Manaaki Whenua
Landcare Research

The habitat value of mānuka for invertebrates following the establishment of a mānuka plantation: project plan

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The habitat value of mānuka for invertebrates following the establishment of a mānuka plantation: project plan

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1 Introduction

Mānuka (*Leptospermum scoparium*) is a fast-growing and robust plant inhabiting shrubland and forest margins throughout New Zealand and will thrive in almost all land types including geothermal areas and dry hill slopes. It has a long history of medicinal uses, especially for Māori (Walls 1998), and recently bioactive compounds have been found in mānuka oil and in the honey produced from its nectar (Douglas et al. 2004; Williams et al. 2014). Mānuka products are highly valued internationally and demand for them is rapidly increasing. In the last 10 years, there has been strong interest in growing mānuka in New Zealand to diversify or supplement farm incomes, or as a new primary income (Saunders 2017).

Planting mānuka offers opportunities to increase the returns on marginal land on farms, while provding a number of environmental benefits, including erosion control, reducing sediment and nutrient loads to waterways, and restoring native habitat for a diverse range of plants and animals over large areas at low cost. Income can also be earned from other sources such as carbon credits, and some regional councils may provide funding assistance for new plantations, particularly on land susceptible to erosion (Saunders 2017).

In July 2017, planting of over 100 ha of mānuka occurred on Taurapa Station, Hawke's Bay. This provides an opportunity to establish a baseline of the habitat value for biodiversity (e.g. soil microbial community, soil fauna, and other invertebrates) of the land under its existing use for pastoral grazing, and measure how this changes over time as the mānuka plantation matures. Changes in the physical structure of the habitat (e.g. vegetation) will be measured along with specific invertebrate groups and their responses to the habitat change. DNA metabarcoding technology (also known as 'environmental DNA' or 'eDNA') is rapidly advancing and previous work shows it is a cost-effective method to address these research questions (Watts et al. in press). However, this method is limited due to the lack of DNA reference barcodes at the species level and can only detect changes in relative abundance of invertebrates. Conventional invertebrate sampling and counting techniques provide a method of rapidly collecting a diverse range of invertebrates and the ability to determine changes in the species richness and absolute abundances of taxa. In addition, specimens can be identified to species level allowing new invertebrate DNA sequences to be added to the reference database.

In October 2018, the Government set a goal to plant one billion trees between 2018 and 2027 (https://www.mpi.govt.nz/funding-and-programmes/forestry/planting-one-billion-trees/ accessed 18 October 2018). This goal has a range of benefits: diversifying farm incomes; improving land productivity, land stability, and water quality; reducing the effects of climate change; enhancing the natural landscapes; and creating jobs. However, there are very few longitudinal datasets that measure soil carbon over time and how the soil changes from pasture to native vegetation.

This project will sample invertebrates in three habitat types on Taurapa Station, including 1) recently (July 2017) planted mānuka, 2) adjacent rank pastoral habitat, and 3) old-growth mānuka/kānuka. Identified species from conventional sampling will be DNA

barcoded to add to the reference sequence database. In addition, soil samples will be collected to measure soil carbon over time.

2 Objectives

- Use conventional and DNA metabarcoding methods to monitor invertebrate community change over time in three habitat types (recently planted mānuka, adjacent rank pasture, and old-growth mānuka/kānuka) on Taurapa Station.
- Quantify the co-benefits of mānuka planting for above- and below-ground carbon.
- Barcode the DNA of at least 150 identified species to increase the number of barcodes for species in reference sequence databases.

3 Methods

3.1 Study area and sampling design

Taurapa Station (1,948 ha) has approximately 1,700 cattle and 7,500 sheep on hill country located near Ocean Beach, Hawke's Bay. Environmental work, including Maraetotara Stream restoration, planting mānuka, threatened bird conservation, pest and weed control, and engaging with the Cape to City programme also occurs on Taurapa. In July 2017, approximately 100 ha on Taurapa Station were retired for grazing and planted with mānuka primarily for hill-slope stabilisation but also for future mānuka honey production (Hayden Rees-Jones, Horizons Farming Limited, pers. comm., 2018). In September 2018, mānuka plants were approximately 30−50 cm high among 30-cm-high rank pasture (Fig. 1). In addition, there are fragments of 'old growth' mānuka/kānuka present on Taurapa (Fig. 2) and these will be used as 'target' or 'reference' communities allowing for comparison with the planted mānuka. The old growth mānuka/kānuka is approximately 80–100 years old and is 5−8 m high. The proportion of mānuka/kānuka is the fragments was approximately 90% with a ground cover of rank pasture species. These fragments were retired from grazing in July 2017. Both the planted mānuka blocks and old growth mānuka/kānuka fragments are on steep south-facing slopes (Fig. 2).

Four 10×10 m sampling plots will be located within 1) recently (July 2017) planted mānuka, 2) adjacent rank pastoral habitat, and 3) old-growth mānuka/kānuka on Taurapa Station. Plots will be at least 50 m apart.

Figure 1. Mānuka, approximately 30-50 cm high (as at Sept 2018), was planted in retired pasture in July 2017.

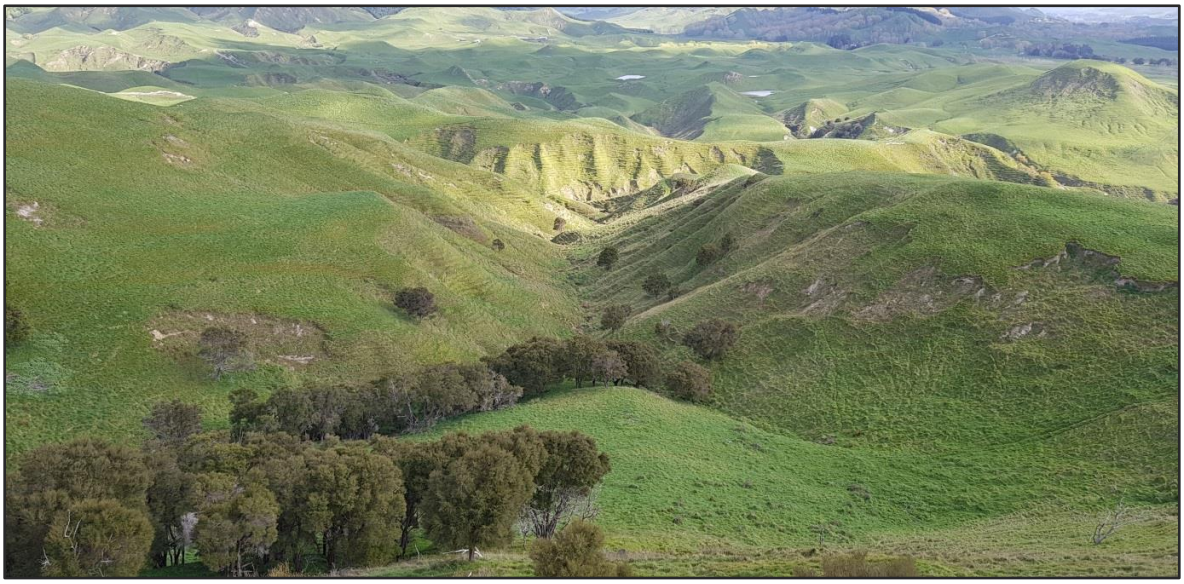

Figure 2. The majority of the gullies with slips in the photograph have been retired from grazing and planted with mānuka in July 2017. The old growth mānuka/kānuka fragments can be seen in the bottom left of the photograph.

Within each 10 x 10m square plot, vegetation composition will be estimated in height tiers (<0.3 m, 0.3–2 m, 2–5 m, 5–12 m, and 12–25 m) using the RECCE method outlined by Hurst & Allen (2007). Vegetation biomass estimates at each plot will be characterised annually using standard methods (i.e., heights and two orthogonal widths of individuals, diameters at breast height once individuals are large enough; use of allometric relationships to estimate biomass; Holdaway et al 2017). The physiography (ridge, face, gully or terrace), slope (convex, concave or linear) and drainage (good, moderate, poor or

very poor) will also be determined. In addition, soil carbon will be measured in each plot by collecting five soil cores across each plot, separating the organic horizon from the top 100 mm of the mineral horizon, pooling within horizons across the five cores, and analysing the homogenised samples for total carbon.

3.2 Conventional invertebrate collection and identification

Invertebrates will be sampled using a modified malaise trap (Watts et al. 2012) and pitfall traps placed in the centre of each 10 ×10 m plot. The malaise trap collecting jar will contain 150 ml of 100% monopropylene glycol and the jar will be orientated northward. Four pitfall traps (each a 105-mm diameter cup containing 75 ml of 100% monopropylene glycol), will be dug 3 m from the four corners of the malaise trap within each plot. Traps will be set for 1 month from mid-December 2018 to mid-January 2019. Samples will be taken annually for 10 years (and then possibly every 5 years after that). Invertebrates will be preserved in 100% ethanol.

Invertebrates will be sorted and counted to Order level using a binocular microscope. Coleoptera, Diptera, and Hymenoptera will be sorted to recognised taxonomic units (hereafter referred to as species) and, where possible, given generic and species-level identifications by an expert diagnostician (Stephen Thorpe, independent diagnostic researcher). After sorting and counting, the specimens from the malaise trap and four pitfall traps from each plot will be pooled into a single bulk invertebrate sample for DNA analyses.

3.3 DNA collection and analyses

DNA barcoding of invertebrate specimens

Representative specimens will be selected for DNA barcoding during the sorting and counting process. Specimens and groups will be prioritised for DNA barcoding based on criteria such as taxonomic representativeness, database representation, and abundance. These specimens (or fragments thereof) will be subjected to DNA extraction followed by PCR amplification of the COI subunit *c* DNA barcode region (Folmer et al. 1994). The PCR amplicons will be sequenced either using conventional Sanger sequencing methods, or high-throughput sequencing techniques. The resulting sequences will be error-corrected and incorporated into reference sequence databases.

DNA metabarcoding of soil samples and bulk invertebrates

Soil is a well-known repository for environmental DNA from both soil-dwelling invertebrates (mites, nematodes, earthworms, larvae) and terrestrial invertebrates (dead carcases, exoskeletons, frass) (Drummond et al. 2015). As such, soil samples could provide a single easy way to collect sample media for eDNA invertebrate analysis. In addition to the bulk invertebrate sample, soil samples will be collected form each plot. A total of 4 soil cores (organic and mineral horizons; 4–6 kg in total) will be collected per plot, with samples randomly located following standard sampling protocols (Watts et al. in press)

using sterilised trowels. This soil will be stored at 4 degrees and transferred to the lab within 5 days of collection for DNA extraction.

Each bulk invertebrate sample will be ground into a paste and homogenised, after which DNA will be extracted from 300 mg of the material using standard methods. The soil samples from each plot will be homogenised, and a 10-g sample taken from which DNA will be extracted following standard protocol. Invertebrate DNA from both the soil and bulk invertebrate samples will be isolated and amplified by PCR using invertebrate-targeted primers and will follow standard methods. The PCR amplicons will be sequenced on an Illumina MiSeq system.

Processing of DNA metabarcoding data

Raw DNA sequences will be merged, demultiplexed, trimmed of adapters and primers, filtered for errors, and grouped into OTUs (or denoised) using USEARCH and/or VSEARCH. Sequences and OTUs will be assigned a taxonomic identity by comparison against reference sequence databases, and against the specimen DNA barcodes.

3.4 Analysis

The biodiversity and composition of above- and below-ground invertebrates (and microbes) will be determined and compared among the different plot/vegetation types. Shifts in community composition in response to vegetation growth will be determined by comparing results over time; for example, which species or groups are consistently present, and which groups increase or decrease with mānuka growth?

4 Milestones planned for 2018 and 2019

It is anticipated that this project will be a multiyear research project. Table 1 outlines the milestones to be completed in 2018 and 2019.

Table 1. Milestones to be completed in 2018 and 2019.

Milestone	Completion date
Project set up	21 Dec 2018
Year 1 conventional invertebrate and DNA sampling	20 Jan 2019
Conventional invertebrate identification	30 Mar 2019
Invertebrate taxa barcoding	30 June 2019
Year 1 DNA metabarcoding analyses	30 Oct 2019
Set conventional invertebrate traps for Year 2 sampling	31 Dec 2019

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6 References

Douglas MH, van Klink JW, Smallfield BM, Perry NB, Anderson RE, Johnstone P, Weavers RT 2004. Essential oils from New Zealand manuka: triketone and other chemotypes of *Leptospermum scoparium*. Phytochemistry 65: 1255–1264.

Drummond AJ, Newcomb RD, Buckley TR, Xie D, Dopheide A, Potter BCM, Heled J, Ross HA, Tooman L, Grosser S, Park D, Demetras NJ, Stevens MI, Russell JC, Anderson SH, Carter A, Nelson N 2015. Evaluating a multigene environmental DNA approach for biodiversity assessment. GigaScience 4: 46.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Hurst J, Allen R. 2007. The Reece method for describing New Zealand vegetation – Field protocols. Manaaki Whenua – Landcare Research, Lincoln. 41 p.

Saunders L 2017. The mānuka and kānuka plantation guide: April 2017. Unpublished report by Boffa Miskell New Zealand.

Walls G 1998. Threatened plants of Hawke's Bay. Conservation Advisory Science Notes No. 200. Wellington, Department of Conservation. 32 p.

Watts C, Rohan M, Thornburrow D 2012. Beetle community responses to grey willow (*Salix cinerea*) invasion within three New Zealand wetlands. New Zealand Journal of Zoology 39: 209–227.

Watts C, Dopheide A, Holdaway R, Davis C, Wood J, Thornburrow D, Dickie I. In press. DNA metabarcoding as a tool for invertebrate community monitoring – a case study comparison with conventional techniques. Austral Entomology.

Williams S, King J, Revell M, Manley-Harris M, Balks M, Janusch F, Kiefer M, Clearwater M, Brooks P, Dawson M 2014. Regional, annual, and individual variations in the dihydroxyacetone content of the nectar of mānuka (*Leptospermum scoparium*) in New Zealand. Journal of Agricultural and Food Chemistry 62: 10332.