PCR control	391	206551

Table S2.4. Complete clamtest analysis results for ASVs classified as specific to lactating and pregnant females. Taxonomic assignments include Class, Order, Genus, and Species where available. Multiple ASVs may correspond to the same taxonomic identification.

Females (lactating)				
ASV	**Class**	**Order**	**Genus**	**Species**
ASV_13	Insecta	Lepidoptera	*Plutella*	*Plutella xylostella*
ASV_26, ASV_954	Insecta	Diptera	NA	*Psychodidae sp.*
ASV_27, ASV_47	Insecta	Coleoptera	NA	*Coleoptera sp.*
ASV_46, ASV_1001	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03013845
ASV_112	Insecta	Siphonaptera	*Nosopsyllus*	*Nosopsyllus laeviceps*
ASV_130, ASV_212	Arachnida	Mesostigmata	NA	*Parasitidae* sp. BIOUG24479-C11
ASV_141, ASV_453	Insecta	Coleoptera	*Odontria*	*Odontria* sp.
ASV_153	Insecta	Hymenoptera	NA	*Hymenoptera* sp.
ASV_215	Arachnida	Trombidiformes	NA	Trombidiformes sp. BOLD:ABW1146
ASV_241	Insecta	Lepidoptera	*Arichanna*	*Arichanna flavomacularia*
ASV_243	Insecta	Diptera	*Rhamphomyia*	*Rhamphomyia helleni*
ASV_278, ASV_942	Insecta	Diptera	*Ebenia*	*Ebenia* sp. ASCRT205-10
ASV_279	Insecta	Diptera	*Tipula*	*Tipula furca*
ASV_329, ASV_1745	Insecta	Diptera	*Shannoniana*	*Shannoniana moralesi*
ASV_343	Insecta	Lepidoptera	*Eudonia*	*Eudonia dinodes*
ASV_347	Insecta	Lepidoptera	*Wiseana*	*Wiseana cervinata*
ASV_356, ASV_711, ASV_1287	Insecta	Lepidoptera	*Declana*	*Declana floccosa*
ASV_383	Insecta	Lepidoptera	*Agrotis*	*Agrotis ipsilon*
ASV_395	Insecta	Diptera	*Colocasiomyia*	Colocasiomyia sp. gr JJG-2023a
ASV_396, ASV_1248, ASV_8281	Insecta	Lepidoptera	*Ourapteryx*	*Ourapteryx thibetaria*
ASV_397	Insecta	Diptera	NA	*Diptera* sp. NZAC 03012179
ASV_487, ASV_1166	Insecta	Diptera	*Oxysarcodexia*	*Oxysarcodexia terminalis*
ASV_511	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03012484
ASV_528, ASV_1017	Insecta	Diptera	*Sympotthastia*	*Sympotthastia fulva*
ASV_557	Insecta	Diptera	*Protomiltogramma*	*Protomiltogramma cincta*
ASV_561, ASV_750	Insecta	Lepidoptera	*Chloroclystis*	*Chloroclystis filata*
ASV_567	Insecta	Lepidoptera	*Agrotis*	*Agrotis lasserrei*
ASV_612	Arachnida	Araneae	*Desis*	*Desis formidabilis*

ASV_626	Insecta	Diptera	NA	*Limoniidae* sp. sc
ASV_643	Insecta	Diptera	NA	*Sciaridae* sp.
ASV_717	Insecta	Lepidoptera	*Barea*	*Barea bathrochorda*
ASV_731	Insecta	Diptera	NA	*Diptera* sp. NZAC 03009179
ASV_738, ASV_815	Insecta	Diptera	*Nephrotoma*	*Nephrotoma submaculosa*
ASV_741	Insecta	Coleoptera	*Cyloma*	*Cyloma guttulatus*
ASV_761	Insecta	Diptera	*Limnophyes*	*Limnophyes minimus*
ASV_804	Insecta	Coleoptera	NA	*Staphylinidae* sp.
ASV_853	Insecta	Diptera	*Culex*	*Culex pervigilans*
ASV_900	Insecta	Diptera	*Anopheles*	*Anopheles darlingi*
ASV_939	Insecta	Hymenoptera	*Diaeretiella*	*Diaeretiella rapae*
ASV_947	Arachnida	Trombidiformes	NA	*Eupodidae* sp. BIOUG27600-C10
ASV_981, ASV_1098, ASV_7676	Insecta	Lepidoptera	*Ctenopseustis*	*Ctenopseustis fraterna*
ASV_1036, ASV_2113, ASV_2290	Insecta	Coleoptera	*Arhopalus*	*Arhopalus ferus*
ASV_1041	Insecta	Diptera	*Forcipomyia*	*Forcipomyia alacris*
ASV_1144	Insecta	Hymenoptera	*Tenthredo*	*Tenthredo* sp. 01gatineau
ASV_1151	Insecta	Lepidoptera	*Choristoneura*	*Choristoneura rosaceana*
ASV_1153	Insecta	Diptera	NA	*Limosininae* sp. BIOUG23680-E01
ASV_1243, ASV_2181	Insecta	Diptera	NA	*Ceratopogonidae* sp.
ASV_1251	Insecta	Diptera	*Anopheles*	*Anopheles tessellatus*
ASV_1280, ASV_1486	Insecta	Coleoptera	*Quedius*	*Quedius fuliginosus*
ASV_1320	Insecta	Hymenoptera	*Euceros*	*Euceros coxalis*
ASV_1379	Insecta	Diptera	*Paraleucophenga*	*Paraleucophenga longiseta*
ASV_1427	Insecta	Lepidoptera	*Lozotaenia*	*Lozotaenia capensana*
ASV_1481	Insecta	Psocoptera	*Enderleinella*	*Enderleinella obsoleta*
ASV_1620	Insecta	Diptera	NA	*Mycetophilidae* sp. BOLD-2016
ASV_1662	Insecta	Diptera	NA	*Mycetophilidae* sp.
ASV_1691	Insecta	Diptera	NA	*Cecidomyiidae* sp. BOLD:ACA9586
ASV_1692	Arachnida	Trombidiformes	NA	*Tarsonemidae* sp. BIOUG35077-G10
ASV_1944	Insecta	Diptera	*Prionocera*	*Prionocera turcica*
ASV_2145	Insecta	Lepidoptera	*Izatha*	*Izatha peroneanella*
ASV_2214, ASV_2294, ASV_2396, ASV_7320	Arachnida	Mesostigmata	NA	*Mesostigmata* sp. BOLD:ACL7934
ASV_2240	Insecta	Diptera	NA	*Diptera* sp. BOLD:AAF9004
ASV_2580	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03009242

ASV_2686	Insecta	Coleoptera	*Uloma*	*Uloma tenebrionoides*
ASV_2993	Insecta	Coleoptera	*Nicrophorus*	*Nicrophorus obscurus*
ASV_3235	Insecta	Lepidoptera	*Gymnobathra*	*Gymnobathra flavidella*
ASV_3336	Insecta	Lepidoptera	*Conoeca*	*Conoeca guildingi*
ASV_3755	Insecta	Diptera	*Ablabesmyia*	*Ablabesmyia* sp. TAS17.4.3
ASV_6723	Insecta	Coleoptera	*Elaphropus*	*Elaphropus parvulus*
ASV_7177	Arachnida	Sarcoptiformes	NA	Scheloribatidae sp. BIOUG24577-B12
ASV_7381	Insecta	Hemiptera	*Aspidiotus*	*Aspidiotus nerii*
ASV_8368	Arachnida	Trombidiformes	NA	*Tarsonemidae* sp. BOLD:ADE1624
ASV_8382	Insecta	Coleoptera	*Agrypnus*	*Agrypnus scutellaris*
ASV_8693	Arachnida	Araneae	*Anelosimus*	*Anelosimus wallacei*
Females (pregnant)				
ASV	**Class**	**Order**	**Genus**	**Species**
ASV_12	Insecta	Diptera	*Symplecta*	*Symplecta stictica*
ASV_122	Insecta	Diptera	NA	*Dixidae* sp. BOLD-2016
ASV_173	Insecta	Trichoptera	*Triplectides*	*Triplectides dolichos*
ASV_180, ASV_404	Insecta	Lepidoptera	*Opogona*	*Opogona omoscopa*
ASV_192	Arachnida	Araneae	*Colaranea*	*Colaranea viriditas*
ASV_193, ASV_376, ASV_388, ASV_555, ASV_656, ASV_866, ASV_1307, ASV_2621, ASV_2740	Insecta	Diptera	NA	*Tipulidae* sp. BOLD:AAF9030
ASV_210	Insecta	Lepidoptera	*Orocrambus*	*Orocrambus flexuosellus*
ASV_239	Insecta	Diptera	*Cricotopus*	*Cricotopus metatibialis*
ASV_267	Insecta	Diptera	*Exorista*	*Exorista civilis*
ASV_270	Insecta	Diptera	*Cheilosia*	*Cheilosia matsumurana*
ASV_314	Insecta	Orthoptera	*Talitropsis*	*Talitropsis sedilloti*
ASV_319	Insecta	Diptera	*Limnophyes*	*Limnophyes minimus*
ASV_328	Arachnida	Sarcoptiformes	*Lepidoglyphus*	*Lepidoglyphus destructor*
ASV_373	Insecta	Diptera	NA	*Diptera* sp. NZAC 03013255
ASV_408	Insecta	Psocoptera	*Trogium*	*Trogium pulsatorium*
ASV_517	Insecta	Diptera	NA	*Orthocladiinae* sp. BOLD-2016
ASV_523	Arachnida	Trombidiformes	*Acalitus*	*Acalitus vaccinii*
ASV_536, ASV_2033	Insecta	NA	NA	*Endopterygota* sp. NZAC 03012222
ASV_566, ASV_1095	Insecta	Diptera	*Boletina*	*Boletina* sp. BOLD-2016

ASV_572, ASV_909, ASV_1187, ASV_3775	Arachnida	Araneae	NA	*Araneae* sp. NZAC 03010896
ASV_608	Arachnida	Trombidiformes	NA	*Tarsonemidae* sp. BOLD:ABV3249
ASV_619	Insecta	Lepidoptera	*Stigmella*	*Stigmella malella*
ASV_635	Insecta	Diptera	*Isomyia*	*Isomyia complantenna*
ASV_637	Insecta	Coleoptera	*Costelytra*	*Costelytra zealandica*
ASV_672, ASV_2537	Insecta	Lepidoptera	*Tingena*	*Tingena armigerella*
ASV_682	Arachnida	Sarcoptiformes	NA	*Sarcoptiformes* sp. BOLD:ACI7088
ASV_754, ASV_1863, ASV_4513, ASV_4730	Insecta	Diptera	NA	*Phoridae* sp.
ASV_778	Insecta	Diptera	NA	*Sarcophaginae* sp. BIOUG24445-B09
ASV_852	Insecta	Diptera	*Molophilus*	*Molophilus fergusonianus*
ASV_872, ASV_1908	Insecta	Trichoptera	*Oeconesus*	*Oeconesus maori*
ASV_889	Insecta	Trichoptera	*Triplectidina*	*Triplectidina oreolimnetes*
ASV_895	Insecta	Diptera	*Hydrotaea*	*Hydrotaea irritans*
ASV_902, ASV_1733	Insecta	Coleoptera	*Mitrastethus*	*Mitrastethus baridioides*
ASV_915	Insecta	Trichoptera	*Psilochorema*	*Psilochorema mimicum*
ASV_962	Arachnida	Sarcoptiformes	NA	*Acaridae* sp. BOLD:AAL8123
ASV_979	Arachnida	Sarcoptiformes	*Tyrophagus*	*Tyrophagus curvipenis*
ASV_1035	Arachnida	Sarcoptiformes	NA	*Sarcoptiformes* sp. BIOUG17145-F05
ASV_1052	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03012619
ASV_1148, ASV_1455, ASV_1930	Insecta	Diptera	NA	*Diptera* sp. NZAC 03010770
ASV_1238	Insecta	Ephemeroptera	*Zephlebia*	*Zephlebia pirongia*
ASV_1334	Insecta	Diptera	*Rhagoletis*	*Rhagoletis basiola*
ASV_1339	Arachnida	Sarcoptiformes	*Zetomimus*	*Zetomimus furcatus*
ASV_1404	Insecta	Lepidoptera	*Aenetus*	*Aenetus dulcis*
ASV_1508	Insecta	Diptera	*Tipula*	*Tipula arctica*
ASV_1556, ASV_1608	Insecta	Diptera	*Platypalpus*	*Platypalpus* sp. BARS
ASV_1575	Insecta	Lepidoptera	NA	*Lepidoptera* sp. BOLD:AAH5252
ASV_1603	Insecta	Lepidoptera	NA	*Noctuidae* sp. BOLD:AAM8658
ASV_1647	Insecta	Lepidoptera	*Chatamla*	*Chatamla flavescens*
ASV_1746	Arachnida	Araneae	NA	*Araneae* sp. NZAC 03013804
ASV_1776	Insecta	Diptera	*Agromyza*	Agromyza sp. JSDIP192-10
ASV_1778	Insecta	Diptera	*Leucophenga*	*Leucophenga* sp. HWC-2012i
ASV_1907	Insecta	Lepidoptera	*Forsterinaria*	*Forsterinaria rustica*

ASV_1962, ASV_6009	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03011148
ASV_1965	Arachnida	Trombidiformes	*Cunaxa*	*Cunaxa setirostris*
ASV_2298	Arachnida	Trombidiformes	NA	*Eupodidae* sp. BOLD:AAM8013
ASV_2615	Insecta	NA	NA	*Endopterygota* sp. NZAC 03012337
ASV_3174	Insecta	Lepidoptera	*Oidaematophorus*	*Oidaematophorus mathewianus*
ASV_3394	Arachnida	Sarcoptiformes	*Anachipteria*	*Anachipteria* sp. BOLD:AAM3565
ASV_4119	Arachnida	Sarcoptiformes	*Oribatella*	*Oribatella* sp. BOLD:ACI5304
ASV_4550	Insecta	Coleoptera	*Sharpius*	*Sharpius* sp. NZAC 03037669
ASV_4680	Insecta	Lepidoptera	*Atrytonopsis*	*Atrytonopsis hianna*
ASV_6001	Insecta	Diptera	NA	*Phoridae* sp. BOLD:AAP3611
ASV_8047	Insecta	Diptera	*Verticia*	*Verticia orientalis*
ASV_9394	Insecta	Diptera	*Diamesa*	*Diamesa bohemani*

Table S2.5. Complete clamtest analysis results for ASVs classified as specific to pre- and post-harvest. Taxonomic assignments include Class, Order, Genus, and Species where available. Multiple ASVs may correspond to the same taxonomic identification.

Pre-harvest				
ASV	**Class**	**Order**	**Genus**	**Species**
ASV_26, ASV_954	Insecta	Diptera	NA	*Psychodidae* sp.
ASV_32, ASV_210, ASV_325, ASV_576, ASV_788, ASV_1966, ASV_4353	Insecta	Lepidoptera	*Orocrambus*	*Orocrambus flexuosellus*
ASV_68, ASV_168	Insecta	Hemiptera	*Colobopyga*	*Colobopyga pritchardiae*
ASV_79	Insecta	Diptera	*Euhybus*	*Euhybus* sp. BIOUG14781-C02
ASV_112	Insecta	Siphonaptera	*Nosopsyllus*	*Nosopsyllus laeviceps*
ASV_130, ASV_212, ASV_1385	Arachnida	Mesostigmata	NA	*Parasitidae* sp. BIOUG24479-C11
ASV_153	Insecta	Hymenoptera	NA	*Hymenoptera* sp.
ASV_181, ASV_1526, ASV_2100	Insecta	Lepidoptera	*Toulgoetia*	*Toulgoetia cauteriata*
ASV_215	Arachnida	Trombidiformes	NA	*Trombidiformes* sp. BOLD:ABW1146
ASV_243, ASV_6505	Insecta	Diptera	*Rhamphomyia*	*Rhamphomyia helleni*
ASV_277	Insecta	Lepidoptera	NA	*Gracillariidae* sp. BOLD:ABV0573
ASV_279	Insecta	Diptera	*Tipula*	*Tipula furca*
ASV_320	Insecta	Lepidoptera	NA	*Noctuidae* sp.
ASV_329, ASV_1745	Insecta	Diptera	*Shannoniana*	*Shannoniana moralesi*
ASV_330, ASV_453	Insecta	Coleoptera	*Odontria*	Odontria sp.
ASV_343	Insecta	Lepidoptera	*Eudonia*	*Eudonia dinodes*
ASV_364	Insecta	Orthoptera	*Pachyrhamma*	*Pachyrhamma longipes*
ASV_386	Insecta	Hymenoptera	*Phytodietus*	*Phytodietus* sp. NZ
ASV_395	Insecta	Diptera	*Colocasiomyia*	*Colocasiomyia* sp. gr JJG-2023a
ASV_397	Insecta	Diptera	NA	*Diptera* sp. NZAC 03012179
ASV_400, ASV_1534	Insecta	Lepidoptera	*Scopula*	*Scopula rubraria*
ASV_429	Insecta	Neuroptera	*Wesmaelius*	*Wesmaelius subnebulosus*
ASV_440	Insecta	Diptera	NA	*Diptera* sp.
ASV_475	Insecta	Lepidoptera	NA	*Crambidae* sp. sc
ASV_504	Insecta	Lepidoptera	*Capua*	*Capua* sp. ANIC8

ASV_528, ASV_1017, ASV_1119	Insecta	Diptera	*Sympotthastia*	*Sympotthastia fulva*
ASV_557	Insecta	Diptera	*Protomiltogramma*	*Protomiltogramma cincta*
ASV_566, ASV_1220	Insecta	Diptera	*Boletina*	*Boletina* sp. BOLD-2016
ASV_612	Arachnida	Araneae	*Desis*	*Desis formidabilis*
ASV_626	Insecta	Diptera	NA	*Limoniidae* sp. sc
ASV_646	Insecta	Lepidoptera	*Scoparia*	*Scoparia aphrodes*
ASV_690	Arachnida	Sarcoptiformes	NA	*Sarcoptiformes* sp. BOLD:ACG2525
ASV_709, ASV_1178, ASV_2794	Malacostraca	Cumacea	*Campylaspis*	*Campylaspis sulcata*
ASV_715	Insecta	Lepidoptera	*Eutorna*	*Eutorna phaulocosma*
ASV_731, ASV_2176, ASV_2212, ASV_2230, ASV_10150	Insecta	Diptera	NA	*Diptera* sp. NZAC 03009179
ASV_741	Insecta	Coleoptera	*Cyloma*	*Cyloma guttulatus*
ASV_805	Insecta	Diptera	*Calliphora*	*Calliphora vicina*
ASV_853	Insecta	Diptera	*Culex*	*Culex pervigilans*
ASV_870	Insecta	Lepidoptera	*Mompha*	*Mompha* sp. BOLD:AAY6975
ASV_900	Insecta	Diptera	*Anopheles*	*Anopheles darlingi*
ASV_974	Arachnida	Sarcoptiformes	*Achipteria*	*Achipteria* sp. BOLD:AAF1479
ASV_1004	Arachnida	Trombidiformes	NA	*Trombidiformes* sp. BOLD:ABZ0007
ASV_1066, ASV_2575	Insecta	Coleoptera	NA	*Cerambycinae* sp. BOLD:AAQ0001
ASV_1071	Insecta	Lepidoptera	*Xanthorhoe*	*Xanthorhoe decoloraria*
ASV_1125	Insecta	Lepidoptera	*Peripyra*	*Peripyra sanguinipuncta*
ASV_1140	Insecta	Coleoptera	*Neorrhina*	*Neorrhina octopunctata*
ASV_1144	Insecta	Hymenoptera	*Tenthredo*	*Tenthredo* sp. 01gatineau
ASV_1147	Insecta	Lepidoptera	NA	*Lepidoptera* sp. sc
ASV_1153	Insecta	Diptera	NA	*Limosininae* sp. BIOUG23680-E01
ASV_1251	Insecta	Diptera	*Anopheles*	*Anopheles tessellatus*
ASV_1349	Insecta	Diptera	*Limnophyes*	*Limnophyes aagaardi*
ASV_1379	Insecta	Diptera	*Paraleucophenga*	*Paraleucophenga longiseta*
ASV_1401, ASV_2382	Insecta	Lepidoptera	*Acrobasis*	*Acrobasis demotella*
ASV_1444	Insecta	Diptera	*Melanostoma*	*Melanostoma mellinum*
ASV_1481	Insecta	Psocoptera	*Enderleinella*	*Enderleinella obsoleta*
ASV_1483	Insecta	NA	NA	*Mandibulata* sp. NZAC 03012118
ASV_1620	Insecta	Diptera	NA	*Mycetophilidae* sp. BOLD-2016
ASV_1692	Arachnida	Trombidiformes	NA	*Tarsonemidae* sp. BIOUG35077-G10

ASV_1726	Insecta	Diptera	NA	*Chironomidae* sp. BACAZ-118
ASV_1786, ASV_2575	Insecta	Coleoptera	*Anomala*	*Anomala* sp.
ASV_1800	Insecta	Coleoptera	*Torridincola*	*Torridincola rhodesica*
ASV_1823	Insecta	Diptera	*Leucophenga*	*Leucophenga cultella*
ASV_1869	Insecta	Diptera	NA	*Limoniidae* sp.
ASV_1944	Insecta	Diptera	*Prionocera*	*Prionocera turcica*
ASV_2050	Insecta	Coleoptera	*Arhopalus*	*Arhopalus* sp. UAM Ento 288332
ASV_2136	Insecta	Diptera	*Zygomyia*	*Zygomyia pictipennis*
ASV_2181	Insecta	Diptera	NA	*Ceratopogonidae* sp.
ASV_2186	Insecta	Lepidoptera	*Cryptaspasma*	*Cryptaspasma sordida*
ASV_2232, ASV_2937	Insecta	Lepidoptera	*Agrotis*	*Agrotis lasserrei*
ASV_2321	Arachnida	Sarcoptiformes	*Avenzoaria*	*Avenzoaria totani*
ASV_2322	Insecta	Lepidoptera	*Opogona*	*Opogona comptella*
ASV_2331	Insecta	Diptera	NA	*Dolichopodidae* sp.
ASV_2530	Insecta	Lepidoptera	*Ormetica*	*Ormetica sicilia*
ASV_2765	Insecta	Orthoptera	*Hemideina*	*Hemideina thoracica*
ASV_2963	Insecta	Lepidoptera	*Stigmella*	*Stigmella freyella*
ASV_2993	Insecta	Coleoptera	*Nicrophorus*	*Nicrophorus obscurus*
ASV_3021	Insecta	Diptera	*Simulium*	*Simulium ngaoense*
ASV_3039	Insecta	Lepidoptera	*Stathmopoda*	*Stathmopoda melanochra*
ASV_3079	Insecta	Coleoptera	*Stenolophus*	*Stenolophus* sp. BBCCM546-10
ASV_3080	Insecta	Diptera	NA	*Tipulidae* sp. BOLD:AAO3950
ASV_3182	Arachnida	Sarcoptiformes	NA	*Oppiidae* sp. MIONB076-10
ASV_3323	Insecta	Coleoptera	*Enochrus*	*Enochrus* (Hugoscottia) sp. IBE-AB88
ASV_3340	Insecta	Hemiptera	NA	*Hemiptera* sp. CNKOG015-14
ASV_3444	Insecta	Lepidoptera	*Operophtera*	*Operophtera bruceata*
ASV_3476	Hexanauplia	Calanoida	*Temora*	*Temora* sp.
ASV_3590	Insecta	Lepidoptera	*Agrotis*	*Agrotis ipsilon*
ASV_3750	Insecta	Diptera	*Boletina*	*Boletina kurilensis*
ASV_3755	Insecta	Diptera	*Ablabesmyia*	*Ablabesmyia* sp. TAS17.4.3
ASV_3777	Insecta	Lepidoptera	*Agriophara*	*Agriophara* sp. ANIC3
ASV_3922	Insecta	Diptera	*Anopheles*	*Anopheles galvaoi*
ASV_3989	Insecta	Diptera	*Phortica*	*Phortica symmetria*
ASV_4148	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03011178
ASV_4232	Insecta	Diptera	NA	*Cecidomyiidae* sp.
ASV_4328	Insecta	Lepidoptera	*Gymnobathra*	*Gymnobathra omphalota*
ASV_4448	Insecta	Diptera	NA	*Diptera* sp. NZAC 03013914
ASV_4672	Insecta	Lepidoptera	*Choristoneura*	*Choristoneura fractivittana*

ASV_4690	Insecta	Diptera	*Pselliophora*	*Pselliophora guangxiensis*
ASV_4759	Insecta	Coleoptera	*Prionoplus*	*Prionoplus reticularis*
ASV_4904	Insecta	Lepidoptera	*Hierodoris*	*Hierodoris atychioides*
ASV_5028	Insecta	Lepidoptera	*Barea*	*Barea* sp. ANIC27
ASV_5086	Arachnida	Trombidiformes	*Acalitus*	*Acalitus vaccinii*
ASV_5649	Insecta	Lepidoptera	*Capua*	*Capua dura*
ASV_6014	Arachnida	Sarcoptiformes	*Eueremaeus*	*Eueremaeus* sp. BOLD:ADI4824
ASV_6788	Insecta	Psocoptera	*Valenzuela*	*Valenzuela atricornis*
ASV_8060	Insecta	Coleoptera	NA	*Staphylinidae* sp.
Post-harvest				
ASV	**Class**	**Order**	**Genus**	**Species**
ASV_10	Insecta	Hemiptera	*Petrusa*	*Petrusa epilepsis*
ASV_12, ASV_3187	Insecta	Diptera	*Symplecta*	*Symplecta stictica*
ASV_13	Insecta	Lepidoptera	*Plutella*	*Plutella xylostella*
ASV_27, ASV_47	Insecta	Coleoptera	NA	*Coleoptera* sp. INDOBIOSYS-CCDB26091-D10
ASV_46, ASV_2536	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03013845
ASV_122	Insecta	Diptera	NA	*Dixidae* sp. BOLD-2016
ASV_192	Arachnida	Araneae	*Colaranea*	*Colaranea viriditas*
ASV_193, ASV_376, ASV_388, ASV_555, ASV_656, ASV_866, ASV_1307, ASV_2740	Insecta	Diptera	NA	*Tipulidae* sp. BOLD:AAF9030
ASV_239	Insecta	Diptera	*Cricotopus*	*Cricotopus metatibialis*
ASV_241, ASV_1479	Insecta	Lepidoptera	*Arichanna*	*Arichanna flavomacularia*
ASV_251	Insecta	Coleoptera	*Maladera*	*Maladera dubia*
ASV_267	Insecta	Diptera	*Exorista*	*Exorista civilis*
ASV_268	Insecta	Diptera	*Culex*	*Culex rotoruae*
ASV_270	Insecta	Diptera	*Cheilosia*	*Cheilosia matsumurana*
ASV_319, ASV_761	Insecta	Diptera	*Limnophyes*	*Limnophyes minimus*
ASV_328	Arachnida	Sarcoptiformes	*Lepidoglyphus*	*Lepidoglyphus destructor*
ASV_373	Insecta	Diptera	NA	*Diptera* sp. NZAC 03013255
ASV_401, ASV_672	Insecta	Lepidoptera	*Tingena*	*Tingena armigerella*
ASV_404	Insecta	Lepidoptera	*Opogona*	*Opogona omoscopa*
ASV_487	Insecta	Diptera	*Oxysarcodexia*	*Oxysarcodexia terminalis*

ASV_517, ASV_8115	Insecta	Diptera	NA	*Orthocladiinae* sp. BOLD-2016
ASV_536	Insecta	NA	NA	*Endopterygota* sp. NZAC 03012222
ASV_562, ASV_1336, ASV_2492	Insecta	Diptera	NA	*Mycetophilidae* sp. AgR-A5
ASV_572, ASV_909, ASV_1187, ASV_3775	Arachnida	Araneae	NA	*Araneae* sp. NZAC 03010896
ASV_608	Arachnida	Trombidiformes	NA	*Tarsonemidae* sp. BOLD:ABV3249
ASV_619	Insecta	Lepidoptera	*Stigmella*	*Stigmella malella*
ASV_635	Insecta	Diptera	*Isomyia*	*Isomyia complantenna*
ASV_637	Insecta	Coleoptera	*Costelytra*	*Costelytra zealandica*
ASV_643	Insecta	Diptera	NA	*Sciaridae* sp.
ASV_644	Malacostraca	Decapoda	NA	*Anomura* sp. LPdivOTU103
ASV_682	Arachnida	Sarcoptiformes	NA	*Sarcoptiformes* sp. BOLD:ACI7088
ASV_738, ASV_815	Insecta	Diptera	*Nephrotoma*	*Nephrotoma submaculosa*
ASV_754, ASV_1863, ASV_4513, ASV_4730	Insecta	Diptera	NA	*Phoridae* sp.
ASV_778	Insecta	Diptera	NA	*Sarcophaginae* sp. BIOUG24445-B09
ASV_843	Arachnida	Sarcoptiformes	*Phalacrodectes*	*Phalacrodectes gaudi*
ASV_852	Insecta	Diptera	*Molophilus*	*Molophilus fergusonianus*
ASV_872, ASV_1908	Insecta	Trichoptera	*Oeconesus*	*Oeconesus maori*
ASV_889	Insecta	Trichoptera	*Triplectidina*	*Triplectidina oreolimnetes*
ASV_895	Insecta	Diptera	*Hydrotaea*	*Hydrotaea irritans*
ASV_915	Insecta	Trichoptera	*Psilochorema*	*Psilochorema mimicum*
ASV_939	Insecta	Hymenoptera	*Diaeretiella*	*Diaeretiella rapae*
ASV_958, ASV_1323, ASV_2135	Insecta	Lepidoptera	*Barea*	*Barea meridarcha*
ASV_962	Arachnida	Sarcoptiformes	NA	*Acaridae* sp. BOLD:AAL8123
ASV_979	Arachnida	Sarcoptiformes	*Tyrophagus*	*Tyrophagus curvipenis*
ASV_1052	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03012619
ASV_1148, ASV_1455, ASV_1930, ASV_3732	Insecta	Diptera	NA	*Diptera* sp. NZAC 03010770
ASV_1194	Insecta	Thysanoptera	*Haplothrips*	*Haplothrips tenuipennis*
ASV_1280	Insecta	Coleoptera	*Quedius*	*Quedius fuliginosus*
ASV_1334	Insecta	Diptera	*Rhagoletis*	*Rhagoletis basiola*
ASV_1339	Arachnida	Sarcoptiformes	*Zetomimus*	*Zetomimus furcatus*

ASV_1404	Insecta	Lepidoptera	*Aenetus*	*Aenetus dulcis*
ASV_1556, ASV_1608	Insecta	Diptera	*Platypalpus*	*Platypalpus* sp. BARS
ASV_1575	Insecta	Lepidoptera	NA	*Lepidoptera* sp. BOLD:AAH5252
ASV_1603	Insecta	Lepidoptera	NA	*Noctuidae* sp. BOLD:AAM8658
ASV_1625	Arachnida	Araneae	*Pandava*	*Pandava sarasvati*
ASV_1647	Insecta	Lepidoptera	*Chatamla*	*Chatamla flavescens*
ASV_1691	Insecta	Diptera	NA	*Cecidomyiidae* sp. BOLD:ACA9586
ASV_1733	Insecta	Coleoptera	*Mitrastethus*	*Mitrastethus baridioides*
ASV_1778	Insecta	Diptera	*Leucophenga*	*Leucophenga* sp. HWC-2012i
ASV_1907	Insecta	Lepidoptera	*Forsterinaria*	*Forsterinaria rustica*
ASV_1962, ASV_6009	Insecta	Lepidoptera	NA	*Lepidoptera* sp. NZAC 03011148
ASV_1965	Arachnida	Trombidiformes	*Cunaxa*	*Cunaxa setirostris*
ASV_2016	Insecta	Coleoptera	*Sericoderus*	*Sericoderus lateralis*
ASV_2036	Insecta	Psocoptera	*Ectopsocus*	*Ectopsocus* sp.
ASV_2145	Insecta	Lepidoptera	*Izatha*	*Izatha peroneanella*
ASV_2298	Arachnida	Trombidiformes	NA	*Eupodidae* sp. BOLD:AAM8013
ASV_2615	Insecta	NA	NA	*Endopterygota* sp. NZAC 03012337
ASV_2686	Insecta	Coleoptera	*Uloma*	*Uloma tenebrionoides*
ASV_2755	Arachnida	Mesostigmata	*Amblyseius*	*Amblyseius adhatodae*
ASV_3069	Arachnida	Araneae	*Australaena*	*Australaena zimmermani*
ASV_3238	Insecta	Lepidoptera	NA	*Lepidoptera* sp. BOLD:AAC7093
ASV_3300	Insecta	Lepidoptera	*Carposina*	*Carposina eriphylla*
ASV_3336	Insecta	Lepidoptera	*Conoeca*	*Conoeca guildingi*
ASV_4074	Insecta	Lepidoptera	NA	*Lepidoptera* sp. BOLD:AAA1106
ASV_4786	Insecta	Lepidoptera	*Metaphrastis*	*Metaphrastis acrochalca*
ASV_6260	Insecta	Coleoptera	NA	*Staphylinidae* sp. INDOBIOSYS-CCBD26991-D01
ASV_6723	Insecta	Coleoptera	*Elaphropus*	*Elaphropus parvulus*
ASV_7177	Arachnida	Sarcoptiformes	NA	*Scheloribatidae* sp. BIOUG24577-B12
ASV_7381	Insecta	Hemiptera	*Aspidiotus*	*Aspidiotus nerii*
ASV_8368	Arachnida	Trombidiformes	NA	*Tarsonemidae* sp. BOLD:ADE1624
ASV_8382	Insecta	Coleoptera	*Agrypnus*	*Agrypnus scutellaris*
ASV_8693	Arachnida	Araneae	*Anelosimus*	*Anelosimus wallacei*
ASV_9372	Insecta	Hemiptera	*Scolops*	*Scolops viridis*

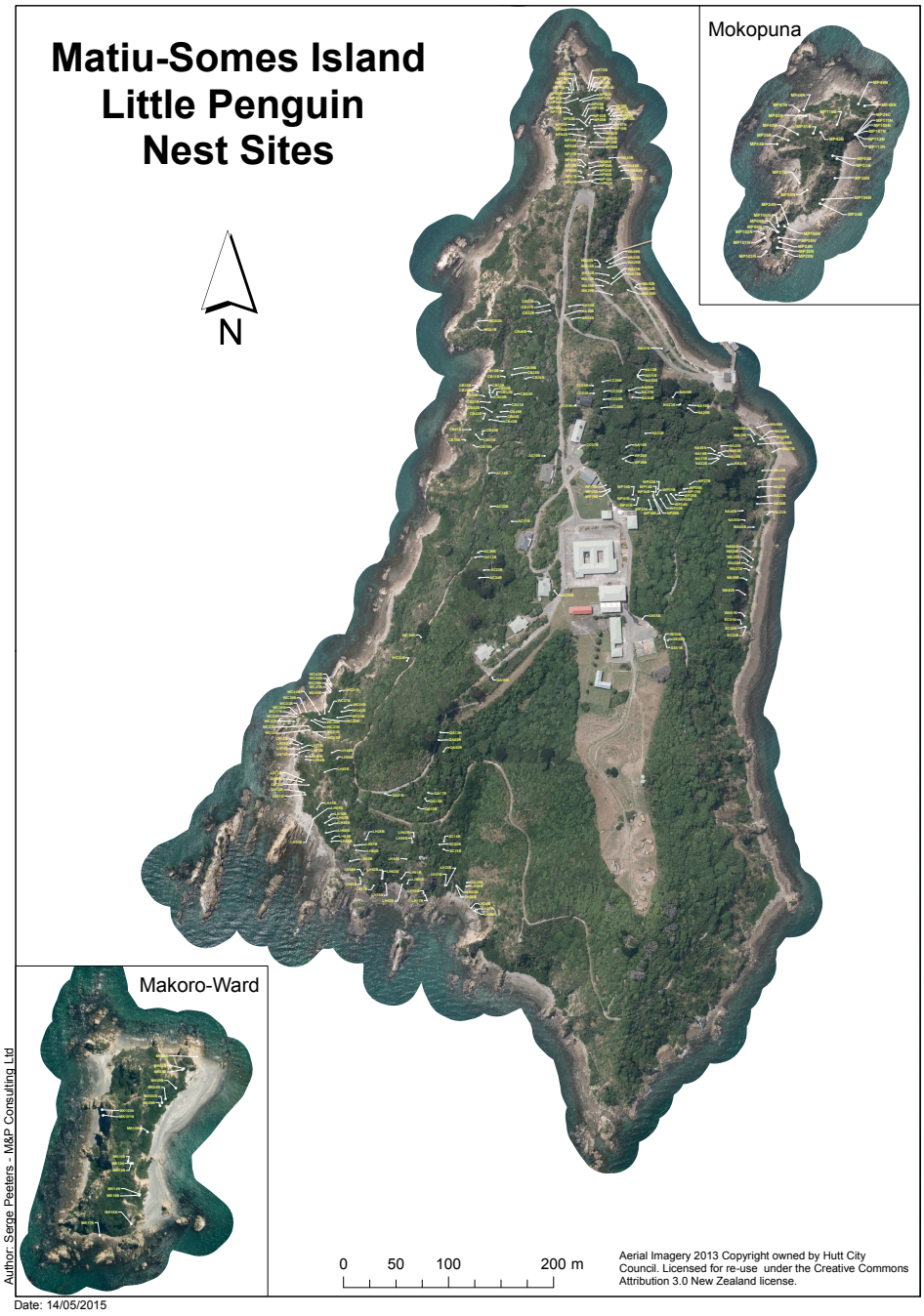

Figure S3.1. Map of Matiu-Somes Island and surrounding islets (Mokopuna and Makoro-Ward) showing the distribution of little penguin (*Eudyptula minor*) nest sites. Yellow markers indicate recorded nest locations. Aerial imagery sourced from Hutt City Council (2013) and licensed under a Creative Commons Attribution 3.0 New Zealand license.

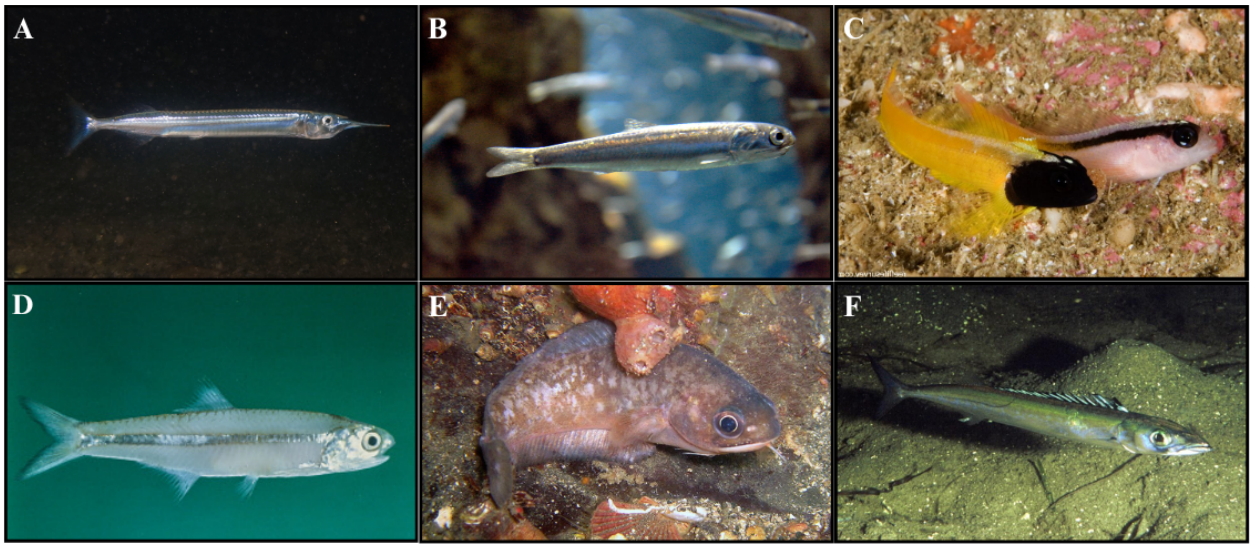

Figure S3.2. Potential diet species of *Eudyptula minor* identified through eDNA analysis: **A** *Hyporhamphus ihi* (garfish, piper, takeke) (Colmer, 2023); **B** *Engraulis japonicus* (Japanese anchovy) (Kingfisher, 2014); **C** *Fosterygion flavonigrum* (yellow-black triplefin) (Green, 2025); **D** *Stolephorus* sp. (anchovy species) (Randall, 1997); **E** *Pseudophycis bachus* (red codling) (White, 2010); **F** *Thyrsites atun* (snoek, barracouta) (Edgar, 2008). Images not to scale.

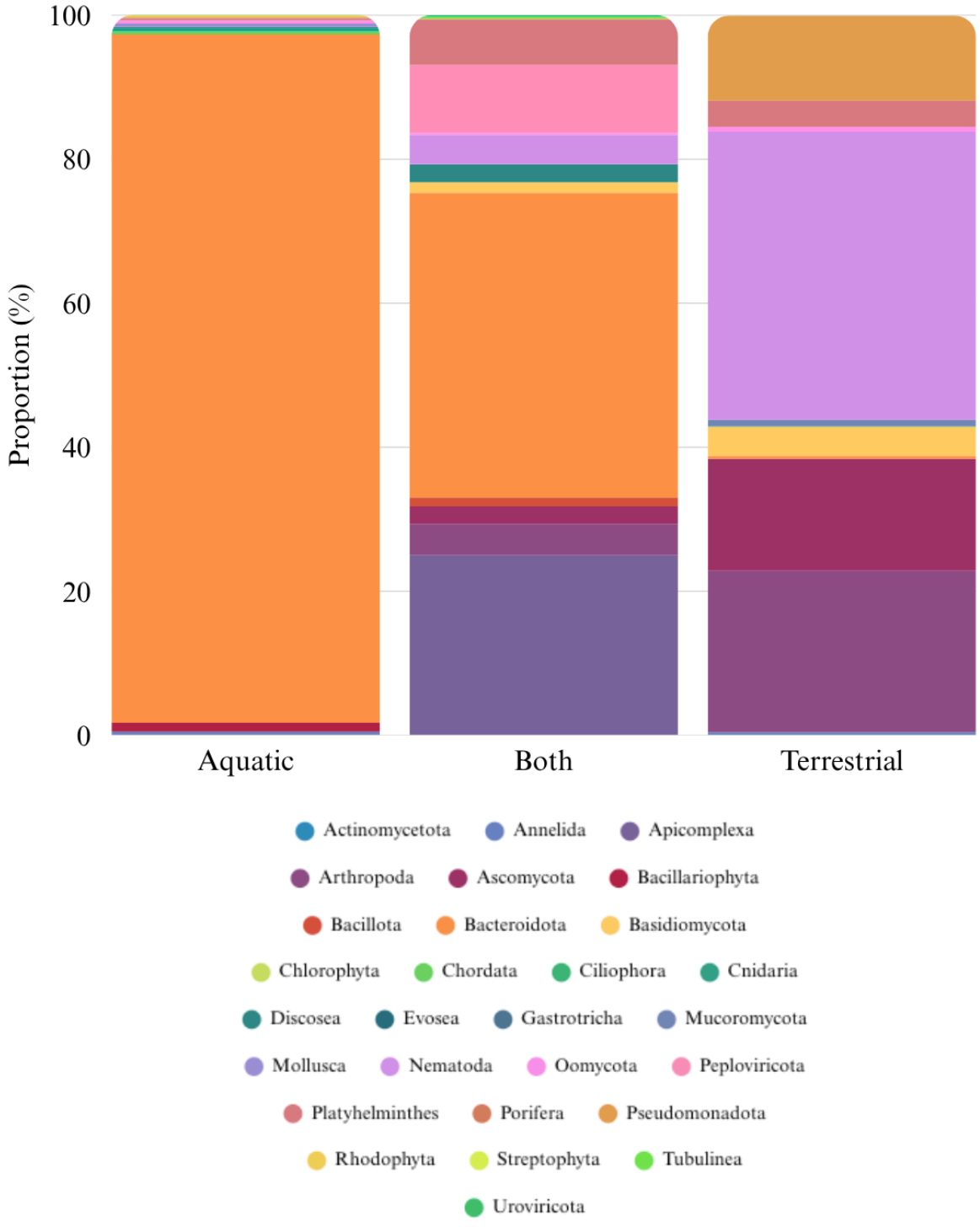

Figure S3.3. Relative abundance of ASVs at the phylum level across three habitat types: Aquatic, Both (organisms inhabiting both terrestrial and aquatic habitats), and Terrestrial. Bars represent the percentage contribution of each phylum to the total ASV reads within each habitat category, corresponding to the values presented in Table 3.2. Colours represent individual phyla.

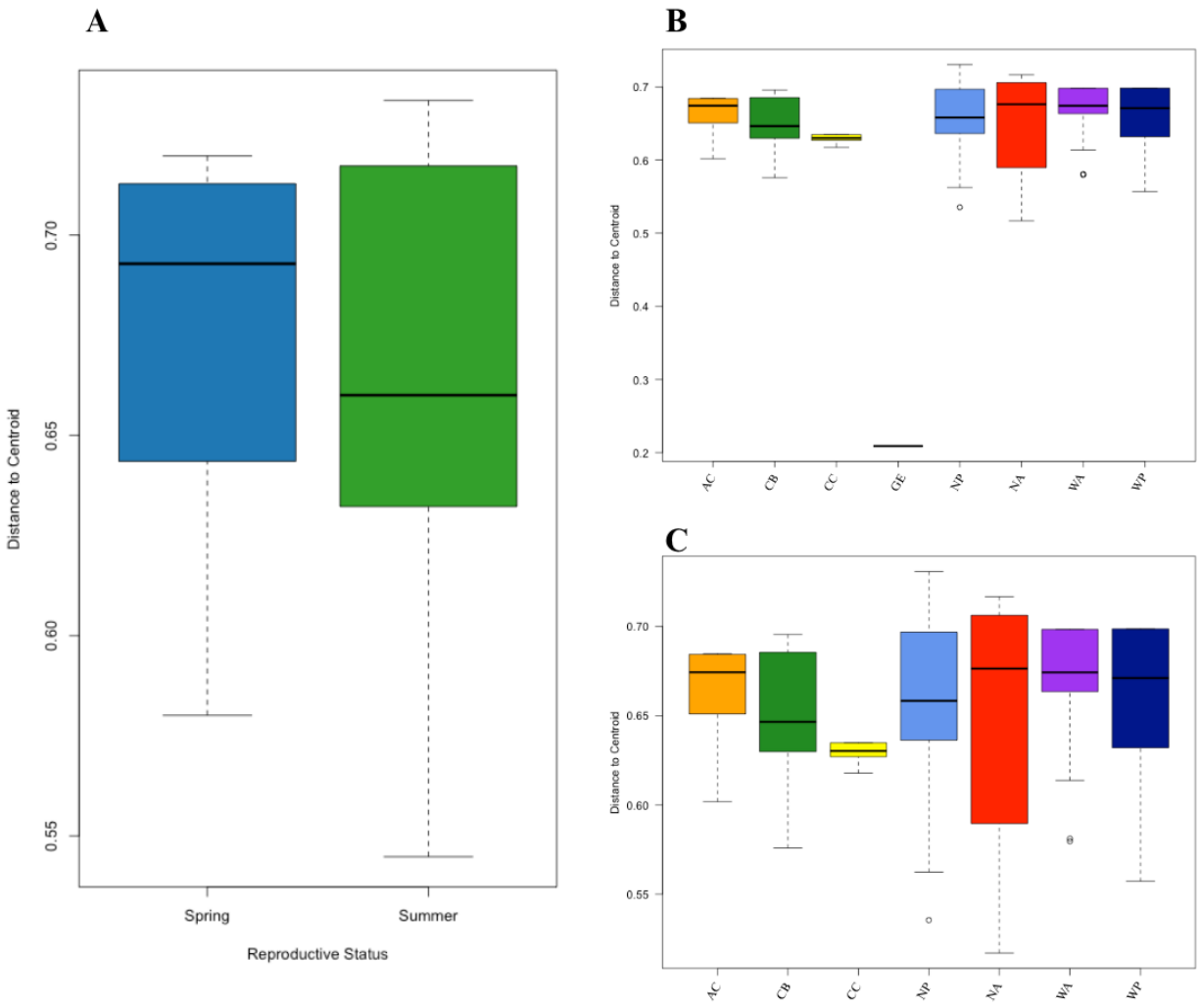

Figure S3.4. Multivariate homogeneity of group dispersions (betadisper analysis) based on COI amplicon data, grouped by location: **A** seasonal, **B** Includes all locations, including Gun Emplacement (GE); **B** Shows results with GE excluded due to insufficient replication (n < 3). Boxplots represent the distance of each sample to its group centroid; bold horizontal lines indicate group medians.

Table S3.1. Metadata for *Eudyptula minor* faecal and environmental composite samples, including sample ID, sample name, season, nest location, and island orientation, where available.

Sample ID	Sample name	Season	Location	Island orientation
UOW_25_04_001	CC04_SP	Spring	Caretakers Cottage (CC)	East
UOW_25_04_003	CC07_SP	Spring	Caretakers Cottage (CC)	East
UOW_25_04_004	GE01_SP	Spring	Gun Emplacement (GE)	East
UOW_25_04_005	GE05_SP	Spring	Gun Emplacement (GE)	East
UOW_25_04_006	NA18_SP	Spring	Nursery (NA)	East
UOW_25_04_007	NA29_SP	Spring	Nursery (NA)	East
UOW_25_04_008	NA32_SP	Spring	Nursery (NA)	East
UOW_25_04_010	NA50_SP	Spring	Nursery (NA)	East
UOW_25_04_011	NA55_SP	Spring	Nursery (NA)	East
UOW_25_04_012	NP01_SP	Spring	North Point (NP)	East
UOW_25_04_013	NP02_SP	Spring	North Point (NP)	East
UOW_25_04_014	NP07_SP	Spring	North Point (NP)	East
UOW_25_04_015	NP10_SP	Spring	North Point (NP)	East
UOW_25_04_016	NP15_SP	Spring	North Point (NP)	East
UOW_25_04_017	NP23_SP	Spring	North Point (NP)	East
UOW_25_04_018	NP61_SP	Spring	North Point (NP)	East
UOW_25_04_019	NP66_SP	Spring	North Point (NP)	East
UOW_25_04_020	NP80_SP	Spring	North Point (NP)	East
UOW_25_04_021	WA13_SP	Spring	Wharf (WA)	East
UOW_25_04_022	WA29_SP	Spring	Wharf (WA)	East
UOW_25_04_023	WA60_SP	Spring	Wharf (WA)	East
UOW_25_04_024	WP16_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_025	WP17_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_026	WP19_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_027	WP23_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_028	WP24_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_029	WP28_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_031	AC10_SP	Spring	Accommodation (AC)	West
UOW_25_04_032	AC23_SP	Spring	Accommodation (AC)	West
UOW_25_04_033	NP78_SP	Spring	North Point (NP)	East
UOW_25_04_034	NP36_SP	Spring	North Point (NP)	East
UOW_25_04_035	AC11_SP	Spring	Accommodation (AC)	West
UOW_25_04_036	CB11_SP	Spring	Cable Bay (CB)	West
UOW_25_04_037	CB32_SP	Spring	Cable Bay (CB)	West
UOW_25_04_038	AC16_SP	Spring	Accommodation (AC)	West
UOW_25_04_039	AC09_SP	Spring	Accommodation (AC)	West
UOW_25_04_040	CB31_SP	Spring	Cable Bay (CB)	West

UOW_25_04_041	CB23_SP	Spring	Cable Bay (CB)	West
UOW_25_04_042	CB42_SP	Spring	Cable Bay (CB)	West
UOW_25_04_043	CB26_SP	Spring	Cable Bay (CB)	West
UOW_25_04_044	NP38_SP	Spring	North Point (NP)	East
UOW_25_04_045	NP58_SP	Spring	North Point (NP)	East
UOW_25_04_047	NA27_SP	Spring	Nursery (NA)	East
UOW_25_04_048	NA11_SP	Spring	Nursery (NA)	East
UOW_25_04_049	WA54A_SP	Spring	Wharf (WA)	East
UOW_25_04_050	WA61_SP	Spring	Wharf (WA)	East
UOW_25_04_051	WA65_SP	Spring	Wharf (WA)	East
UOW_25_04_052	WP04_SP	Spring	Workshop Paddock (WP)	East
UOW_25_04_053	WA22_SP	Spring	Wharf (WA)	East
UOW_25_04_055	WA54B_SP	Spring	Wharf (WA)	East
UOW_25_04_056	WA21_SP	Spring	Wharf (WA)	East
UOW_25_04_057	CB32_SU	Summer	Cable Bay (CB)	West
UOW_25_04_058	WP28_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_059	CB23_SU	Summer	Cable Bay (CB)	West
UOW_25_04_060	NP15_SU	Summer	North Point (NP)	East
UOW_25_04_061	NP07_SU	Summer	North Point (NP)	East
UOW_25_04_062	NP10_SU	Summer	North Point (NP)	East
UOW_25_04_063	NP80_SU	Summer	North Point (NP)	East
UOW_25_04_064	WP16_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_065	CB42_SU	Summer	Cable Bay (CB)	West
UOW_25_04_066	NP23_SU	Summer	North Point (NP)	East
UOW_25_04_067	CC05_SU	Summer	Caretakers Cottage (CC)	East
UOW_25_04_068	AC16_SU	Summer	Accommodation (AC)	West
UOW_25_04_069	WA27_SU	Summer	Wharf (WA)	East
UOW_25_04_070	NA55_SU	Summer	Nursery (NA)	East
UOW_25_04_071	NA32_SU	Summer	Nursery (NA)	East
UOW_25_04_072	WA54_SU	Summer	Wharf (WA)	East
UOW_25_04_073	NP02_SU	Summer	North Point (NP)	East
UOW_25_04_074	WP19_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_075	WP17_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_076	WA22_SU	Summer	Wharf (WA)	East
UOW_25_04_077	CC07_SU	Summer	Caretakers Cottage (CC)	East
UOW_25_04_078	WP04_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_079	AC23_SU	Summer	Accommodation (AC)	West
UOW_25_04_080	AC11_SU	Summer	Accommodation (AC)	West
UOW_25_04_082	WP11_SU	Summer	Nursery (NA)	East

UOW_25_04_083	CB34_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_081	NA18_SU	Summer	Cable Bay (CB)	West
UOW_25_04_084	CC04_SU	Summer	Caretakers Cottage (CC)	East
UOW_25_04_085	NP66_SU	Summer	North Point (NP)	East
UOW_25_04_086	WA60_SU	Summer	Wharf (WA)	East
UOW_25_04_087	NA37_SU	Summer	Nursery (NA)	East
UOW_25_04_088	NP61_SU	Summer	North Point (NP)	East
UOW_25_04_089	AC10_SU	Summer	Accommodation (AC)	West
UOW_25_04_090	NP01_SU	Summer	North Point (NP)	East
UOW_25_04_091	WA29_SU	Summer	Wharf (WA)	East
UOW_25_04_092	WA61_SU	Summer	Wharf (WA)	East
UOW_25_04_093	WP23_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_094	WP21_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_095	AC09_SU	Summer	Accommodation (AC)	West
UOW_25_04_096	NP58_SU	Summer	North Point (NP)	East
UOW_25_04_187	NA29_SU	Summer	Nursery (NA)	East
UOW_25_04_188	NP36_SU	Summer	North Point (NP)	East
UOW_25_04_189	CB11_SU	Summer	Cable Bay (CB)	West
UOW_25_04_190	WP25_SU	Summer	Workshop Paddock (WP)	East
UOW_25_04_191	CB42_SU	Summer	Cable Bay (CB)	West
UOW_25_01_030	Extraction control	NA	NA	NA
UOW_25_01_046	PCR control	NA	NA	NA

Table S3.2. Per-amplicon sequencing statistics for each sample, including the number of raw, filtered, denoised (F, R; for forward and reverse reads, respectively), merged, and non-chimera reads.

Sample	Raw	Filtered	Denoised (F)	Denoised (R)	Merged	Non-chimera
UOW-25-04-001	130866	106004	105980	105984	104003	104003
UOW-25-04-003	147808	118949	118764	118623	116639	116610
UOW-25-04-004	99255	80375	80277	80274	79332	79332
UOW-25-04-005	142034	114678	114550	114536	112951	112951
UOW-25-04-006	114643	92812	92742	92548	90018	90018
UOW-25-04-007	105375	85600	85516	85546	85020	84925
UOW-25-04-008	160379	130314	130174	129990	127317	127317
UOW-25-04-010	161218	129852	129316	129694	127304	124073
UOW-25-04-011	151035	121893	121727	121837	119880	119880
UOW-25-04-012	188099	151586	151201	151118	148118	148118
UOW-25-04-013	56601	45454	45439	45436	44846	44846
UOW-25-04-014	87441	70891	70670	70777	69654	69654
UOW-25-04-015	117737	95332	95163	95206	94318	94313
UOW-25-04-016	66290	53495	53400	53424	52677	52677
UOW-25-04-017	117968	95540	95471	95497	94014	94014
UOW-25-04-018	120068	97385	97289	97115	95653	95653
UOW-25-04-019	88291	71580	71361	71443	70276	70276
UOW-25-04-020	169637	137118	136986	137002	134641	134641
UOW-25-04-021	110176	88620	88296	88414	87857	87857
UOW-25-04-022	272047	219633	219448	219063	210278	210278
UOW-25-04-023	154630	125196	124357	124692	123247	123228
UOW-25-04-024	227793	184237	184135	183373	178815	178815
UOW-25-04-025	11	7	6	7	0	0
UOW-25-04-026	120136	97072	97037	96786	96174	96174
UOW-25-04-027	95928	76206	75838	75971	75405	75402
UOW-25-04-028	60589	49256	48934	49040	48452	48386
UOW-25-04-029	26872	21649	21546	21547	20045	20002
UOW-25-04-030	10340	8237	8200	8234	7971	7935
UOW-25-04-031	139735	113374	112946	112967	111445	108217
UOW-25-04-032	124907	101182	100919	101059	99599	99598
UOW-25-04-033	121471	96780	96574	96538	95120	94873
UOW-25-04-034	206319	166443	166241	166353	162885	162669
UOW-25-04-035	147472	119358	118797	119069	116587	116587
UOW-25-04-036	155323	125065	124745	124947	122659	122659
UOW-25-04-037	138292	111526	111444	111390	109971	109971
UOW-25-04-038	178206	143547	143112	143239	140860	140763
UOW-25-04-039	103983	83793	83603	83618	82220	82220

UOW-25-04-040	129946	105060	104685	104805	103995	102955
UOW-25-04-041	152396	122969	122882	122931	120485	120485
UOW-25-04-042	131578	106621	106122	106335	104613	104536
UOW-25-04-043	112339	90735	90624	90395	89012	89012
UOW-25-04-044	131754	106303	105633	106021	104391	104324
UOW-25-04-045	120921	97662	97333	97127	96242	96242
UOW-25-04-046	95641	75419	75360	74474	52563	48091
UOW-25-04-047	165602	134101	133864	133929	131156	131156
UOW-25-04-048	141702	113766	113184	113162	111202	111195
UOW-25-04-049	128704	104466	103853	103996	102974	102935
UOW-25-04-050	123065	98639	98141	98351	96799	96708
UOW-25-04-051	191323	155226	152812	154147	149989	149775
UOW-25-04-052	42	37	21	35	21	21
UOW-25-04-053	2	2	1	1	0	0
UOW-25-04-055	809	621	579	617	577	577
UOW-25-04-056	26397	21465	21397	21407	20365	20365
UOW-25-04-057	144419	116895	116481	116415	115523	115229
UOW-25-04-058	72539	57474	57185	56978	27263	26134
UOW-25-04-059	17691	13844	12403	13394	0	0
UOW-25-04-060	145621	117746	117067	117345	110932	110906
UOW-25-04-061	24533	19579	19292	19401	15760	14799
UOW-25-04-062	139849	113476	113113	113201	109824	107563
UOW-25-04-063	98718	79837	79593	79705	78561	78449
UOW-25-04-064	76530	61935	61677	61706	60657	60657
UOW-25-04-065	94250	76880	75266	76109	72470	71846
UOW-25-04-066	127424	102689	102557	102631	100937	100937
UOW-25-04-067	117338	94020	94004	93967	92102	92102
UOW-25-04-068	196429	159661	158390	159165	155991	154972
UOW-25-04-069	75666	58844	55712	58032	20324	15208
UOW-25-04-070	151430	118512	117624	117189	44704	21197
UOW-25-04-071	6362	5042	4993	4988	3577	3577
UOW-25-04-072	112361	88106	87759	87329	61013	56177
UOW-25-04-073	123255	98985	97947	98951	95266	95222
UOW-25-04-074	116596	93831	93758	93798	92449	92449
UOW-25-04-075	1959	1506	1496	1466	548	548
UOW-25-04-076	74521	58116	57285	57262	8592	3267
UOW-25-04-077	99012	79880	79785	79801	78596	78596
UOW-25-04-078	73845	57567	56531	56673	9478	3073
UOW-25-04-079	89508	71852	71809	71809	70705	70705

UOW-25-04-080	19288	14916	14645	14696	5872	3610
UOW-25-04-081	135570	107775	105698	107358	86991	85628
UOW-25-04-082	96418	77702	77516	77469	76238	76236
UOW-25-04-083	942	733	653	694	0	0
UOW-25-04-084	107819	87277	87228	87060	85863	85863
UOW-25-04-085	137792	110702	109458	110512	98157	94654
UOW-25-04-086	112450	90722	90411	90618	89411	89411
UOW-25-04-087	142204	114763	113443	114252	109039	109034
UOW-25-04-088	111665	90309	88369	89952	83286	82934
UOW-25-04-089	137374	111908	111308	111513	107848	106893
UOW-25-04-090	167103	135348	135115	134929	132105	132105
UOW-25-04-091	659	492	182	448	179	179
UOW-25-04-092	119508	97140	96948	96901	95880	95634
UOW-25-04-093	109377	84611	82610	83491	35535	11953
UOW-25-04-094	166866	135016	134397	134677	127729	127729
UOW-25-04-095	71802	55023	54295	54177	11545	3688
UOW-25-04-096	128193	104051	103327	103323	98765	98748
UOW-25-04-187	1296	1047	1045	1042	862	862
UOW-25-04-188	18017	14442	13555	14373	12112	12061
UOW-25-04-189	121785	97282	96631	96847	90352	90284
UOW-25-04-190	133421	107804	107706	107588	105835	105736
UOW-25-04-191	131558	105645	104410	105442	97368	95634

Table S3.3. Abundance of phylum-level specialist species counts across four sampling categories (spring, summer, east, and west). Values represent the number of specialist species counts observed per phylum, with column totals indicating seasonal or spatial variation.

Phylum	Spring species	Summer species	East species	West species
Annelida	1	2	0	0
Apicomplexa	0	1	0	1
Arthropoda	60	49	34	31
Ascomycota	60	31	38	29
Bacillariophyta	1	5	0	4
Bacillota	0	6	1	2
Bacteroidota	54	15	24	15
Basidiomycota	10	9	8	10
Chlorophyta	2	1	0	0
Chordata	5	4	3	4
Ciliophora	0	1	0	1
Cnidaria	4	1	1	0
Discosea	11	5	7	4
Evosea	4	1	3	1
Gastrotricha	0	1	0	1
Mollusca	2	0	3	0
Mucoromycota	1	2	3	0
Nematoda	11	4	9	1
Oomycota	15	3	6	2
Platyhelminthes	5	2	2	3
Porifera	3	0	3	0
Pseudomonadota	4	5	1	7
Rhodophyta	3	3	1	1
Rotifera	2	6	2	5
Streptophyta	1	0	0	0
Tubulinea	3	0	2	0
Uroviricota	1	0	1	0
Total	**263**	**157**	**152**	**122**

Table S3.4. Complete clamtest analysis results for ASVs classified as specific to spring and summer seasons. Taxonomic assignments include phylum, class, genus, and species, where available. Multiple ASVs may correspond to the same taxonomic identification, as indicated.

Spring				
ASV	**Phylum**	**Class**	**Genus**	**Species**
ASV_15 ASV_300 ASV_2647	Ascomycota	Dothideomycetes	*Corynespora*	*Corynespora cassiicola*
ASV_26 ASV_1199	Basidiomycota	Agaricomycetes	*Fomitopsis*	*Fomitopsis palustris*
ASV_34 ASV_317 ASV_718	Arthropoda	Collembola	*Entomobrya*	*Entomobrya* sp. HBNA852 3
ASV_39 ASV_93 ASV_753 ASV_770 ASV_1024 ASV_3212	Ascomycota	Sordariomycetes	*Simplicillium*	*Simplicillium lamellicola*
ASV_41 ASV_346 ASV_1028 ASV_1118 ASV_2003 ASV_2011 ASV_2322 ASV_3552 ASV_4633	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum fructicola*
ASV_42 ASV_1928 ASV_2106	Platyhelminthes	Cestoda	NA	*Tetrabothriidae* sp. BOLD:AAM7874
ASV_44 ASV_112	Oomycota	NA	*Globisporangium*	*Globisporangium cystogenes*
ASV_45 ASV_856 ASV_961 ASV_993 ASV_1457	Bacteroidota	Sphingobacteriia	*Sphingobacterium*	*Sphingobacterium* sp. ML3W
ASV_46	Nematoda	Chromadorea	*Adineta*	*Adineta* sp. MX.1.1
ASV_49	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium inviolabile*
ASV_76	Arthropoda	Elardia	*Cucurbitella*	*Cucurbitella mespiliformis*
ASV_81 ASV_110 ASV_140 ASV_1141 ASV_1954 ASV_1977 ASV_3144	Mucoromycota	Mucoromycetes	*Lichtheimia*	*Lichtheimia hongkongensis*
ASV_94	Nematoda	Chromadorea	*Koerneria*	*Koerneria* sp. RS1982
ASV_98	Nematoda	Chromadorea	*Teratorhabditis*	*Teratorhabditis synpapillata*
ASV_136	Ascomycota	Eurotiomycetes	*Exophiala*	*Exophiala lecanii-corni*

ASV_141 ASV_731	Ascomycota	Leotiomycetes	*Tetracladium*	*Tetracladium breve*
ASV_142 ASV_562 ASV_675 ASV_692	Arthropoda	Insecta	*Xestocephalus*	*Xestocephalus desertorum*
ASV_149	Porifera	Demospongiae	*Cyamon*	*Cyamon neon*
ASV_159	Ascomycota	Sordariomycetes	*Fusarium*	*Fusarium delphinoides*
ASV_173 ASV_526 ASV_1565 ASV_3112	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. CFS9
ASV_178	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium alkalisoli*
ASV_206 ASV_2156	Nematoda	Chromadorea	*Parapristionchus*	*Parapristionchus giblindavisi*
ASV_222 ASV_1069 ASV_1244 ASV_2286 ASV_2873 ASV_3039 ASV_3113 ASV_3717	Ascomycota	Dothideomycetes	*Aureobasidium*	*Aureobasidium pullulans*
ASV_227	Ascomycota	Sordariomycetes	*Sporothrix*	*Sporothrix schenckii*
ASV_252 ASV_2037 ASV_2066 ASV_2727 ASV_2798 ASV_4082	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium flavipigmentatum*
ASV_257 ASV_1283	Ascomycota	Eurotiomycetes	*Arachnomyces*	*Arachnomyces peruvianus*
ASV_278	Arthropoda	Arachnida	*Miratemnus*	*Miratemnus* sp. JM-2NANA8
ASV_283 ASV_789	Oomycota	NA	*Globisporangium*	*Globisporangium kandovanense*
ASV_291	Chordata	Actinopteri	*Hyporhamphus*	*Hyporhamphus ihi*
ASV_296	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Candidatus Pedobacter colombiensis*
ASV_304	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium sangjuense*
ASV_357	Discosea	Flabellinia	*Vannella*	*Vannella samoroda*
ASV_372	Arthropoda	Arachnida	NA	*Ereynetidae* sp. BOLD:ABV186NA
ASV_375	Arthropoda	Insecta	*Boletina*	*Boletina* sp. BOLD:AAVNA669
ASV_398 ASV_672 ASV_3570	Arthropoda	Insecta	*Mimeoma*	*Mimeoma maculata*
ASV_413 ASV_1951	Basidiomycota	Agaricomycetes	*Russula*	*Russula virescens*
ASV_419 ASV_821 ASV_1217	Arthropoda	Insecta	*Culex*	*Culex erraticus*

ASV_493 ASV_3569 ASV_3597	Ascomycota	Dothideomycetes	*Periconia*	*Periconia digitata*
ASV_501	Discosea	Flabellinia	*Vannella*	*Vannella pentlandii*
ASV_516	Ascomycota	Leotiomycetes	*Blumeria*	*Blumeria graminis*
ASV_519	Bacteroidota	Flavobacteriia	*Winogradskyella*	*Winogradskyella* sp. HaHa 3 26
ASV_598	Chordata	Actinopteri	*Engraulis*	*Engraulis japonicus*
ASV_609	Bacteroidota	Flavobacteriia	*Lacinutrix*	*Lacinutrix* sp. WUR7
ASV_615	Arthropoda	Arachnida	*Sundochernes*	*Sundochernes* sp. PSENA9NA
ASV_616	Uroviricota	Caudoviricetes	*Phapecoctavirus*	*Escherichia* phage vB EcoM NA1
ASV_663 ASV_892 ASV_3216	Ascomycota	Dothideomycetes	*Cladosporium*	*Cladosporium cladosporioides*
ASV_674 ASV_1112 ASV_1233 ASV_1324 ASV_2035	Ascomycota	Sordariomycetes	*Hapsidospora*	*Hapsidospora chrysogena*
ASV_688	Rotifera	Eurotatoria	*Philodina*	*Philodina corrugata*
ASV_728	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium acetivorans*
ASV_772 ASV_1792	Ascomycota	Sordariomycetes	*Verticillium*	*Verticillium dahliae*
ASV_816 ASV_1079	Nematoda	Chromadorea	*Angiostoma*	*Angiostoma gandavense*
ASV_828	Arthropoda	Insecta	*Lophosceles*	*Lophosceles* sp. BIOUG24473-HNA9
ASV_865 ASV_2374 ASV_3232 ASV_4076	Ascomycota	Sordariomycetes	*Gliomastix*	*Gliomastix murorum*
ASV_867	Discosea	Flabellinia	*Vexillifera*	*Vexillifera* sp. AKu-2NA2NAa
ASV_871	Arthropoda	Insecta	*Simulium*	*Simulium lemborense*
ASV_890	Porifera	Demospongiae	NA	*Raspailiidae* sp. Po.25592
ASV_891	Nematoda	Chromadorea	*Caenorhabditis*	*Caenorhabditis elegans*
ASV_903	Bacteroidota	Flavobacteriia	*Cellulophaga*	*Cellulophaga algicola*
ASV_944	Ascomycota	Saccharomycetes	*Torulaspora*	*Torulaspora delbrueckii*
ASV_982	Oomycota	NA	*Globisporangium*	*Globisporangium intermedium*
ASV_987	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. K5-23
ASV_1000	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. D749
ASV_1027	Bacteroidota	Flavobacteriia	NA	uncultured *Flavobacteriaceae* bacterium
ASV_1095 ASV_1632 ASV_2074	Ascomycota	Dothideomycetes	*Cercospora*	*Cercospora beticola*
ASV_1116	Ascomycota	Sordariomycetes	*Verticillium*	*Verticillium nonalfalfae*

ASV_1147 ASV_2638	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. NG2
ASV_1148	Platyhelminthes	Trematoda	NA	*Schistosomatidae* sp. W829
ASV_1175	Arthropoda	Insecta	*Monopis*	*Monopis crocicapitella*
ASV_1183	Arthropoda	Arachnida	NA	*Sarcoptiformes* sp. BOLD:AAN6699
ASV_1187 ASV_3703	Bacteroidota	Flavobacteriia	*Fluviicola*	*Fluviicola taffensis*
ASV_1188	Ascomycota	Sordariomycetes	*Apiospora*	*Apiospora arundinis*
ASV_1193	Arthropoda	Insecta	*Parapsyllus*	*Parapsyllus humboldti*
ASV_1235	Platyhelminthes	Cestoda	*Versteria*	*Versteria mustelae*
ASV_1282 ASV_3718	Mollusca	Gastropoda	*Weltersia*	*Weltersia obscura*
ASV_1295	Annelida	Polychaeta	*Dasybranchus*	*Dasybranchus* sp. DH1
ASV_1305	Oomycota	NA	*Nothophytophthora*	*Nothophytophthora chlamydospora*
ASV_1318	Tubulinea	Elardia	*Plagiopyxis*	*Plagiopyxis callida*
ASV_1320	Bacteroidota	Sphingobacteriia	NA	*Sphingobacteriaceae* bacterium GW46NA-11-11-14-LB5
ASV_1343	Oomycota	NA	*Achlya*	*Achlya hypogyna*
ASV_1372	Discosea	NA	*Cochliopodium*	*Cochliopodium minus*
ASV_1373 ASV_3433	Ascomycota	Sordariomycetes	*Hirsutella*	*Hirsutella vermicola*
ASV_1390	Ascomycota	Sordariomycetes	*Madurella*	*Madurella* sp.
ASV_1392 ASV_2194	Arthropoda	Insecta	*Coelocrossa*	*Coelocrossa hypocrocea*
ASV_1405	Ascomycota	Sordariomycetes	*Emericellopsis*	*Emericellopsis* sp. 1a5
ASV_1415 ASV_3763	Ascomycota	Dothideomycetes	*Parastagonospora*	*Parastagonospora nodorum*
ASV_1416	Bacteroidota	Flavobacteriia	*Flavobacterium*	Flav+H2277obacterium psychrophilum
ASV_1425 ASV_2229 ASV_3771	Arthropoda	Arachnida	*Trouessartia*	*Trouessartia* sp. 2 LGP-2NA21a
ASV_1426	Chordata	Actinopteri	*Stolephorus*	*Stolephorus* sp.
ASV_1438	Tubulinea	Elardia	*Copromyxa*	*Copromyxa protea*
ASV_1452	Nematoda	Chromadorea	*Bursaphelenchus*	*Bursaphelenchus tadamiensis*
ASV_1453	Ascomycota	Lecanoromycetes	*Parmotrema*	*Parmotrema ultralucens*
ASV_1456 ASV_2494	Arthropoda	Arachnida	NA	*Ereynetidae* sp. BOLD:AAU6279
ASV_1458	Arthropoda	Insecta	NA	*Elachistidae* gen. elachBioLepNA1 sp. BioLep834
ASV_1459	Ascomycota	Sordariomycetes	*Scopulariopsis*	*Scopulariopsis brevicaulis*
ASV_1466	Basidiomycota	Agaricomycetes	*Lactarius*	*Lactarius hatsudake*
ASV_1467	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. FW3NA5-3-2-15-E-R2A2

ASV_1468 ASV_2072 ASV_2287 ASV_4443	Arthropoda	Arachnida	*Euzetes*	*Euzetes globulus*
ASV_1470	Bacteroidota	Flavobacteriia	*Flavobacterium*	uncultured *Flavobacterium* sp.
ASV_1476 ASV_1634 ASV_2503 ASV_3713	Platyhelminthes	Trematoda	*Cercaria*	*Cercaria pythionike*
ASV_1479	Arthropoda	Insecta	NA	*Limoniidae* sp. BBDCQ925-1NA
ASV_1514	Bacteroidota	Flavobacteriia	*Flagellimonas*	*Flagellimonas* sp. MMGNA31
ASV_1538 ASV_3055	Bacteroidota	Flavobacteriia	*Mesoflavibacter*	*Mesoflavibacter* sp. HG37
ASV_1552	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium sclerotiorum*
ASV_1570	Ascomycota	Sordariomycetes	*Niesslia*	*Niesslia mucida*
ASV_1571 ASV_2485	Oomycota	NA	*Paralagenidium*	*Paralagenidium karlingii*
ASV_1595	Cnidaria	Hydrozoa	*Margelopsis*	*Margelopsis haeckelii*
ASV_1605	Rhodophyta	Florideophyceae	*Gloiophyllis*	*Gloiophyllis barkerae*
ASV_1590 ASV_3220 ASV_3709 ASV_4042	Pseudomonadota	Gammaproteobacteria	*Vibrio*	*Vibrio rumoiensis*
ASV_1606	Mollusca	Gastropoda	*Biomphalaria*	*Biomphalaria schrammi*
ASV_1616 ASV_2633	Arthropoda	Insecta	NA	*Eupodidae* sp. BOLD:ACX7636
ASV_1629	Nematoda	Chromadorea	*Halicephalobus*	*Halicephalobus* sp. AA5
ASV_1642	Ascomycota	Sordariomycetes	*Cordyceps*	*Cordyceps pruinosa*
ASV_1643	Discosea	Flabellinia	*Vannella*	*Vannella sp.*
ASV_1645	Ascomycota	Lecanoromycetes	*Xanthoria*	*Xanthoria parietina*
ASV_1660	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. xlx-214
ASV_1677	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum lindemuthianum*
ASV_1711	Ascomycota	Sordariomycetes	*Plectosphaerella*	*Plectosphaerella* sp.
ASV_1737	Evosea	Eumycetozoa	*Didymium*	*Didymium nigripes*
ASV_1770	Arthropoda	Arachnida	NA	*Eupodidae* sp. BOLD:AAZ5836
ASV_1776 ASV_2946	Ascomycota	Eurotiomycetes	*Aspergillus*	*Aspergillus citrinoterreus*
ASV_1794 ASV_2119 ASV_3139	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter schmidteae*
ASV_1795	Arthropoda	Thecostraca	*Parasacculina*	*Parasacculina* sp. 1 JJ-2NA23a
ASV_1845	Ascomycota	Dothideomycetes	NA	*Dothideomycetes* sp. NU2NANA
ASV_1848	Discosea	NA	*Acanthamoeba*	*Acanthamoeba comandoni*

ASV_1849	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. N2NA38
ASV_1868 ASV_2233 ASV_3347	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium verrucosum*
ASV_1873 ASV_2234	Arthropoda	Collembola	*Desoria*	*Desoria* sp. BOLD:AAA7162
ASV_1874	Basidiomycota	Agaricomycetes	*Clitocybe*	*Clitocybe subditopoda*
ASV_1876	Discosea	Flabellinia	*Vannella*	*Vannella navicula*
ASV_1877 ASV_4383	Bacillariophyta	Bacillariophyceae	*Frustulia*	*Frustulia vulgaris*
ASV_1894	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter mucosus*
ASV_1895 ASV_1978 ASV_3699	Ascomycota	Dothideomycetes	*Zasmidium*	*Zasmidium cellare*
ASV_1897 ASV_3249	Arthropoda	Arachnida	*Pleuropoma*	*Pleuropoma jana*
ASV_1901	Evosea	Eumycetozoa	*Didymium*	*Didymium trachysporum*
ASV_1929 ASV_2039 ASV_2498 ASV_2872	Porifera	Demospongiae	*Abyssocladia*	*Abyssocladia* sp. SMF<DEU> 1175NA
ASV_1930	Basidiomycota	Agaricomycetes	*Clitocybe*	*Clitocybe robusta*
ASV_1952	Oomycota	NA	*Phytophthora*	*Phytophthora rhizophorae*
ASV_2001	Bacteroidota	Flavobacteriia	*Aestuariibaculum*	*Aestuariibaculum lutulentum*
ASV_2006	Ascomycota	Sordariomycetes	*Zelopaecilomyces*	*Zelopaecilomyces penicillatus*
ASV_2009	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter riviphilus*
ASV_2010	Tubulinea	Elardia	*Hyalosphenia*	*Hyalosphenia papilio*
ASV_2026	Streptophyta	Magnoliopsida	*Quercus*	*Quercus suber*
ASV_2028	Arthropoda	Insecta	*Dodona*	*Dodona dipoea*
ASV_2036	Arthropoda	Insecta	*Meimuna*	*Meimuna opalifera*
ASV_2063 ASV_2226 ASV_3240	Ascomycota	Dothideomycetes	*Cercospora*	*Cercospora sojina*
ASV_2064	Arthropoda	Insecta	*Euproctis*	*Euproctis cervina*
ASV_2070	Ascomycota	Sordariomycetes	*Hirsutella*	*Hirsutella thompsonii*
ASV_2071	Chlorophyta	Trebouxiophyceae	*Koliella*	*Koliella longiseta*
ASV_2108 ASV_3468	Cnidaria	Hydrozoa	*Praya*	*Praya dubia*
ASV_2109	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium pectinovorum*
ASV_2110	Ascomycota	NA	NA	*Ascomycota* sp.
ASV_2113 ASV_2431	Chordata	Actinopteri	*Forsterygion*	*Forsterygion flavonigrum*
ASV_2114 ASV_2227	Arthropoda	Arachnida	NA	*Oribatellidae* sp. BIOUGNA8437-GNA8
ASV_2116	Bacteroidota	Flavobacteriia	*Nonlabens*	*Nonlabens ulvanivorans*
ASV_2117	Basidiomycota	Agaricomycetes	*Floccularia*	*Floccularia luteovirens*

ASV_2159 ASV_2709 ASV_3030	Basidiomycota	Tremellomycetes	*Filobasidium*	*Filobasidium floriforme*
ASV_2164 ASV_2330 ASV_3353	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum salicis*
ASV_2165	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. ZE23DGluNA8
ASV_2197 ASV_2279	Ascomycota	Sordariomycetes	*Cordyceps*	*Cordyceps militaris*
ASV_2199 ASV_2379 ASV_2956	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter lusitanus*
ASV_2232	Oomycota	NA	*Phytophthora*	*Phytophthora* sp. 'zentmyerii x Peru4-like'
ASV_2237	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. CLA17
ASV_2275	Arthropoda	Insecta	NA	*Cecidomyiidae* sp. BOLD:ACC8NA68
ASV_2276	Rhodophyta	Florideophyceae	*Portieria*	*Portieria* cf. hornemannii sp.64 FL-2NA18
ASV_2331	Discosea	NA	*Cochliopodium*	*Cochliopodium larifeili*
ASV_2366	Ascomycota	Sordariomycetes	*Orbiocrella*	*Orbiocrella petchii*
ASV_2372	Arthropoda	Insecta	*Tatuidris*	*Tatuidris tatusia*
ASV_2373 ASV_3672	Evosea	Eumycetozoa	*Didymium*	*Didymium difforme*
ASV_2420	Arthropoda	Collembola	*Xenylla*	*Xenylla boerneri*
ASV_2421	Arthropoda	Arachnida	NA	*Tydeidae* sp. BOLD:ADE32NA4
ASV_2433	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. KACC 22758
ASV_2436	Ascomycota	Eurotiomycetes	*Talaromyces*	*Talaromyces funiculosus*
ASV_2491 ASV_2720	Ascomycota	Dothideomycetes	*Edenia*	*Edenia gomezpompae*
ASV_2492	Nematoda	Chromadorea	*Micoletzkya*	*Micoletzkya palliati*
ASV_2496	Ascomycota	Dothideomycetes	*Pseudocercospora*	*Pseudocercospora zelkovae*
ASV_2500 ASV_3045 ASV_3047	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter foliorum*
ASV_2504	Bacteroidota	Flavobacteriia	*Zhouia*	*Zhouia spongiae*
ASV_2566 ASV_3591	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. HDW13
ASV_2567	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. PACM 27299
ASV_2632 ASV_3248	Bacteroidota	Flavobacteriia	*Polaribacter*	*Polaribacter* sp. L3A8
ASV_2648	Arthropoda	Hexanauplia	*Temora*	*Temora* sp.
ASV_2650	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. 14NA616W15
ASV_2653	Pseudomonadota	Gammaproteobacteria	*Psychrobacter*	*Psychrobacter* sp. van23A
ASV_2655	Arthropoda	Insecta	*Paraplea*	*Paraplea halei*

ASV_2706	Arthropoda	Arachnida	*Eueremaeus*	*Eueremaeus* sp. BOLD:ADI4824
ASV_2707	Oomycota	NA	*Globisporangium*	*Globisporangium irregulare*
ASV_2713	Cnidaria	Hydrozoa	*Amphicaryon*	*Amphicaryon* sp. USNM IZ 1449593
ASV_2721	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium psychrophilum*
ASV_2728	Bacteroidota	Flavobacteriia	*Flavivirga*	*Flavivirga eckloniae*
ASV_2784	Arthropoda	Insecta	*Myzocallis*	*Myzocallis* sp. A RGF-2NANA8
ASV_2799	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium panacagri*
ASV_2801	Oomycota	NA	*Globisporangium*	*Globisporangium barbulae*
ASV_2847	Ascomycota	Lecanoromycetes	*Parmotrema*	*Parmotrema stuppeum*
ASV_2851	Nematoda	Chromadorea	*Hemicriconemoides*	*Hemicriconemoides californianus*
ASV_2864	Discosea	NA	*Cochliopodium*	*Cochliopodium kieliense*
ASV_2866	Arthropoda	Arachnida	*Mesalgoides*	*Mesalgoides* sp. BIOUG3NA847-GNA9
ASV_2868	Arthropoda	Collembola	NA	*Tomocerinae* sp. BIOUG24412-F11
ASV_2869	Arthropoda	Arachnida	*Analges*	*Analges bidentatus*
ASV_2870	Arthropoda	Insecta	NA	*Mycetophilidae* sp. BOLD:AAM8972
ASV_2874 ASV_3052	Pseudomonadota	Betaproteobacteria	*Methylotenera*	*Methylotenera* sp.
ASV_2939	Discosea	Flabellinia	*Vannella*	*Vannella robusta*
ASV_2944	Oomycota	NA	*Aphanomyces*	*Aphanomyces salsuginosus*
ASV_2963	Ascomycota	Sordariomycetes	*Beauveria*	*Beauveria brongniartii*
ASV_2967	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter cryoconitis*
ASV_2969	Rhodophyta	Florideophyceae	*Herposiphonia*	*Herposiphonia* sp. 1Tristan TDCRH1NA2-11
ASV_3024	Oomycota	NA	*Phytophthora*	*Phytophthora falcata*
ASV_3046	Ascomycota	Sordariomycetes	*Pleurocordyceps*	*Pleurocordyceps sinensis*
ASV_3051 ASV_3119	Cnidaria	Hydrozoa	*Zanclea*	*Zanclea implexa*
ASV_3118	Ascomycota	Sordariomycetes	*Metarhizium*	*Metarhizium robertsii*
ASV_3130	Arthropoda	Insecta	*Heterarthrus*	*Heterarthrus* sp. 1 Daocheng
ASV_3145	Oomycota	NA	*Saprolegnia*	*Saprolegnia monilifera*
ASV_3146	Arthropoda	Insecta	*Bucculatrix*	*Bucculatrix ulmella*
ASV_3147	Arthropoda	Arachnida	*Thyreophagus*	*Thyreophagus corticalis*
ASV_3213	Arthropoda	Arachnida	*Ixodes*	*Ixodes* sp. KM-2NA14a
ASV_3218	Arthropoda	Arachnida	*Clubiona*	*Clubiona cochlearis*
ASV_3242	Arthropoda	Insecta	*Cirrhochrista*	*Cirrhochrista* cf. brizoalis BOLD:AAF3NA65
ASV_3243	Arthropoda	Arachnida	*Epidamaeus*	*Epidamaeus* sp. BOLD:ACZNA7NA5
ASV_3246	Bacteroidota	Sphingobacteriia	NA	*Sphingobacteriales* bacterium JAD PAG5NA586 3

ASV_3250	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium crocinum*
ASV_3252	Evosea	Eumycetozoa	*Didymium*	*Didymium crustaceum*
ASV_3325	Basidiomycota	Ustilaginomycetes	*Sporisorium*	*Sporisorium scitamineum*
ASV_3327	Oomycota	NA	*Globisporangium*	*Globisporangium acanthophoron*
ASV_3357	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. SLBNA2
ASV_3359	Discosea	NA	*Parvamoeba*	*Parvamoeba rugata*
ASV_3360	Nematoda	Chromadorea	*Paraquimperia*	*Paraquimperia* sp. ALS-2NA19
ASV_3362	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. Sr18
ASV_3435	Arthropoda	Insecta	*Limnophyes*	*Limnophyes* sp. 12ES
ASV_3438	Ascomycota	Sordariomycetes	*Beauveria*	*Beauveria bassiana*
ASV_3439 ASV_4198	Arthropoda	Insecta	*Claustropyga*	*Claustropyga acanthostyla*
ASV_3442	Bacteroidota	Flavobacteriia	*Muriicola*	*Muriicola soli*
ASV_3454	Arthropoda	Arachnida	NA	*Eupodidae* sp. BOLD:ADU4475
ASV_3465	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium eburneipallidum*
ASV_3469	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. UC225 65
ASV_3472	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium brevicompactum*
ASV_3473	Ascomycota	Leotiomycetes	*Tetracladium*	*Tetracladium apiense*
ASV_3477	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. N2469
ASV_3479	Arthropoda	Arachnida	*Eueremaeus*	*Eueremaeus* sp. BOLD:AAE296NA
ASV_3549	Ascomycota	Eurotiomycetes	*Aspergillus*	*Aspergillus puulaauensis*
ASV_3558	Arthropoda	Insecta	NA	*Coleoptera* sp. NZAC NA3NANA9NA96
ASV_3586	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter roseus*
ASV_3593	Arthropoda	Insecta	*Bradysia*	*Bradysia* sp. BIOUG25629-G11
ASV_3595	Ascomycota	Leotiomycetes	*Antarctomyces*	*Antarctomyces pellizariae*
ASV_3652	Ascomycota	Dothideomycetes	*Phoma*	*Phoma* sp. 1 OB-2NA14
ASV_3654	Arthropoda	Insecta	*Spilogona*	*Spilogona* sp. BOLD:AAP25NA7
ASV_3674	Ascomycota	Sordariomycetes	*Madurella*	*Madurella mycetomatis*
ASV_3701	Basidiomycota	Agaricomycetes	*Hygrocybe*	*Hygrocybe persistens*
ASV_3702	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. N5NA331NA
ASV_3704	Arthropoda	Insecta	*Melitaea*	*Melitaea trivia*
ASV_3705	Rotifera	Eurotatoria	NA	*Bdelloidea* sp. Bd34 NA2
ASV_3708	Ascomycota	Sordariomycetes	*Sarocladium*	*Sarocladium implicatum*
ASV_3761	Platyhelminthes	Cestoda	*Dipylidium*	*Dipylidium caninum*
ASV_3762	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum scovillei*
ASV_3786	Ascomycota	Lecanoromycetes	*Graphis*	*Graphis lineola*

ASV_3899	Chordata	Actinopteri	*Pseudophycis*	*Pseudophycis bachus*
ASV_3928	Arthropoda	Arachnida	*Anachipteria*	*Anachipteria* sp. BIOUG25375-D12
ASV_3933	Pseudomonadota	Gammaproteobacteria	*Escherichia*	*Escherichia coli*
ASV_4142	Arthropoda	Insecta	NA	*Diptera* sp.
ASV_4197	Chlorophyta	Trebouxiophyceae	*Chloroidium*	*Chloroidium* sp. UTEX 3NA77
ASV_4236	Arthropoda	Arachnida	NA	*Trombidiformes* sp. BOLD:AAHNA995
ASV_4438	Ascomycota	Lecanoromycetes	*Hypogymnia*	*Hypogymnia vittata*
ASV_4439	Basidiomycota	Malasseziomycetes	*Malassezia*	*Malassezia restricta*
ASV_4475	Oomycota	NA	*Phytophthora*	*Phytophthora pseudokernoviae*
ASV_4559	Arthropoda	Arachnida	*Typhlodromus*	*Typhlodromus transvaalensis*
Summer				
ASV	**Phylum**	**Class**	**Genus**	**Species**
ASV_10 ASV_249 ASV_409 ASV_1128	Nematoda	Chromadorea	*Rhabditis*	*Rhabditis* sp.
ASV_17 ASV_548 ASV_1573 ASV_2041	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. KBSNA721
ASV_24 ASV_1493 ASV_4518	Bacteroidota	Flavobacteriia	*Aggregatimonas*	*Aggregatimonas sangjinii*
ASV_36 ASV_192 ASV_287 ASV_344 ASV_351 ASV_380 ASV_423 ASV_433 ASV_459 ASV_518 ASV_560 ASV_636 ASV_699 ASV_715 ASV_767 ASV_768 ASV_849 ASV_1207 ASV_1259 ASV_1746	Basidiomycota	Agaricomycetes	*Rigidoporus*	*Rigidoporus microporus*
ASV_75	Ascomycota	Sordariomycetes	*Niveomyces*	*Niveomyces insectorum*
ASV_84 ASV_207 ASV_646 ASV_700	Mucoromycota	Mucoromycetes	*Apophysomyces*	*Apophysomyces elegans*

ASV_1276 ASV_4888 ASV_4888				
ASV_86	Arthropoda	Insecta	NA	*Chloropidae* sp.
ASV_102 ASV_353 ASV_531 ASV_749 ASV_4520	Ascomycota	Leotiomycetes	*Leohumicola*	*Leohumicola incrustata*
ASV_109 ASV_2881 ASV_3729	Ascomycota	Insecta	*Curculio*	*Curculio* sp. 2 ZM-2NA22a
ASV_113 ASV_422 ASV_1362 ASV_1449 ASV_1815	Bacillariophyta	Coscinodiscophyceae	*Chaetoceros*	*Chaetoceros* sp. MBTD-CMFRI-SNA45
ASV_133 ASV_591 ASV_886 ASV_937 ASV_941 ASV_989 ASV_1022 ASV_1075 ASV_1102 ASV_1404	Annelida	Clitellata	*Bimastos*	*Bimastos parvus*
ASV_144 ASV_709 ASV_1125 ASV_1841	Arthropoda	Catenulida	*Stenostomum*	*Stenostomum* cf. simplex AW-2NA18
ASV_147	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium citrinum*
ASV_163 ASV_276	Ascomycota	Leotiomycetes	*Leohumicola*	*Leohumicola* sp. DAOM 239516
ASV_165 ASV_340 ASV_416 ASV_622 ASV_853	Ascomycota	Leotiomycetes	Leohumicola	*Leohumicola levissima*
ASV_170 ASV_2439	Arthropoda	Arachnida	NA	*Acaridae* sp. BIOUG3NA847-BNA2
ASV_171	Ascomycota	Sordariomycetes	*Zarea*	*Zarea fungicola*
ASV_191 ASV_263 ASV_386 ASV_722 ASV_777 ASV_3302 ASV_3378	Arthropoda	Insecta	NA	*Endopterygota* sp. NZAC NA3NANA9123
ASV_199 ASV_297 ASV_382 ASV_390 ASV_1527	Ascomycota	Pichiomycetes	*Clavispora*	*Clavispora lusitaniae*

ASV_204 ASV_226	Ascomycota	Sordariomycetes	*Trichoderma*	*Trichoderma atroviride*
ASV_217 ASV_374	Arthropoda	Insecta	*Petrusa*	*Petrusa epilepsis*
ASV_234	Arthropoda	NA	NA	*Mandibulata* sp. NZAC NA3NA11NA75
ASV_238	Ascomycota	Leotiomycetes	*Rhynchobrunnera*	*Rhynchobrunnera orthospora*
ASV_243 ASV_1696	Ascomycota	Sordariomycetes	*Tolypocladium*	*Tolypocladium ophioglossoides*
ASV_244 ASV_447	Ascomycota	Leotiomycetes	*Myxotrichum*	*Myxotrichum deflexum*
ASV_266 ASV_270 ASV_475 ASV_521 ASV_580 ASV_696	Rotifera	Eurotatoria	*Macrotrachela*	*Macrotrachela quadricornifera*
ASV_277	Ascomycota	Leotiomycetes	*Acephala*	*Acephala applanata*
ASV_293	Ascomycota	Dothideomycetes	*Cladosporium*	*Cladosporium* sp.
ASV_315	Nematoda	Chromadorea	*Fergusobia*	*Fergusobia* sp. 1NA37 FC5NA8
ASV_319	Arthropoda	Insecta	*Exorista*	*Exorista civilis*
ASV_343 ASV_691 ASV_819	Basidiomycota	Agaricomycetes	*Russula*	*Russula compacta*
ASV_367 ASV_506 ASV_513 ASV_629 ASV_1383 ASV_1541 ASV_1956	Ascomycota	Pichiomycetes	*Candida*	*Candida buenavistaensis*
ASV_373 ASV_590	Arthropoda	Insecta	*Schinia*	*Schinia sordidus*
ASV_381	Basidiomycota	Agaricomycetes	*Entoloma*	*Entoloma clypeatum*
ASV_383	Arthropoda	Insecta	*Symplecta*	*Symplecta pilipes*
ASV_391 ASV_579 ASV_671	Ascomycota	Leotiomycetes	*Pseudofabraea*	*Pseudofabraea citricarpa*
ASV_415 ASV_3321	Cnidaria	Hydrozoa	*Hydra*	*Hydra vulgaris*
ASV_417	Arthropoda	Insecta	*Diaeretiella*	*Diaeretiella rapae*
ASV_430	Arthropoda	Insecta	*Paralucilia*	*Paralucilia* sp. ACA1NA6NA
ASV_440	Arthropoda	Insecta	NA	*Cecidomyiidae* sp. BOLD:ACA9586
ASV_442 ASV_573 ASV_605	Arthropoda	Insecta	*Aracynthus*	*Aracynthus* sp. CCDB-32968-BNA9
ASV_443	Evosea	Eumycetozoa	*Trichia*	*Trichia scabra*
ASV_444 ASV_596	Arthropoda	Insecta	NA	*Lepidoptera* sp. NZAC NA3NA11646

ASV_450	Nematoda	Chromadorea	*Angiostrongylus*	*Angiostrongylus costaricensis*
ASV_456 ASV_3880	Arthropoda	Insecta	NA	*Coleoptera* sp. INDOBIOSYS-CCDB26NA91-D1NA
ASV_466 ASV_515 ASV_2396	Arthropoda	Insecta	*Symplecta*	*Symplecta stictica*
ASV_473 ASV_601	Rotifera	Eurotatoria	*Philodina*	*Philodina hongcheonensis*
ASV_484	Ascomycota	Lecanoromycetes	*Phyllopsora*	*Phyllopsora corallina*
ASV_529	Basidiomycota	Agaricomycetes	*Aureoboletus*	*Aureoboletus raphanaceus*
ASV_539 ASV_721 ASV_735	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium* sp. ShG4C
ASV_542	Rhodophyta	Florideophyceae	*Dipterocladia*	*Dipterocladia arabiensis*
ASV_545	Ascomycota	Eurotiomycetes	*Nannizziopsis*	*Nannizziopsis barbatae*
ASV_546 ASV_708 ASV_737	Ascomycota	Sordariomycetes	*Ceratocystiopsis*	*Ceratocystiopsis pallidobrunnea*
ASV_559 ASV_925	Arthropoda	Insecta	NA	*Anisopodidae* sp. BOLD:AAM639NA
ASV_575	Arthropoda	Arachnida	*Acalitus*	*Acalitus vaccinii*
ASV_577 ASV_1186	Arthropoda	Insecta	*Notonecta*	*Notonecta lutea*
ASV_588	Oomycota	NA	*Pythium*	*Pythium* sp. CAL-2NA11c
ASV_593 ASV_3534 ASV_4194	Arthropoda	Arachnida	NA	*Arachnida* sp. BOLD:ACI5923
ASV_614 ASV_997	Bacillota	Bacilli	*Oceanobacillus*	*Oceanobacillus* sp. FSL K6-NA127
ASV_627	Basidiomycota	Agaricomycetes	*Russula*	*Russula nigricans*
ASV_631	Arthropoda	Insecta	*Colobopyga*	*Colobopyga pritchardiae*
ASV_635 ASV_869 ASV_1884	Arthropoda	Insecta	*Leptocera*	*Leptocera erythrocera*
ASV_639 ASV_1650	Bacteroidota	Flavobacteriia	*Tamlana*	*Tamlana crocina*
ASV_642 ASV_4167	Ascomycota	Leotiomycetes	*Phialocephala*	*Phialocephala europaea*
ASV_682 ASV_1224	Oomycota	NA	*Phytopythium*	*Phytopythium* sp. UZ612-like
ASV_687	Arthropoda	Insecta	*Opogona*	*Opogona omoscopa*
ASV_698	Ascomycota	Dothideomycetes	*Aureobasidium*	*Aureobasidium pullulans*
ASV_712	Oomycota	NA	*Nothophytophthora*	*Nothophytophthora* sp. 'caduca-like'
ASV_714	Arthropoda	Insecta	*Rachispoda*	*Rachispoda anceps*
ASV_730	Ascomycota	Dothideomycetes	*Cercospora*	*Cercospora nicotianae*
ASV_736 ASV_747 ASV_850	Arthropoda	Insecta	*Plutella*	*Plutella xylostella*

ASV_744	Bacillariophyta	Bacillariophyceae	*Gomphonema*	*Gomphonema parvulum*
ASV_792	Ascomycota	Sordariomycetes	*Ophiostoma*	*Ophiostoma* sp.
ASV_793	Basidiomycota	Agaricomycetes	*Psathyrella*	*Psathyrella* aff. gracilis TRTC155531
ASV_794 ASV_1523 ASV_2734	Arthropoda	Arachnida	*Dermatophagoides*	*Dermatophagoides pteronyssinus*
ASV_796	Ascomycota	Sordariomycetes	*Thelonectria*	*Thelonectria veuillotiana*
ASV_805	Bacillariophyta	Bacillariophyceae	*Nitzschia*	*Nitzschia* sp. BOLD:AAX5147
ASV_820	Bacteroidota	Chitinophagia	*Lacibacter*	*Lacibacter sediminis*
ASV_833	Gastrotricha	NA	*Chaetonotus*	*Chaetonotus* aff. persimilis MK-2NA19
ASV_847	Arthropoda	Insecta	*Mirax*	*Mirax* sp.
ASV_855	Bacteroidota	Flavobacteriia	*Mangrovimonas*	*Mangrovimonas* sp. YM274
ASV_857	Ascomycota	Leotiomycetes	*Leohumicola*	*Leohumicola terminalis*
ASV_859	Platyhelminthes	Catenulida	*Stenostomum*	*Stenostomum leucops*
ASV_862	Arthropoda	Insecta	NA	*Chironomidae* sp.
ASV_889	Bacteroidota	Flavobacteriia	*Myroides*	*Myroides fluvii*
ASV_894	Bacillariophyta	Coscinodiscophyceae	*Cyclotella*	*Cyclotella cryptica*
ASV_927 ASV_954	Bacteroidota	Flavobacteriia	*Arenibacter*	*Arenibacter antarcticus*
ASV_936 ASV_1472	Basidiomycota	Agaricomycetes	*Peniophora*	*Peniophora lycii*
ASV_996	Rotifera	Eurotatoria	*Keratella*	*Keratella tropica*
ASV_998	Arthropoda	Arachnida	*Mesalgoides*	*Mesalgoides* sp. BIOUG15124-BNA2
ASV_1016	Basidiomycota	Agaricomycetes	*Amanita*	*Amanita basii*
ASV_1019	Rhodophyta	Florideophyceae	*Amansia*	*Amansia glomerata*
ASV_1023	Rotifera	Eurotatoria	*Rotaria*	*Rotaria neptunia*
ASV_1030	Arthropoda	Collembola	*Entomobrya*	*Entomobrya nivalis*
ASV_1038	Ciliophora	Oligohymenophorea	*Carchesium*	*Carchesium polypinum*
ASV_1047 ASV_1054 ASV_1143 ASV_1504 ASV_1574 ASV_1618 ASV_1721 ASV_5060 ASV_5060	Bacillota	Bacilli	*Carnobacterium*	*Carnobacterium divergens*
ASV_1062 ASV_4728	Pseudomonadota	Alphaproteobacteria	*Peteryoungia*	*Peteryoungia desertarenae*
ASV_1132	Ascomycota	Leotiomycetes	*Podosphaera*	*Podosphaera xanthii*
ASV_1159	Arthropoda	Insecta	NA	*Chironominae* sp. BOLD:AAJ4272
ASV_1161	Bacteroidota	Flavobacteriia	*Maribacter*	*Maribacter* sp. R77961

ASV_1173 ASV_1511	Pseudomonadota	Alphaproteobacteria	*Candidatus_Megaera*	*Candidatus Megaera polyxenophila*
ASV_1180	Arthropoda	Insecta	*Anax*	*Anax guttatus*
ASV_1231	Ascomycota	Dothideomycetes	*Ascochyta*	*Ascochyta pisi*
ASV_1242	Arthropoda	Insecta	*Paratanytarsus*	*Paratanytarsus grimmii*
ASV_1292 ASV_1813	Bacillariophyta	Fragilariophyceae	*Ulnaria*	*Ulnaria acus*
ASV_1313	Discosea	Flabellinia	*Korotnevella*	*Korotnevella fousta*
ASV_1345	Basidiomycota	Malasseziomycetes	*Malassezia*	*Malassezia arunalokei*
ASV_1429	Chlorophyta	Trebouxiophyceae	*Picochlorum*	*Picochlorum* sp. SENEW3
ASV_1446	Discosea	NA	*Acanthamoeba*	*Acanthamoeba astronyxis*
ASV_1463	Rotifera	Eurotatoria	*Rotaria*	*Rotaria rotatoria*
ASV_1484	Arthropoda	Insecta	*Glenea*	*Glenea relicta*
ASV_1536	Mucoromycota	Mucoromycetes	*Mucor*	*Mucor piriformis*
ASV_1549	Discosea	Flabellinia	*Clydonella*	*Clydonella sawyeri*
ASV_1576	Arthropoda	Insecta	*Aploneura*	*Aploneura lentisci*
ASV_1596	Discosea	Flabellinia	*Ripella*	*Ripella decalvata*
ASV_1651	Pseudomonadota	Betaproteobacteria	*Nitrosomonas*	*Nitrosomonas ureae*
ASV_1665	Bacillota	Bacilli	*Virgibacillus*	*Virgibacillus* sp. Bac332
ASV_1699	Chordata	Aves	*Porphyrio*	*Porphyrio melanotus*
ASV_1704	Rotifera	Eurotatoria	*Trichocerca*	*Trichocerca* sp. WM-2NA17e
ASV_1717	Bacillota	Bacilli	*Carnobacterium*	*Carnobacterium maltaromaticum*
ASV_1782 ASV_2245	Bacteroidota	Flavobacteriia	*Maribacter*	*Maribacter* sp. R86514
ASV_1799	Arthropoda	Insecta	*Calliphora*	*Calliphora hilli*
ASV_1800	Bacteroidota	Flavobacteriia	*Formosa*	*Formosa agariphila*
ASV_1802	Ascomycota	Eurotiomycetes	*Aspergillus*	*Aspergillus magnivesiculatus*
ASV_1822	Arthropoda	Insecta	*Bactrocera*	*Bactrocera correcta*
ASV_1913	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. Sr18
ASV_2002	Arthropoda	Insecta	NA	*Hymenoptera* sp. BOLD:ACJ31NANA
ASV_2294	Bacillota	Tissierellia	*Tissierella*	*Tissierella* sp. MB52-C2
ASV_2365	Arthropoda	Collembola	*Dicranocentrus*	*Dicranocentrus pallidus*
ASV_2438	Bacteroidota	Flavobacteriia	*Myroides*	*Myroides odoratimimus*
ASV_2506	Chordata	Aves	*Cyanoramphus*	*Cyanoramphus novaezelandiae*
ASV_2507	Apicomplexa	Conoidasida	*Goussia*	*Goussia bayae*
ASV_2660	Arthropoda	Insecta	*Telomerina*	*Telomerina flavipes*
ASV_2689	Nematoda	Chromadorea	*Ficophagus*	*Ficophagus virens*
ASV_2934	Chordata	Actinopteri	*Thyrsites*	*Thyrsites atun*
ASV_3012	Ascomycota	Dothideomycetes	*Cladosporium*	*Cladosporium cladosporioides*
ASV_3093	Bacillota	Bacilli	*Enterococcus*	*Enterococcus faecalis*

ASV_3257	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum jinshuiense*
ASV_3320	Discosea	NA	*Acanthamoeba*	*Acanthamoeba* sp.
ASV_3374	Pseudomonadota	Gammaproteobacteria	*Oceanisphaera*	*Oceanisphaera avium*
ASV_3498	Bacteroidota	Flavobacteriia	*Cellulophaga*	*Cellulophaga* sp. HaHaR 3 176
ASV_3501	Bacteroidota	Flavobacteriia	*Oceanihabitans*	*Oceanihabitans* sp. IOP 32
ASV_3503 ASV_4168	Chordata	Aves	*Pygoscelis*	*Pygoscelis adeliae*
ASV_3535	Arthropoda	Insecta	*Tingena*	*Tingena armigerella*
ASV_3727	Arthropoda	Collembola	*Orthonychiurus*	*Orthonychiurus folsomi*
ASV_3861	Bacteroidota	Flavobacteriia	*Lacinutrix*	*Lacinutrix* sp. WUR7
ASV_3862	Pseudomonadota	Alphaproteobacteria	*Rhizobium*	*Rhizobium leguminosarum*
ASV_3879	Annelida	Polychaeta	*Parandalia*	*Parandalia* sp. 1 DH1
ASV_4002	Arthropoda	Arachnida	*Propelops*	*Propelops* sp. BOLD:ADI4824
ASV_4036	Arthropoda	Collembola	*Parisotoma*	*Parisotoma* aff. notabilis L1
ASV_4183	Arthropoda	Insecta	*Diamesa*	*Diamesa bertrami*
ASV_4189	Arthropoda	Arachnida	*Telamonia*	*Telamonia festiva*
ASV_4192	Arthropoda	Arachnida	*Analges*	*Analges* aff. passerinus B MD-2NA2NA
ASV_4382	Arthropoda	Ostracoda	*Cypridopsis*	*Cypridopsis vidua*
ASV_4557	Rhodophyta	Florideophyceae	*Capreolia*	*Capreolia implexa*

Table S3.5. Complete clamtest analysis results for ASVs classified as specific to east and west island orientations. Taxonomic assignments include phylum, class, genus, and species, where available. Multiple ASVs may correspond to the same taxonomic identification as indicated.

East				
ASV	**Phylum**	**Class**	**Genus**	**Species**
ASV_15 ASV_300	Arthropoda	Insecta	NA	*Eupodidae* sp. BOLD:ACX7636
ASV_27 ASV_808 ASV_1247	Arthropoda	Arachnida	*Tyrophagus*	*Tyrophagus curvipenis*
ASV_34 ASV_317 ASV_718	Arthropoda	Collembola	*Entomobrya*	*Entomobrya* sp. HBNA852 3
ASV_37 ASV_424	Bacteroidota	Flavobacteriia	*Winogradskyella*	*Winogradskyella schleiferi*
ASV_44 ASV_112	Platyhelminthes	Trematoda	NA	*Schistosomatidae* sp. W829
ASV_46	Nematoda	Chromadorea	*Adineta*	*Adineta* sp. MX.1.1
ASV_49	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium inviolabile*
ASV_55 ASV_202 ASV_279 ASV_851	Rotifera	Eurotatoria	NA	*Bdelloidea* environmental sample
ASV_76	Arthropoda	Elardia	*Cucurbitella*	*Cucurbitella mespiliformis*
ASV_86	Arthropoda	Insecta	NA	*Chloropidae* sp.
ASV_87 ASV_1512	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium marginilacus*
ASV_88 ASV_837 ASV_1337	Arthropoda	Insecta	*Psychoda*	*Psychoda sigma*
ASV_94	Nematoda	Chromadorea	*Koerneria*	*Koerneria* sp. RS1982
ASV_98	Nematoda	Chromadorea	*Teratorhabditis*	*Teratorhabditis synpapillata*
ASV_130	Basidiomycota	Agaricomycetes	*Amanita*	*Amanita thiersii*
ASV_136	Arthropoda	Arachnida	*Pleuropoma*	*Pleuropoma jana*
ASV_141 ASV_731	Arthropoda	Collembola	*Desoria*	*Desoria* sp. BOLD:AAA7162
ASV_142 ASV_562 ASV_675 ASV_692	Discosea	NA	*Acanthamoeba*	*Acanthamoeba comandoni*
ASV_147	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium citrinum*
ASV_149	Nematoda	Chromadorea	*Halicephalobus*	*Halicephalobus* sp. AA5
ASV_154 ASV_843	Basidiomycota	Microbotryomycetes	*Rhodotorula*	*Rhodotorula mucilaginosa*

ASV_159	Oomycota	NA	*Paralagenidium*	*Paralagenidium karlingii*
ASV_170	Arthropoda	Arachnida	NA	*Acaridae* sp. BIOUG3NA847-BNA2
ASV_171	Ascomycota	Sordariomycetes	*Zarea*	*Zarea fungicola*
ASV_178	Nematoda	Chromadorea	*Bursaphelenchus*	*Bursaphelenchus tadamiensis*
ASV_199 ASV_1527	Ascomycota	Pichiomycetes	*Clavispora*	*Clavispora lusitaniae*
ASV_204 ASV_226	Ascomycota	Sordariomycetes	*Trichoderma*	*Trichoderma atroviride*
ASV_206 ASV_2156	Nematoda	Chromadorea	*Parapristionchus*	*Parapristionchus giblindavisi*
ASV_217	Arthropoda	Insecta	*Petrusa*	*Petrusa epilepsis*
ASV_234	Arthropoda	NA	NA	*Mandibulata* sp. NZAC NA3NA11NA75
ASV_237	Ascomycota	Sordariomycetes	*Sporothrix*	*Sporothrix brasiliensis*
ASV_238	Ascomycota	Leotiomycetes	*Rhynchobrunnera*	*Rhynchobrunnera orthospora*
ASV_245	Ascomycota	Lecanoromycetes	*Phaeoplaca*	*Phaeoplaca camptidia*
ASV_257 ASV_1283	Mollusca	Gastropoda	*Weltersia*	*Weltersia obscura*
ASV_277	Ascomycota	Leotiomycetes	*Acephala*	*Acephala applanata*
ASV_278	Arthropoda	Arachnida	*Miratemnus*	*Miratemnus* sp. JM-2NANA8
ASV_282	Ascomycota	Sordariomycetes	*Fusarium*	*Fusarium haematococcum*
ASV_291	Chordata	Actinopteri	*Hyporhamphus*	*Hyporhamphus ihi*
ASV_292 ASV_977 ASV_2230	Ascomycota	Dothideomycetes	*Sydowia*	*Sydowia polyspora*
ASV_296	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Candidatus Pedobacter colombiensis*
ASV_304	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium sangjuense*
ASV_357	Discosea	Flabellinia	*Vannella*	*Vannella samoroda*
ASV_372	Arthropoda	Arachnida	NA	*Ereynetidae* sp. BOLD:ABV186NA
ASV_375	Arthropoda	Insecta	*Boletina*	*Boletina* sp. BOLD:AAVNA669
ASV_401 ASV_773	Ascomycota	Leotiomycetes	*Synchaetomella*	*Synchaetomella acerina*
ASV_413 ASV_1951	Bacteroidota	Flavobacteriia	*Fluviicola*	*Fluviicola taffensis*
ASV_419 ASV_821 ASV_1217	Arthropoda	Arachnida	NA	*Sarcoptiformes* sp. BOLD:AAN6699
ASV_430	Arthropoda	Insecta	*Paralucilia*	*Paralucilia* sp. ACA1NA6NA
ASV_485 ASV_1330	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium digitatum*
ASV_501	Discosea	Flabellinia	*Vannella*	*Vannella pentlandii*

ASV_529	Basidiomycota	Agaricomycetes	*Aureoboletus*	*Aureoboletus raphanaceus*
ASV_614	Bacillota	Bacilli	*Oceanobacillus*	*Oceanobacillus* sp. FSL K6-NA127
ASV_615	Arthropoda	Arachnida	*Sundochernes*	*Sundochernes* sp. PSENA9NA
ASV_616	Uroviricota	Caudoviricetes	*Phapecoctavirus*	*Escherichia* phage vB EcoM NA1
ASV_688	Rotifera	Eurotatoria	*Philodina*	*Philodina corrugata*
ASV_703	Chordata	Aves	*Pygoscelis*	*Pygoscelis adeliae*
ASV_728	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium acetivorans*
ASV_772	Ascomycota	Dothideomycetes	*Cercospora*	*Cercospora beticola*
ASV_781	Arthropoda	Arachnida	*Ixodes*	*Ixodes* sp. KM-2NA14a
ASV_789	Oomycota	NA	*Globisporangium*	*Globisporangium kandovanense*
ASV_792	Ascomycota	Sordariomycetes	*Ophiostoma*	*Ophiostoma* sp.
ASV_806 ASV_836	Mucoromycota	Mucoromycetes	*Mucor*	*Mucor lusitanicus*
ASV_816 ASV_1079	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. D749
ASV_828	Arthropoda	Insecta	*Lophosceles*	*Lophosceles* sp. BIOUG24473-HNA9
ASV_844	Mollusca	Gastropoda	*Glyptophysa*	*Glyptophysa* sp. 2 PGA-2NA18
ASV_867	Discosea	Flabellinia	*Vexillifera*	*Vexillifera* sp. AKu-2NA2NAa
ASV_889	Bacteroidota	Flavobacteriia	*Myroides*	*Myroides fluvii*
ASV_890	Porifera	Demospongiae	NA	*Raspailiidae* sp. Po.25592
ASV_891	Nematoda	Chromadorea	*Caenorhabditis*	*Caenorhabditis elegans*
ASV_892 ASV_1662	Ascomycota	Dothideomycetes	*Cladosporium*	*Cladosporium cladosporioides*
ASV_912 ASV_1106 ASV_1631	Ascomycota	Eurotiomycetes	*Arthroderma*	*Arthroderma uncinatum*
ASV_951 ASV_1590	Ascomycota	Sordariomycetes	*Niesslia*	*Niesslia mucida*
ASV_969	Arthropoda	Arachnida	*Trouessartia*	*Trouessartia* sp. 2 LGP-2NA21a
ASV_982	Oomycota	NA	*Globisporangium*	*Globisporangium intermedium*
ASV_987	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. K5-23
ASV_1000	Nematoda	Chromadorea	*Angiostoma*	*Angiostoma gandavense*
ASV_1027	Mucoromycota	Mucoromycetes	*Lichtheimia*	*Lichtheimia hongkongensis*
ASV_1035 ASV_1344	Ascomycota	Dothideomycetes	*Cladosporium*	*Cladosporium sphaerospermum*
ASV_1095 ASV_1632	Ascomycota	Sordariomycetes	*Verticillium*	*Verticillium dahliae*
ASV_1116	Ascomycota	Sordariomycetes	*Hapsidospora*	*Hapsidospora chrysogena*
ASV_1148	Oomycota	NA	*Globisporangium*	*Globisporangium cystogenes*

ASV_1175	Platyhelminthes	Cestoda	NA	*Tetrabothriidae* sp. BOLD:AAM7874
ASV_1183	Arthropoda	Insecta	*Culex*	*Culex erraticus*
ASV_1187	Basidiomycota	Agaricomycetes	*Russula*	*Russula virescens*
ASV_1188	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum fructicola*
ASV_1193	Ascomycota	Sordariomycetes	*Simplicillium*	*Simplicillium lamellicola*
ASV_1235	Basidiomycota	Agaricomycetes	*Fomitopsis*	*Fomitopsis palustris*
ASV_1305	Oomycota	NA	*Phytophthora*	*Phytophthora rhizophorae*
ASV_1318	Basidiomycota	Agaricomycetes	*Clitocybe*	*Clitocybe robusta*
ASV_1320	Porifera	Demospongiae	*Abyssocladia*	*Abyssocladia* sp. SMF<DEU> 1175NA
ASV_1327	Bacteroidota	Flavobacteriia	*Kordia*	*Kordia* sp. SMS9
ASV_1343	Evosea	Eumycetozoa	*Didymium*	*Didymium trachysporum*
ASV_1373	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter mucosus*
ASV_1392	Discosea	Flabellinia	*Vannella*	*Vannella navicula*
ASV_1405	Basidiomycota	Agaricomycetes	*Clitocybe*	*Clitocybe subditopoda*
ASV_1416	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. N2NA38
ASV_1425	Ascomycota	Dothideomycetes	NA	*Dothideomycetes* sp. NU2NANA
ASV_1426	Arthropoda	Thecostraca	*Parasacculina*	*Parasacculina* sp. 1 JJ-2NA23a
ASV_1438	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter schmidteae*
ASV_1452	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium alkalisoli*
ASV_1453	Ascomycota	Eurotiomycetes	*Aspergillus*	*Aspergillus citrinoterreus*
ASV_1456	Arthropoda	Arachnida	NA	*Eupodidae* sp. BOLD:AAZ5836
ASV_1458	Evosea	Eumycetozoa	*Didymium*	*Didymium nigripes*
ASV_1459	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. CFS9
ASV_1466	Ascomycota	Sordariomycetes	*Plectosphaerella*	*Plectosphaerella* sp.
ASV_1467	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum lindemuthianum*
ASV_1468	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. xlx-214
ASV_1470	Ascomycota	Lecanoromycetes	*Xanthoria*	*Xanthoria parietina*
ASV_1476 ASV_1634	Discosea	Flabellinia	*Vannella*	*Vannella* sp.
ASV_1479	Ascomycota	Sordariomycetes	*Cordyceps*	*Cordyceps pruinosa*
ASV_1514	Mollusca	Gastropoda	*Biomphalaria*	*Biomphalaria schrammi*
ASV_1536	Mucoromycota	Mucoromycetes	*Mucor*	*Mucor piriformis*
ASV_1538	Rhodophyta	Florideophyceae	*Gloiophyllis*	*Gloiophyllis barkerae*
ASV_1552	Cnidaria	Hydrozoa	*Margelopsis*	*Margelopsis haeckelii*
ASV_1565	Ascomycota	Sordariomycetes	*Scopulariopsis*	*Scopulariopsis brevicaulis*
ASV_1570	Pseudomonadota	Gammaproteobacteria	*Vibrio*	*Vibrio rumoiensis*
ASV_1571	Ascomycota	Sordariomycetes	*Fusarium*	*Fusarium delphinoides*

ASV_1595	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium sclerotiorum*
ASV_1605	Bacteroidota	Flavobacteriia	*Mesoflavibacter*	*Mesoflavibacter* sp. HG37
ASV_1629	Porifera	Demospongiae	*Cyamon*	*Cyamon neon*
ASV_1642	Arthropoda	Insecta	NA	*Limoniidae* sp. BBDCQ925-1NA
ASV_1677	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter* sp. FW3NA5-3-2-15-E-R2A2
ASV_1699	Chordata	Aves	*Porphyrio*	*Porphyrio melanotus*
ASV_1711	Basidiomycota	Agaricomycetes	*Lactarius*	*Lactarius hatsudake*
ASV_1737	Arthropoda	Insecta	NA	*Elachistidae* gen. elachBioLepNA1 sp. BioLep834
ASV_1770	Arthropoda	Arachnida	NA	*Ereynetidae* sp. BOLD:AAU6279
ASV_1776	Ascomycota	Lecanoromycetes	*Parmotrema*	*Parmotrema ultralucens*
ASV_1794	Tubulinea	Elardia	*Copromyxa*	*Copromyxa protea*
ASV_1849	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium psychrophilum*
ASV_1873	Ascomycota	Leotiomycetes	*Tetracladium*	*Tetracladium breve*
ASV_1874	Ascomycota	Sordariomycetes	*Emericellopsis*	*Emericellopsis* sp. 1a5
ASV_1876	Arthropoda	Insecta	*Coelocrossa*	*Coelocrossa hypocrocea*
ASV_1877	Ascomycota	Sordariomycetes	*Madurella*	*Madurella* sp.
ASV_1894	Ascomycota	Sordariomycetes	*Hirsutella*	*Hirsutella vermicola*
ASV_1895 ASV_1978	Discosea	NA	*Cochliopodium*	*Cochliopodium minus*
ASV_1929	Bacteroidota	Sphingobacteriia	NA	*Sphingobacteriaceae* bacterium GW46NA-11-11-14-LB5
ASV_1952	Oomycota	NA	*Nothophytophthora*	*Nothophytophthora chlamydospora*
ASV_2002	Arthropoda	Insecta	NA	*Hymenoptera* sp. BOLD:ACJ31NANA
ASV_2009	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter riviphilus*
ASV_2010	Tubulinea	Elardia	*Hyalosphenia*	*Hyalosphenia papilio*
ASV_2028	Arthropoda	Insecta	*Dodona*	*Dodona dipoea*
ASV_2063	Ascomycota	Dothideomycetes	*Cercospora*	*Cercospora sojina*
ASV_2064	Arthropoda	Insecta	*Euproctis*	*Euproctis cervina*
ASV_2070	Ascomycota	Sordariomycetes	*Hirsutella*	*Hirsutella thompsonii*
ASV_2116	Bacteroidota	Flavobacteriia	*Nonlabens*	*Nonlabens ulvanivorans*
ASV_2365	Arthropoda	Collembola	*Dicranocentrus*	*Dicranocentrus pallidus*
ASV_2372	Arthropoda	Insecta	*Tatuidris*	*Tatuidris tatusia*
ASV_2373	Evosea	Eumycetozoa	*Didymium*	*Didymium difforme*
ASV_2420	Arthropoda	Collembola	*Xenylla*	*Xenylla boerneri*
ASV_2492	Nematoda	Chromadorea	*Micoletzkya*	*Micoletzkya palliati*
ASV_2500	Bacteroidota	Sphingobacteriia	*Pedobacter*	*Pedobacter foliorum*

ASV_2706	Arthropoda	Arachnida	*Eueremaeus*	*Eueremaeus* sp. BOLD:ADI4824
West				
ASV	**Phylum**	**Class**	**Genus**	**Species**
ASV_8 ASV_24 ASV_4518	Bacteroidota	Flavobacteriia	*Aggregatimonas*	*Aggregatimonas sangjinii*
ASV_10 ASV_249 ASV_409 ASV_1128	Nematoda	Chromadorea	*Rhabditis*	*Rhabditis* sp.
ASV_17 ASV_548 ASV_1573 ASV_2041	Bacteroidota	Flavobacteriia	*Flavobacterium*	*Flavobacterium* sp. KBSNA721
ASV_26 ASV_1199	Platyhelminthes	Cestoda	*Versteria*	*Versteria mustelae*
ASV_42 ASV_1928	Arthropoda	Insecta	*Monopis*	*Monopis crocicapitella*
ASV_75	Ascomycota	Sordariomycetes	*Niveomyces*	*Niveomyces insectorum*
ASV_102 ASV_353 ASV_531 ASV_1185	Ascomycota	Leotiomycetes	*Leohumicola*	*Leohumicola incrustata*
ASV_144 ASV_709 ASV_1125 ASV_1841	Arthropoda	Catenulida	*Stenostomum*	*Stenostomum* cf. simplex AW-2NA18
ASV_310 ASV_3343	Ascomycota	Sordariomycetes	*Akanthomyces*	*Akanthomyces lecanii*
ASV_335	Ascomycota	Sordariomycetes	*Sporothrix*	*Sporothrix* sp. FH-2NA23a
ASV_343	Basidiomycota	Agaricomycetes	*Russula*	*Russula compacta*
ASV_373 ASV_590	Arthropoda	Insecta	*Schinia*	*Schinia sordidus*
ASV_391 ASV_579 ASV_671	Ascomycota	Leotiomycetes	*Pseudofabraea*	*Pseudofabraea citricarpa*
ASV_397 ASV_2490 ASV_3787	Basidiomycota	Agaricomycetes	*Coprinellus*	*Coprinellus micaceus*
ASV_398 ASV_672	Arthropoda	Insecta	*Mimeoma*	*Mimeoma maculata*
ASV_417	Arthropoda	Insecta	*Diaeretiella*	*Diaeretiella rapae*
ASV_440	Arthropoda	Insecta	NA	*Cecidomyiidae* sp. BOLD:ACA9586
ASV_443	Evosea	Eumycetozoa	*Trichia*	*Trichia scabra*
ASV_444 ASV_596	Arthropoda	Insecta	NA	*Lepidoptera* sp. NZAC NA3NA11646
ASV_452 ASV_826	Ascomycota	Dothideomycetes	*Pseudocercospora*	*Pseudocercospora fuligena*
ASV_473 ASV_601	Rotifera	Eurotatoria	*Philodina*	*Philodina hongcheonensis*

ASV_484	Ascomycota	Lecanoromycetes	*Phyllopsora*	*Phyllopsora corallina*
ASV_493	Ascomycota	Dothideomycetes	*Periconia*	*Periconia digitata*
ASV_517	Ascomycota	Dothideomycetes	*Fulvia*	*Fulvia fulva*
ASV_525 ASV_784 ASV_2430 ASV_4794	Basidiomycota	Cystobasidiomycetes	*Cystobasidium*	*Cystobasidium* sp.
ASV_539 ASV_721 ASV_735	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium* sp. ShG4C
ASV_545	Ascomycota	Eurotiomycetes	*Nannizziopsis*	*Nannizziopsis barbatae*
ASV_546 ASV_708 ASV_737 ASV_1066	Ascomycota	Sordariomycetes	*Ceratocystiopsis*	*Ceratocystiopsis pallidobrunnea*
ASV_559 ASV_925	Arthropoda	Insecta	NA	*Anisopodidae* sp. BOLD:AAM639NA
ASV_561	Arthropoda	Insecta	*Leptopteromyia*	*Leptopteromyia* sp. USNM ENT NANA914399
ASV_575	Arthropoda	Arachnida	*Acalitus*	*Acalitus vaccinii*
ASV_593	Arthropoda	Arachnida	NA	*Arachnida* sp. BOLD:ACI5923
ASV_598	Chordata	Actinopteri	*Engraulis*	*Engraulis japonicus*
ASV_621 ASV_1152	Ascomycota	Leotiomycetes	*Leohumicola*	*Leohumicola lenta*
ASV_627	Basidiomycota	Agaricomycetes	*Russula*	*Russula nigricans*
ASV_631	Arthropoda	Insecta	*Colobopyga*	*Colobopyga pritchardiae*
ASV_639 ASV_1650	Bacteroidota	Flavobacteriia	*Tamlana*	*Tamlana crocina*
ASV_682 ASV_1224	Oomycota	NA	*Phytopythium*	*Phytopythium* sp. 'UZ612-like '
ASV_736 ASV_747 ASV_850	Arthropoda	Insecta	*Plutella*	*Plutella xylostella*
ASV_744	Bacillariophyta	Bacillariophyceae	*Gomphonema*	*Gomphonema parvulum*
ASV_796 ASV_4239	Ascomycota	Sordariomycetes	*Thelonectria*	*Thelonectria veuillotiana*
ASV_805	Bacillariophyta	Bacillariophyceae	*Nitzschia*	*Nitzschia* sp. BOLD:AAX5147
ASV_820	Bacteroidota	Chitinophagia	*Lacibacter*	*Lacibacter sediminis*
ASV_833	Gastrotricha	NA	*Chaetonotus*	*Chaetonotus* aff. persimilis MK-2NA19
ASV_847	Arthropoda	Insecta	*Mirax*	*Mirax* sp.
ASV_855	Bacteroidota	Flavobacteriia	*Mangrovimonas*	*Mangrovimonas* sp. YM274
ASV_857	Ascomycota	Leotiomycetes	*Leohumicola*	*Leohumicola terminalis*
ASV_859	Platyhelminthes	Catenulida	*Stenostomum*	*Stenostomum leucops*
ASV_871	Arthropoda	Insecta	*Simulium*	*Simulium lemborense*
ASV_894	Bacillariophyta	Coscinodiscophyceae	*Cyclotella*	*Cyclotella cryptica*

ASV_927 ASV_954	Bacteroidota	Flavobacteriia	*Arenibacter*	*Arenibacter antarcticus*
ASV_996	Rotifera	Eurotatoria	*Keratella*	*Keratella tropica*
ASV_998	Arthropoda	Arachnida	*Mesalgoides*	*Mesalgoides* sp. BIOUG15124-BNA2
ASV_1016	Basidiomycota	Agaricomycetes	*Amanita*	*Amanita basii*
ASV_1019	Rhodophyta	Florideophyceae	*Amansia*	*Amansia glomerata*
ASV_1023	Rotifera	Eurotatoria	*Rotaria*	*Rotaria neptunia*
ASV_1036	Ascomycota	Eurotiomycetes	*Penicillium*	*Penicillium canescens*
ASV_1038	Ciliophora	Oligohymenophorea	*Carchesium*	*Carchesium polypinum*
ASV_1062	Pseudomonadota	Alphaproteobacteria	*Peteryoungia*	*Peteryoungia desertarenae*
ASV_1082	Discosea	NA	*Acanthamoeba*	*Acanthamoeba tubiashi*
ASV_1147 ASV_2638	Bacteroidota	Sphingobacteriia	*Sphingobacterium*	*Sphingobacterium* sp. ML3W
ASV_1159	Arthropoda	Insecta	NA	*Chironominae* sp. BOLD:AAJ4272
ASV_1161	Bacteroidota	Flavobacteriia	*Maribacter*	*Maribacter* sp. R77961
ASV_1173 ASV_1511	Pseudomonadota	Alphaproteobacteria	*Candidatus Megaera*	*Candidatus Megaera polyxenophila*
ASV_1180	Arthropoda	Insecta	*Anax*	*Anax guttatus*
ASV_1242	Arthropoda	Insecta	*Paratanytarsus*	*Paratanytarsus grimmii*
ASV_1292 ASV_1813	Bacillariophyta	Fragilariophyceae	*Ulnaria*	*Ulnaria acus*
ASV_1313	Discosea	Flabellinia	*Korotnevella*	*Korotnevella fousta*
ASV_1446	Discosea	NA	*Acanthamoeba*	*Acanthamoeba astronyxis*
ASV_1463 ASV_2060	Rotifera	Eurotatoria	*Rotaria*	*Rotaria rotatoria*
ASV_1472 ASV_4355	Basidiomycota	Agaricomycetes	*Peniophora*	*Peniophora lycii*
ASV_1484	Arthropoda	Insecta	*Glenea*	*Glenea relicta*
ASV_1549	Discosea	Flabellinia	*Clydonella*	*Clydonella sawyeri*
ASV_1576	Arthropoda	Insecta	*Aploneura*	*Aploneura lentisci*
ASV_1651	Pseudomonadota	Betaproteobacteria	*Nitrosomonas*	*Nitrosomonas ureae*
ASV_1660	Arthropoda	Arachnida	*Euzetes*	*Euzetes globulus*
ASV_1665	Bacillota	Bacilli	*Virgibacillus*	*Virgibacillus* sp. Bac332
ASV_1690	Ascomycota	Eurotiomycetes	*Onygena*	*Onygena corvina*
ASV_1704	Rotifera	Eurotatoria	*Trichocerca*	*Trichocerca* sp. WM-2NA17e
ASV_1782 ASV_2245	Bacteroidota	Flavobacteriia	*Maribacter*	*Maribacter* sp. R86514
ASV_1795	Chordata	Actinopteri	*Stolephorus*	*Stolephorus* sp.
ASV_1800	Bacteroidota	Flavobacteriia	*Formosa*	*Formosa agariphila*
ASV_1824	Bacillota	Bacilli	*Sporosarcina*	*Sporosarcina* sp. PTS23NA4
ASV_1897	Ascomycota	Eurotiomycetes	*Exophiala*	*Exophiala lecanii-corni*
ASV_2001	Bacteroidota	Flavobacteriia	*Aestuariibaculum*	*Aestuariibaculum lutulentum*

ASV_2022	Pseudomonadota	Gammaproteobacteria	*Methylomonas*	*Methylomonas denitrificans*
ASV_2113 ASV_2431	Chordata	Actinopteri	*Forsterygion*	*Forsterygion flavonigrum*
ASV_2114	Arthropoda	Arachnida	NA	*Oribatellidae* sp. BIOUGNA8437-GNA8
ASV_2491	Ascomycota	Dothideomycetes	*Edenia*	*Edenia gomezpompae*
ASV_2506	Chordata	Aves	*Cyanoramphus*	*Cyanoramphus novaezelandiae*
ASV_2507	Apicomplexa	Conoidasida	*Goussia*	*Goussia bayae*
ASV_2660	Arthropoda	Insecta	*Telomerina*	*Telomerina flavipes*
ASV_2861	Basidiomycota	Agaricomycetes	*Candolleomyces*	*Candolleomyces candolleanus*
ASV_3130	Arthropoda	Insecta	*Heterarthrus*	*Heterarthrus* sp. 1 Daocheng
ASV_3257	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum jinshuiense*
ASV_3340 ASV_4438	Ascomycota	Lecanoromycetes	*Hypogymnia*	*Hypogymnia vittata*
ASV_3374	Pseudomonadota	Gammaproteobacteria	*Oceanisphaera*	*Oceanisphaera avium*
ASV_3454	Arthropoda	Arachnida	NA	*Eupodidae* sp. BOLD:ADU4475
ASV_3498	Bacteroidota	Flavobacteriia	*Cellulophaga*	*Cellulophaga* sp. HaHaR 3 176
ASV_3501	Bacteroidota	Flavobacteriia	*Oceanihabitans*	*Oceanihabitans* sp. IOP 32
ASV_3558	Arthropoda	Insecta	NA	*Coleoptera* sp. NZAC NA3NANA9NA96
ASV_3606	Ascomycota	Dothideomycetes	*Septoria*	*Septoria linicola*
ASV_3674	Ascomycota	Sordariomycetes	*Madurella*	*Madurella mycetomatis*
ASV_3727	Arthropoda	Collembola	*Orthonychiurus*	*Orthonychiurus folsomi*
ASV_3782	Bacteroidota	Sphingobacteriia	*Mucilaginibacter*	*Mucilaginibacter mallensis*
ASV_3786	Ascomycota	Lecanoromycetes	*Graphis*	*Graphis lineola*
ASV_3862	Pseudomonadota	Alphaproteobacteria	*Rhizobium*	*Rhizobium leguminosarum*
ASV_3923	Ascomycota	Leotiomycetes	*Podosphaera*	*Podosphaera xanthii*
ASV_3928	Arthropoda	Arachnida	*Anachipteria*	*Anachipteria* sp. BIOUG25375-D12
ASV_3931	Ascomycota	Eurotiomycetes	*Aspergillus*	*Aspergillus magnivesiculatus*
ASV_4002	Arthropoda	Arachnida	*Propelops*	*Propelops* sp. BOLD:ADI4824
ASV_4079	Oomycota	NA	*Lagenidium*	*Lagenidium* sp. PWL-2NA1NAf
ASV_4173	Basidiomycota	Agaricomycetes	*Porogramme*	*Porogramme epimiltina*
ASV_4175	Pseudomonadota	Alphaproteobacteria	*Palleronia*	*Palleronia* sp. THAF1
ASV_4234	Bacteroidota	Flavobacteriia	*Winogradskyella*	*Winogradskyella* sp. HaHa 3 26
ASV_4236	Arthropoda	Arachnida	NA	*Trombidiformes* sp. BOLD:AAHNA995
ASV_4354	Basidiomycota	Agaricomycetes	*Lactarius*	*Lactarius* sp.
ASV_4439	Basidiomycota	Malasseziomycetes	*Malassezia*	*Malassezia restricta*
ASV_4627	Ascomycota	Sordariomycetes	*Colletotrichum*	*Colletotrichum tamarilloi*

ASV_4628	Ascomycota	NA	NA	*Ascomycota* sp.
ASV_4786	Ascomycota	Sordariomycetes	*Hirsutella*	*Hirsutella rhossiliensis*