New Zealand Journal of Ecology (2025) 49(1): 3621 © 2025 The Author(s), under a CC BY 4.0 licence

NEW ZEALAND JOURNAL OF ECOLOGY

RESEARCH

Assessing the impact of honey bees on invertebrate communities in mānuka shrublands in New Zealand

Corinne H. Watts*[1], Sarah J. Richardson[2], Carina Davis[2], Stephen E. Thorpe[3]†, Danny Thornburrow[1], Brenda Tahi[4], Bubs Smith[5], Catherine Beard[6], Robert J. Holdaway[2] & Andrew Dopheide[7]

[1] Bioeconomy Science Institute, Manaaki Whenua – Landcare Research Group, Private Bag 3127, Hamilton 3216, New Zealand
[2] Bioeconomy Science Institute, Manaaki Whenua – Landcare Research Group, PO Box 69040, Lincoln 7640, New Zealand
[3]Independent researcher, Auckland, New Zealand
[4]Tūhoe Tuawhenua Trust, PO Box 3001, Ruatāhuna, Rotorua, New Zealand
[5]Te Rūnanganui o Ngāti Hikairo ki Tongariro, Tūrangi, New Zealand
[6]Department of Conservation – Te Papa Atawhai, PO Box 10420, Wellington 6143, New Zealand
[7] Bioeconomy Science Institute, Manaaki Whenua – Landcare Research Group, Private Bag 92170, Auckland 1142, New Zealand
†Passed away 24 August 2024
*Author for correspondence (Email: wattsc@landcareresearch.co.nz)

Abstract: The Western honey bee (*Apis mellifera* L.) is an important species for crop pollination and honey production. New Zealand has a major wealth-creation opportunity through the production and export of honeys sourced from native flowers. However, honey bees are not native to New Zealand, and the impacts of commercial honey production and honey bee apiaries on native ecosystems are largely unknown. In this study, we used DNA metabarcoding of bulk flowers and bulk Malaise trapped invertebrate samples alongside conventional specimen identifications to compare invertebrate communities in mānuka (*Leptospermum* spp.) shrublands with and without honey bee apiaries. Diptera, Hemiptera, Hymenoptera, and Lepidoptera each had 44–47% lower amplicon sequence variant (ASV) richness on flowers from apiary sites compared to non-apiary sites, suggesting honey bees disrupt flower visits by these other invertebrates. Conversely, Thysanoptera (thrip) ASV richness was 64% higher on flowers from apiary sites compared to non-apiary sites, suggesting transport of thrips between flowers by honey bees. Apiary sites also had lower abundances of conventionally identified specimens of Lepidoptera and a pool of unidentified small invertebrates compared to non-apiary sites. In contrast, DNA metabarcoding of Malaise-trapped bulk invertebrates suggested that honey bee effects on these communities may be site-dependent, with differing richness of Hymenoptera, Lepidoptera, and Orthoptera ASVs between site pairs but not between sites with and without apiaries. Multivariate analyses of community composition also suggested that honey bees had consistent effects on flower communities, but not on bulk invertebrate communities. We recommend future studies with greater replication of site pairs along key environmental gradients to complement and increase interpretability of the results presented here.

Keywords: *Apis mellifera*, community composition, DNA metabarcoding, honey production, Malaise traps, mānuka vegetation

Introduction

The Western honey bee (*Apis mellifera* L.) has been deliberately introduced into many regions for honey production and improving crop pollination, and the species now has a near-global distribution (Geldmann & Gonzalez-Varo 2018). Introductions of honey bees into New Zealand first occurred in the late 1830s and continued until the 1980s (Beard 2015) with a recent development of a major wealth-creation opportunity through the production and export of honeys sourced from native flowers. The impacts of commercial apiaries and honey extraction on New Zealand's natural ecosystems are largely unknown. Potential impacts include displacement of native pollinators, altered invertebrate, fungal, and bacterial communities, disease transmission, modified pollination and seed set by native plants, and enhanced pollination and seed set of non-native plant species (Beard 2015).

Knowledge of invertebrate species richness, abundance, distributions, and interactions is essential for understanding the potential impacts of honey bees on native communities. There

DOI: https://doi.org/10.20417/nzjecol.49.3621

are increasing examples in the international literature of honey bees outcompeting native invertebrate species (Geldmann & González-Varo 2018; Valido et al. 2019; Page & Williams 2023). For example, solitary or semi-social bees are often outcompeted by honey bees (Beard 2015). In recent studies, honey bees displaced native bees and reduced wildflower pollination in the Sierra Nevada (Page & Williams 2023), and disrupted flower-pollinator networks in Australia (Prendergast & Ollerton 2022) and the Canary Islands (Valido et al. 2019). In New Zealand, there is limited knowledge regarding the impacts of honey bees on the native invertebrate fauna. Donovan (1980) suggested that co-occurrence of native bee species with honey bees indicates that native bees can compete with honey bees. However, more recently, some populations of native bee species have declined substantially, which could be related to competition with honey bees (Donovan 2007; Hart 2016). One study revealed that body size, diversity and abundance of native pollinating invertebrates was significantly lower in sites with honey bees compared to those without honey bees (Jackson 2019). Other studies have shown that the abundances of large flies were negatively correlated with honey bee abundance (Murphy & Robertson 2000; Bennik 2009). In addition, Nepia (2020) found that honey bees were the key driver of differences in flower visitor diversity and the structure of interaction networks in submontane native forest in New Zealand's North Island. In contrast, Iwasaki (2017) found little evidence of competition between honey bees and native bees after beehives were introduced to montane habitat in New Zealand's South Island, attributed to clear preferences for different plant taxa.

Honey bee impacts on native biodiversity are likely to scale with the density of hives, but the nature of those scaling relationships may vary among taxa. The most sensitive taxa may be adversely affected by minimal hive densities and can only be maintained in areas without bee hives. On the other hand, some taxa may be more resilient to bee hives and will only be affected at very high hive densities. In between these extremes, many taxa will be affected in proportion to hive density. Quantifying these effects could enable the maintenance of native invertebrate communities alongside honey production, by allowing managers to select hive densities that minimise biodiversity loss. A first step in this direction is to compare areas with high hive densities to those with none, since we might expect the greatest differences in invertebrate communities under these conditions.

Sampling and identifying invertebrate communities is often time consuming, expensive, and restricted by the limited number of people with taxonomic expertise. DNA-based approaches offer tremendous promise for comprehensive, efficient, and cost-effective sampling of invertebrate communities (Holdaway et al. 2017). These DNA techniques are becoming widely used in ecological studies, but they are imperfect and benefit from validation alongside traditional invertebrate sampling methods. Applications in New Zealand suggest that DNA metabarcoding can detect differences in community composition that are consistent with those achieved by Malaise trapping and specimen counts, while also providing data on a much broader taxonomic range of invertebrates than conventional monitoring for a given cost (Watts et al. 2019). Furthermore, DNA metabarcoding can be used to detect insect DNA upon flowers (Thomsen & Sigsgaard 2019; Johnson et al. 2023), which might provide evidence of honey bee disruption of native insect-flower interactions.

Mānuka (*Leptospermum scoparium*[1], Myrtaceae) is a small tree with a widespread distribution in New Zealand that commonly dominates ex-pastoral secondary successions and waterlogged or infertile soils (Wardle 1991; McCarthy et al. 2021). Honey derived from mānuka nectar is renowned for its therapeutic properties and is highly valued internationally, underpinning a robust apiculture industry in New Zealand (Ministry for Primary Industries 2020; El-Senduny et al. 2021). Due to the large number of honey bee hives being placed into mānuka-dominated shrublands, there is potential for both direct and indirect competitive interactions between honey bees and native invertebrates. However, within New Zealand, there are no published studies of which the authors are aware that examine the impacts of honey bees on broad invertebrate communities; nor studies using DNA metabarcoding methods to investigate these questions.

Apiaries are established in mānuka shrublands with the aim of collecting monofloral mānuka honey, implying that the influence of honey bees might be most apparent in the flower-visiting community and then extend outward to affect the broader community. In this study, therefore, we used DNA metabarcoding to analyse insect DNA on and in mānuka flowers in shrublands with and without honey bee apiaries, to investigate whether there was a discernible effect of honey bees upon flower-visiting communities. Furthermore, we compared invertebrate communities between mānuka shrubland sites with and without honey bee apiaries by applying both DNA metabarcoding and conventional morphological analysis techniques to Malaise-trapped invertebrate communities. Specifically, we examined whether invertebrate communities in sites with honey bees present would have different species richness, abundance, and community composition compared to those in sites without honey bees.

Methods

Study areas

We selected a pair of sample sites with naturally occurring mānuka shrublands in three locations in the central North Island: Tuawhenua, Tongariro Frost Flat, and Tongariro Alpine (Fig. 1; Table 1). In each pair, one site had an apiary, and the other site did not. Apiary sites had between 36 and 55 hives, distributed within a similar area at each pair. Both sites in each pair had otherwise similar attributes, e.g. elevation, mean annual temperature (MAT), mean annual rainfall (MAR), dominant woody species (Table 1). At each site, 3 plots (each 10 × 10 m) were located at least 50 m apart.

A key aspect of our study design was selecting non-apiary areas of mānuka where honey bees were not likely to be dominant; our sites without apiaries were always > 6 km from a site with apiaries, separated by non-mānuka vegetation (Table 1). This distance was based on (1) expert knowledge from local beekeepers that bees rarely travel > 2 km in mānuka; and (2) a United Kingdom study that showed that honey bees

---

[1] The common name mānuka/kahikātoa is applied to several published taxa in NZ including *Leptospermum hoipolloi f. hoipolloi* L.M.H.Schmid & de Lange, *Leptospermum hoipolloi f. incanum* (Cockayne) de Lange & L.M.H.Schmid , *Leptospermum hoipolloi f. procumbens* L.M.H.Schmid & de Lange, *Leptospermum repo* de Lange & L.M.H.Schmid, *Leptospermum tairawhitiense* G.J.Atkins, de Lange & M.A.M.Renner, and *Leptospermum scoparium* J.R.Forst. & G.Forst. Note that this taxonomy is disputed by Chagné et al. (2023).

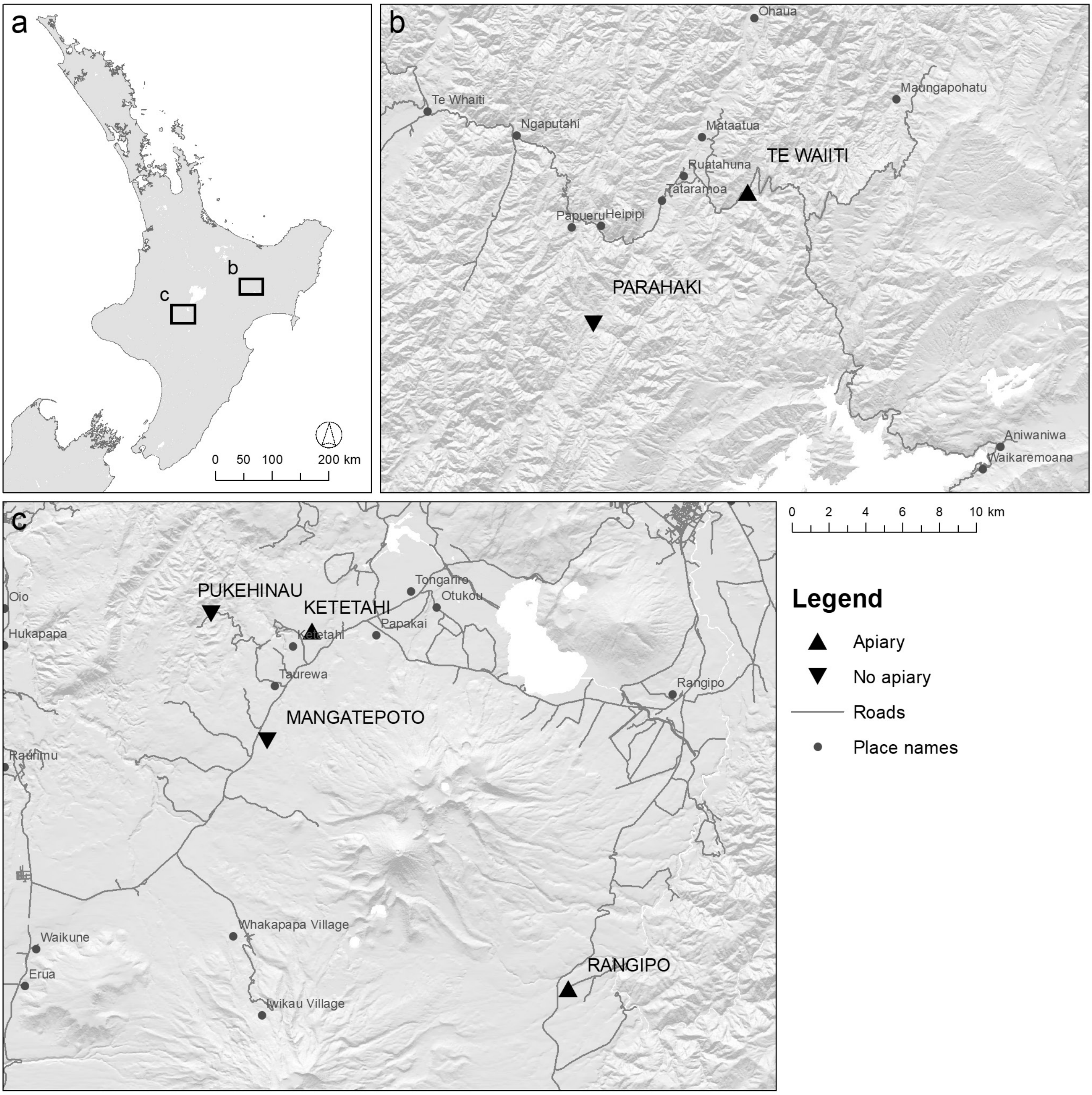

Figure 1. Study site locations: (a) within New Zealand, (b) expanded detail showing Tuawhenua site, and (c) expanded details of the two Tongariro sites: Tongaririo Alpine (Mangatepopo and Rangipo) and Tongariro Frost Flat (Pukehinau and Ketetahi) sample sites. In the expanded images, names in CAPITALS are study site names.

spent most of their time within 1 km of the hive but routinely travelled ≥ 5 km to a profitable nectar supply, while 95% of bees foraged within 10 km from the hive (Beekman & Ratnieks 2000). Apiary sites in mānuka were selected in consultation with local beekeepers; these plots were always situated within 250 m of the hives and measured using a GPS.

Sampling of bulk flowers

In each 10 × 10 m plot, five plants were randomly selected for flower sampling. Two sets of 20 mature flowers were collected from each plant using gloves and sterilised scissors, and pooled together, resulting in two bulk flower samples per plot, each containing 100 flowers. Due to uncertainty about optimal preservation methods, one bulk flower sample per plot was preserved in 95% ethanol, while the other was unpreserved. A total of 3600 flowers (18 bulk flower samples) were collected. The flower samples were kept cool in the field and stored at −20°C in the laboratory until DNA extraction.

Sampling and identification of invertebrate communities

Modified Malaise traps (Watts et al. 2012, 2019) were used to collect the invertebrate fauna associated with mānuka

Table 1. Attributes of sample sites.

Site pair	**Site**	**Number of hives within 2.5 km of apiary sites**	**Location**	**Elevation (m)**	**MAT (°C)**[†]	**MAR (mm)**[†]	**Species with ≥ 25% cover**[††]	**Mean top height**[††] **(m)**
Tuawhenua	Parahaki	None	E1938885 N5710315	565	10.8	1850	*Leptospermum scoparium*	2.7
	Te Waiiti	40	E1947682 N5717820	510	11.1	1844	*L. scoparium*	2.9
Tongariro Frost Flat	Pukehinau	None	E1817274 N5675431	615	10.3	2103	*L. scoparium, Calluna vulgaris,*	2.0
	Ketetahi	36	E1822772 N5674512	730	9.6	2268	*L. scoparium, C. vulgaris, Phormium tenax*	2.0
Tongariro Alpine	Mangatepopo	None	E1820327 N5668526	915	8.5	2520	*L. scoparium, C. vulgaris*	1.6
	Rangipo	55	E1836711 N5655077	950	8.3	2215	*L. scoparium, C. vulgaris, Dracophyllum longifolium*	1.7

[†] Mean Annual Temperature (MAT) and Mean Annual Rainfall (MAR) estimated from the climate layers developed for the LENZ classification of Leathwick et al. (2003)

[††] Cover and top height measured using the RECCE method of Hurst & Allen (2007)

flowers and foliage. A Malaise trap was placed in the centre of each plot (3 locations × 2 sites × 3 plots = 18 Malaise traps in total). In each case, a collection jar containing 300 ml of 100% monopropylene glycol was placed at the northern end of the trap. Traps were set for 1 month, from approximately 19 December 2016 to 17 January 2017, during peak mānuka flowering. The collected invertebrates were preserved in 100% ethanol.

All invertebrate specimens were sorted to taxonomic order level and counted using a binocular microscope. Most specimens (> 90%) belonged to Diptera or Hymenoptera; any of these specimens that were ≥ 5 mm in body length (or easily recognisable) were identified to morphospecies (referred to as 'species' hereafter) based on external morphology, as were all Coleoptera specimens. Where possible, these morphospecies were given genus- and species-level identifications, and classified as native, introduced, or of unknown status. Because of the large numbers of small (< 5 mm) hymenopteran and dipteran specimens, their abundances in each sample were inferred from replicate subsamples of the total specimen pool (see full details of the modified counting technique in Appendix S1 in Supplementary Material). Any orders representing < 0.1 % of specimens were grouped together as 'Others' for analysis; and any specimens that could not be identified to a taxonomic order due to their very small size or other reasons (e.g. specimen damage) were referred to as 'Unidentified'.

Molecular laboratory procedures

DNA was extracted from the unpreserved and ethanol-preserved flower samples using sterile phosphate buffer saline (40 ml) added first to each unpreserved bulk flower sample, but not to the ethanol-preserved bulk flower samples. Both ethanol-preserved and unpreserved bulk flower samples were then each mixed on a horizontal shaker for 3 minutes at c. 2000 revolutions per minute (rpm), centrifuged for 2 minutes at 11 000 relative centrifugal force (rcf), and filtered through a 25 mm polycarbonate hydrophilic membrane with 0.2 µm pore size (Millipore, Merck KgaA, Darmstadt, Germany) by vacuum suction. The filters were then cut into pieces from which DNA was extracted using a Macherey-Nagel Nucleospin 96 Soil DNA extraction kit according to its manufacturer's instructions, with a Janus Automated Liquid Handling System.

Invertebrate specimens from the Malaise traps were pooled into a bulk sample for each plot and frozen in liquid nitrogen and then crushed with a mortar and pestle to a homogenous texture. The equipment used in this procedure was sterilised with 10% bleach, 70% ethanol, and UV light between processing of each sample. Duplicate DNA extractions were carried out from each homogenised invertebrate sample, using a Machery-Nagel NucleoSpin 96 Soil extraction kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany). For each extraction, approximately 350 mg of homogenised invertebrate material was combined with 700 µl of lysis buffer, 150 µl SX enhancer and 10 µl proteinase K (20 mg ml^{-1}), vortexed to mix, and incubated overnight at 56°C with shaking. The extractions were completed according to the manufacturer's instructions using a Janus Automated Liquid Handling System (PerkinElmer, Waltham, MA, USA), and eluted in 2 × 100 µl elution buffer. The duplicate DNA extracts from each sample were pooled together for polymerase chain reaction (PCR) processing.

A 313 base pair (bp) region from the 3′ end of the metazoan cytochrome *c* oxidase subunit I (COI) barcode gene (Folmer et al. 1994) was amplified in duplicate PCRs from each bulk flower DNA extract, and from each bulk invertebrate DNA extract, using the primers miCOIintF (5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′; Leray et al. 2013) and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′; Folmer et al. 1994). The COI PCRs were carried out in 20 µl volumes, each containing 10 µl KAPA 3G Plant 2X buffer, 2 µg rabbit serum albumin (RSA), 200 nM of each primer, 0.5 U KAPA 3G Plant Taq, 0.8 µl water, and 4 µl DNA template. The PCR temperature protocol was 95°C for 180s; 35 cycles of 95°C for 20s, 52°C for 15s, 72°C for 30s; and 72°C for 60s. Sample-specific barcode indices and sequencing adaptors were added to the amplicons from the first PCR in a second round of PCR. Each second-round PCR contained 10 µl KAPA 3G Plant 2X buffer, 2 µg RSA, 0.5 µl additional Mg^{2+} (25 mM, for a final concentration of 2 mM), 200 nM of each primer, 0.5 U KAPA 3G Plant Taq, 2.3 µl of water, and 2 µl of the first PCR amplicons as template. The temperature cycle for the second stage PCRs was: 95°C for 3 minutes; five cycles of 95°C for 20 seconds, 54°C for 15 seconds, 72°C for 30s; 72°C for 1 minute. DNA extraction blanks, and negative and positive PCR controls were processed alongside the bulk flower DNA and bulk invertebrate extracts. The PCR amplicons from all the samples and controls were purified using a Pippen™ Prep size fractionator (Sage Science, Beverley, MA, USA), quantified, and combined into one pool, which was sequenced in a 2 × 250 bp Illumina MiSeq run by the Australian Genomics Research Facility.

Bioinformatic processing of sequence data

Raw forward and reverse sequence reads per sample were merged and relabelled by sample using fastq_mergepairs in VSEARCH v2.21.1 (Rognes et al. 2016). The merged sequences were trimmed of primers using cutadapt v1.11 (Martin 2011), retaining only sequences in which both forward and reverse primers were detected. Using VSEARCH again, sequences with any undetermined nucleotides, more than one expected error, and/or length outside 300 to 320 bp, were discarded by fastx_filter, after which any duplicate sequences were removed. The remaining sequences were denoised into amplicon sequence variants (ASVs) (Callahan et al. 2017) using cluster_unoise, after which the merged and trimmed sequences were mapped to the denoised ASV sequences at the 97% identity threshold using usearch_global, resulting in a table of ASV abundances per sample. ASVs were identified using SINTAX (Edgar 2016) against the UNIQ SP COI GB256 component of the MIDORI2 database (Leray et al. 2022). ASVs identified to an arthropod order with a bootstrap score ≥ 0.7 were retained for subsequent biodiversity analyses. Any orders representing < 0.5% of ASVs were grouped together as 'Others' for analysis.

The sequence data were then curated using lulu v0.1.0 (Frøslev et al. 2017), which merges spurious low-abundance ASVs with higher-abundance ASVs based on patterns of sequence similarity and distribution across samples. Negative control samples were removed and ASV abundances adjusted accordingly using the R package microDecon v1.0.2 (McKnight et al. 2019).

Biodiversity analyses

We used a suite of statistical tools to test for differences in the abundance, species richness, and composition of overall invertebrate communities, orders, and individual species between apiary and non-apiary treatments (apiary status) across the three pairs of sites. All analyses were carried out using R v4.4.2 and Rstudio v2023.06.0, including dplyr v1.1.4 (Wickham et al. 2022) for data manipulation and ggplot2 v3.5.2 (Wickham 2016) for data visualisation. We used analyses of variance to test for richness and abundance differences between sites with and without apiaries and between different pairs of sites, with an interaction between apiary status and site pair to account for potentially varying apiary effects in the different site pairs. We used the same approach to analyse ASV richness of different invertebrate orders identified by DNA metabarcoding of the bulk flower samples and bulk invertebrate samples, abundances of conventionally identified specimens in different orders, and richness of conventionally identified Coleoptera, Diptera, and Hymenoptera species. For the bulk flower samples, we did not observe any obvious differences between ASV richness or sequence abundance per sample from the unpreserved or ethanol-preserved flowers; therefore, we added the ASV abundances resulting from the two preservation methods from each sample together, to make the number of bulk flower samples consistent with the number of invertebrate samples. For the DNA metabarcoding analyses, to account for varying sequencing depths per sample, we used a random subsampling approach to calculate species richness estimates per sample as previously described (Makiola et al. 2019; Dopheide et al. 2021).

We also used the *manyglm* function from the R package mvabund v4.2.1 (Wang et al. 2012), with a negative binomial model and 999 bootstrap replicates, to test for mean abundance differences between sites with and without apiaries, between different site pairs, and for interactions between apiary status and site pairs, for overall invertebrate communities, individual ASVs, and individual Coleoptera, Diptera, and Hymenoptera species. To limit computational demands, we first limited each data set to species or ASVs with > 5 non-zero occurrences across the 18 samples.

We compared multivariate invertebrate community structure between sites with and without apiaries and between site pairs using non-metric multi-dimensional scaling (NMDS) ordinations and permutational multivariate analyses of variance (PERMANOVA), carried out using the R package vegan v2.6-10 (Oksanen et al. 2017). These analyses were based on Bray-Curtis dissimilarities and Jaccard distances (PERMANOVA only) between proportional abundances of bulk flower ASVs, bulk invertebrate ASVs, or conventionally identified invertebrate species in each sample. Any samples with < 20 ASVs or species were excluded.

We also visualised the abundances and distributions of the bulk flower ASVs, the 1000 most abundant bulk invertebrate ASVs, and the conventionally identified invertebrate species among samples and treatments using heatmaps generated using plot_heatmap from the R package phyloseq (Rajaram & Oono 2010; McMurdie & Holmes 2013).

Results

Bulk flower and invertebrate community composition

Over 9000 COI ASVs were detected in total, of which 4697 were identified to an arthropod taxonomic order. Of these, 686 were detected among the bulk flower samples, and 4602 among the bulk invertebrate samples (Appendix S2). Most (86%, 591) of the 686 bulk flower-detected ASVs were also detected among the bulk invertebrate samples. Most ASVs were identified as Diptera (78.4%), followed by Lepidoptera (9.41%), Hymenoptera (6.03%), Thysanoptera (1.81%), Orthoptera (1.60%), Hemiptera (1.19%), and Araneae (0.53%). A further 13 orders, each representing < 0.5% of ASVs, were grouped together as Others for analyses. The taxonomic composition of bulk-flower detected ASVs differed from that of the bulk invertebrate ASVs, with a clearly lower proportion of Diptera ASVs but higher proportions of Thysanoptera and Hemiptera ASVs (56.6%, 12.4%, and 6.71%, compared to 80%, 0.46%, and 0.89%, respectively). The Thysanoptera and Hemiptera ASVs detected on the bulk flowers tended to have higher abundances than Diptera, Lepidoptera, and Hymenoptera ASVs.

Conventionally identified invertebrate community composition based on external morphology

A total of 36 881 invertebrate specimens were collected from 14 orders (Appendix S2). Diptera and Hymenoptera dominated all samples (17 466 and 16 063 individuals, respectively), followed by Coleoptera (839 individuals), Lepidoptera (420 individuals), Orthoptera (161 individuals), Araneae (76 individuals), and Hemiptera (65 individuals). A further seven orders (Opiliones, Blattodea, Ephemeroptera, Mantodea, Neuroptera, Plectoptera, Myriapoda) each represented < 0.1 % of specimens (between 1 and 15 individuals; Others), while 1748 specimens could not be identified to a taxonomic order (Unidentified). More specimens were collected in the Tuawhenua samples (mean of 3180) than in the Tongariro frost flat (1540) or Tongariro alpine (1428) samples. No honey bees were found in Malaise traps at non-apiary sites.

A total of 137 species were identified among the three main insect orders (specimens ≥ 5 mm body length, or otherwise easily recognisable), consisting of 55 species of Coleoptera, 45 species of Diptera, and 37 species of Hymenoptera (Appendix S2; Appendix S3a). Most of these species of Coleoptera, Diptera and Hymenoptera were native (Appendix S3b).

Effects of apiary status and site pair on invertebrate richness and abundance

Bulk flower ASV richness differences

ASV richness trends for taxa detected by DNA metabarcoding of bulk flowers were clearer than those for taxa detected from the bulk invertebrate samples (Fig. 2; Appendix S4a; Appendix S5). The mean ASV richness values for Diptera, Hemiptera, Hymenoptera, and Lepidoptera were each modestly but clearly lower in apiary sites compared with non-apiary sites, by from 2.91 (44%, Hymenoptera) to 11.4 (46.9%, Diptera) ASVs ($F_{1,12}$ = 6.73–15.8, p = 0.024–0.002). Conversely, the mean ASV richness of Thysanoptera was substantially higher in apiary sites than in non-apiary sites, by 17.6 ASVs (63.5%) ($F_{1,12}$ = 109.4, $p < 0.001$). Mean Thysanoptera richness also differed between site pairs, with values ranging from 25.9 ASVs in Tongariro Alpine samples to 50.6 ASVs in Tuawhenua samples ($F_{2,12}$ = 76.9, $p < 0.001$). Additionally, interactions between apiary status and site pair were detected for ASV richness of Diptera, Thysanoptera, and Others detected on bulk flower samples ($F_{2,12}$ = 4.53–27.3, p = 0.034–< 0.001; Appendix S5c).

Bulk invertebrate ASV richness differences

Based on DNA metabarcoding of bulk invertebrate samples, the mean ASV richness of Diptera was higher by 150 ASVs (17.2%) in apiary sites compared to non-apiary sites, but this trend was not statistically significant (Fig. 2; Appendix S4a; Appendix S5b). Otherwise, apiary vs non-apiary differences

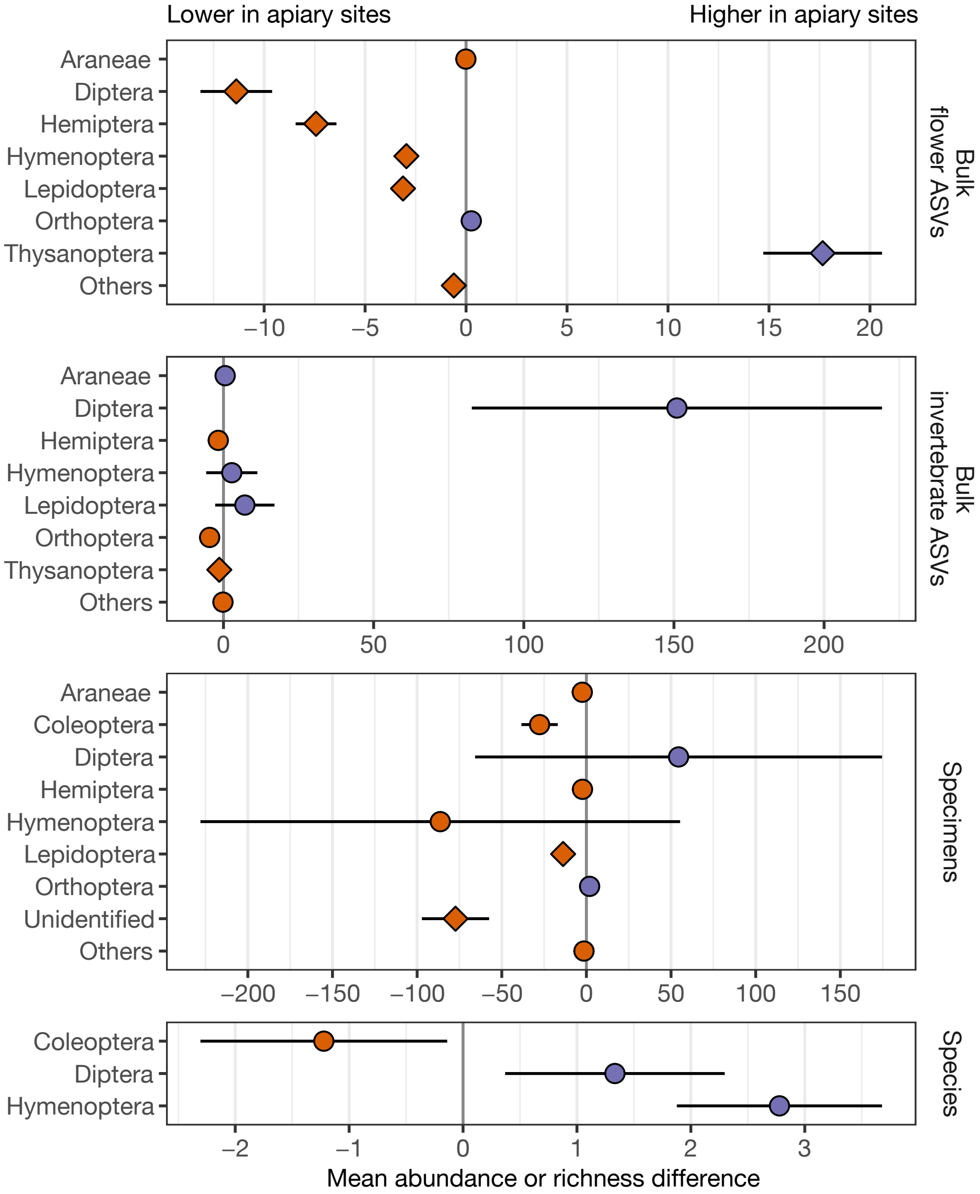

Figure 2. Differences between mean bulk invertebrate and bulk flower amplicon sequence variant (ASV) richness values, and conventionally identified insect specimen abundance and species richness values, in sites with apiaries relative to sites without them. Points above zero (purple) and below zero (orange) represent higher and lower values in sites with apiaries compared to sites without apiaries, respectively. Diamonds indicate p-values ≤ 0.05, circles indicate p-values > 0.05, and horizontal lines represent standard errors. The Unidentified category represents specimens that could not be identified by conventional means due to small size (typically < 5mm). The Others category denotes specimens from orders representing fewer than c. 0.1% of specimens, or ASVs from orders representing fewer than 0.5% of ASVs.

for each taxonomic Order were of small magnitude (≤ 7 ASVs). However, the mean ASV richness of Hymenoptera, Lepidoptera, and Orthoptera each differed substantially between site pairs, with values ranging from 51.8 to 101 Hymenoptera ASVs in Tongariro Frost and Tuawhenua samples, respectively; 52.8 to 112 Lepidoptera ASVs in Tongariro Alpine and Tuawhenua samples, respectively; and 11.5 to 32.3 Orthoptera ASVs in Tongariro Frost and Tongariro Alpine samples, respectively ($F_{2,12}$ = 4.46–5.66, p = 0.036–0.019). An interaction between apiary status and site pair was also detected for Hymenoptera ASV richness ($F_{2,12}$ = 4.19, p = 0.042; Appendix S4a; Appendix S5d).

Conventionally identified specimen abundance and species richness differences

Mean abundances of Lepidoptera and Unidentified specimens were lower in apiary compared to non-apiary sites, by 13.8 specimens (45.6%) and 77.3 specimens (57%), respectively ($F_{1,12}$ = 5.52 and 8.57, p = 0.037 and 0.013, respectively; Fig. 2; Appendix S4b; Appendix S6a). Similarly, mean abundances of Coleoptera and Hymenoptera specimens were lower in apiary compared to non-apiary sites (by 27.7 and 86.3 specimens, respectively), whereas abundance of Diptera was higher in apiary compared to non-apiary sites by 54.4 specimens (5.77%), but these differences lacked statistical support. There were also substantial specimen abundance differences between site pairs for Diptera, Hymenoptera, Lepidoptera, and Unidentified specimens (from 698, 583, 8.33, and 53.3 mean specimens, respectively, in Tongariro Alpine samples, to 1412, 1497, 43.2 and 180 mean specimens respectively in Tuawhenua samples; $F_{2,12} = 4.59$ to 19.3, $p = 0.033$–0.0002). Furthermore, significant interactions between apiary status and site pair were detected for Coleoptera and Lepidoptera specimen abundances ($F_{2,12} = 7.33$ and 3.93, $p = 0.008$ and 0.049; Appendix S4b; Appendix S6c).

The mean species richness of conventionally identified species of Coleoptera, Lepidoptera, and Hymenoptera differed little between samples. Hymenoptera species richness was higher in Tuawhenua samples (16.2) than in the other two site pairs (11), and there was an interaction between apiary status and site pair for Coleoptera species richness ($F_{2,12} = 4.39$ and 6.14, $p = 0.037$ and 0.015; Appendix S4b; Appendix S6b & d).

Effects of apiary status and site pair on community composition

Results of manyglm *tests*

For bulk flower communities, *manyglm* detected an apiary status effect on community composition, as well as a site pair effect and an interaction between apiary status and site pair ($F_{1\text{-}2,12\text{-}16} = 400$–761, $p = 0.003$–≤ 0.001). Three Thysanoptera ASVs showed differing abundances between bulk flower samples from sites with and without apiaries; eleven ASVs (ten Thysanoptera, one Trombidiformes) had differing abundances between samples from different site pairs; and interactions between apiary status and site pair were detected for two Thysanoptera ASVs ($F_{1\text{-}2,12\text{-}16} = 14.48$–60.95, $p.adj = 0.019$–≤ 0.001; Appendix S4c).

For bulk invertebrate communities, manyglm detected a site pair effect on community composition and an interaction between apiary status and site pair ($F_{2,12\text{-}14} = 9876$–14 168, $p = 0.09$–≤ 0.001), but no apiary status effect. Three Diptera ASVs had differing abundances between samples from different site pairs, and interactions between apiary status and site pair were detected for another seven Diptera ASVs ($F_{2,12\text{-}14} = 25.36$–35.47, $p.adj = 0.047$–0.002; Appendix S4c).

As for the bulk invertebrate species, there was a site pair effect on community composition and an interaction between apiary status and site pair ($F_{2,12\text{-}14} = 335.2$–355.7, $p \leq 0.001$), but no apiary status effect, for overall conventionally identified species. No conventionally identified species had differing abundances between samples from sites with and without apiaries, but four species (Braconidae sp., Ichneumonidae sp., Beridinae sp., and *Parentia* sp.) had differing abundances between samples from different site pairs, and interactions between apiary status and site pair were detected for two species (Empididae sp., and *Tachysphex nigerrimus*; $F = 17.72$–22.80, $p.adj = 0.034$–0.017; Appendix S4c).

Results of NMDS ordinations

An NMDS ordination based on bulk flower invertebrate ASVs showed clearer patterns than those based on the bulk invertebrate ASVs or the morphologically identified invertebrate species data, with very clear grouping of samples by plot and a clear and consistent separation of samples from apiary sites from those from non-apiary sites within each site pair (Fig. 3a). A heatmap of ASV relative abundances and distributions among bulk flower samples showed evidence

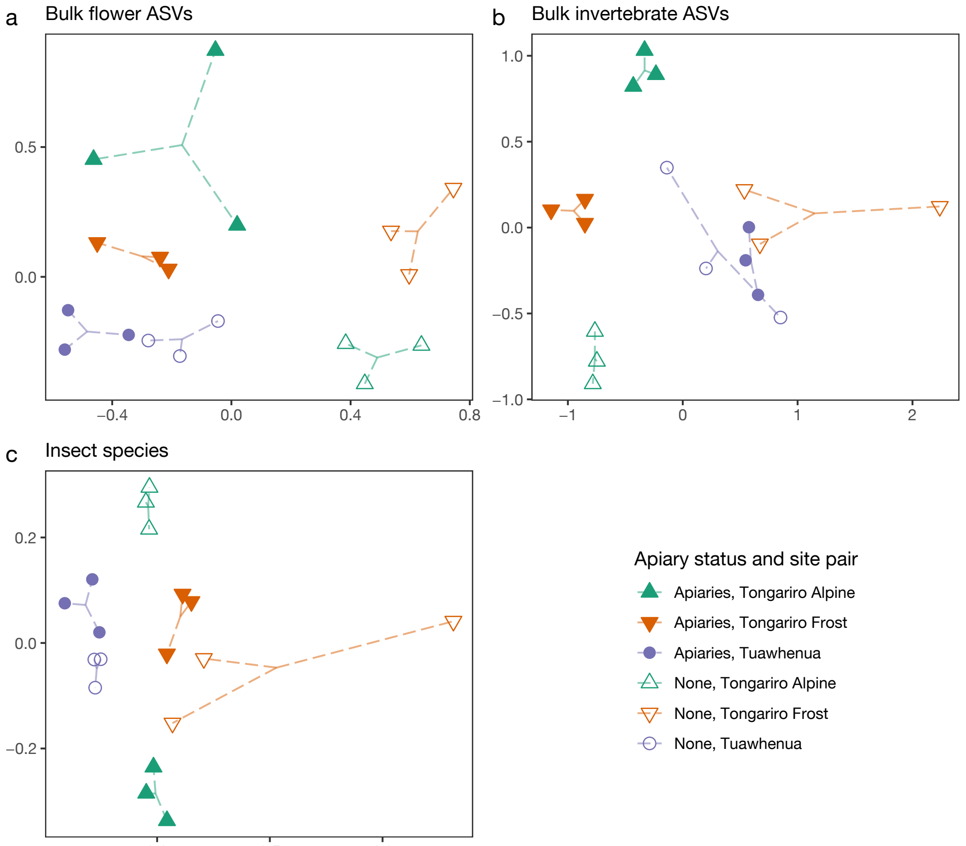

Figure 3. NMDS ordinations showing invertebrate community structure differences between sites with and without apiaries at three different locations, based on (a) amplicon sequence variants (ASVs) detected by DNA metabarcoding of bulk flower samples, (b) ASVs detected by DNA metabarcoding of bulk Malaise-trapped invertebrate samples, and (c) Malaise-trapped insects identified by conventional methods as species of Coleoptera, Diptera, or Hymenoptera; based on Bray-Curtis dissimilarities. Dashed lines link individual samples to centroid points for each site.

of differing assemblages of Diptera ASVs detected with low abundances in each sample, and greater richness and higher abundances of Thysanoptera ASVs detected on apiary samples compared to non-apiary samples from Tongariro Frost and, to a lesser extent, Tongariro Alpine, but not Tuawhenua (Fig. 4).

An NMDS ordination based on the bulk invertebrate ASVs showed evidence of consistent community composition among samples within each of the three apiary sites, and the Tongariro Alpine non-apiary site, but greater heterogeneity among samples from the Tongariro Frost and Tuawhenua non-apiary sites (Fig. 3b). There was clearer separation of apiary from non-apiary samples from the Tongariro Alpine and Tongariro Frost site pairs than for the Tuawhenua site pair. A heatmap showing the relative abundances and distributions of the 1000 most abundant bulk invertebrate ASVs showed clear evidence of shifts in community composition between apiary and non-apiary sites within each of the three site pairs, particularly for Diptera ASVs in the Tongariro Alpine and Tongariro Frost samples, but with limited consistency between the different site pairs (Appendix S7). Apiary and non-apiary ASV assemblages in the Tongariro Alpine site pair were clearly distinct from those in the other two site pairs. ASV assemblages of the Tongariro Frost apiary samples showed some similarity to those of the Tuawhenua non-apiary samples, as did two of the Tongariro Frost non-apiary samples to the Tuawhenua apiary samples.

The NMDS ordinations based on morphologically identified species (of Coleoptera, Diptera, Hymenoptera) showed that community composition tended to be broadly consistent between plots within most (but not all) sites. However, patterns of similarity related to apiary status and site pairs were less clear (Fig. 3c; Appendix S8). Samples from one site pair (Tuawhenua) were clearly distinct from those in the other two site pairs, but there was overlap between one or other site (depending on the taxonomic group) from each of the other two site pairs, and no clear evidence of a consistent shift in community composition due to the presence or absence of apiaries. Similarly, a heatmap of species abundances/distributions among samples showed little evidence of consistent changes in species composition related to apiaries (Appendix S8e).

Results of PERMANOVA tests

PERMANOVA tests of overall bulk flower and bulk invertebrate community composition differences found clear evidence of apiary status differences, site pair differences, and interactions between apiary status and site pair for both data sets, using both Bray-Curtis and Jaccard distance metrics ($F_{1\text{-}2,12}$ = 1.388–4.550, p = 0.017–0.001; Table 2). For orders within the bulk flower data set, there was strong evidence for apiary status differences and site pair differences, and weaker evidence of interactions between apiary status and site pair, for both Thysanoptera (using both metrics) and Hemiptera (interaction with Jaccard distances only) ($F_{1\text{-}2,12}$ = 1.385–5.818, p = 0.029–0.007; Appendix S4d). There was also evidence of site pair differences for Diptera (using Jaccard distances only) and Lepidoptera ($F_{2,12}$ = 1.141–3.181, p = 0.01–0.001), and modest evidence of an interaction between apiary status and site pair for Diptera (using Jaccard distances only) ($F_{2,12}$ = 1.104, p = 0.037). For orders within the bulk invertebrate data set, using both Bray-Curtis and Jaccard distances, there was strong evidence of apiary status and site pair differences, and

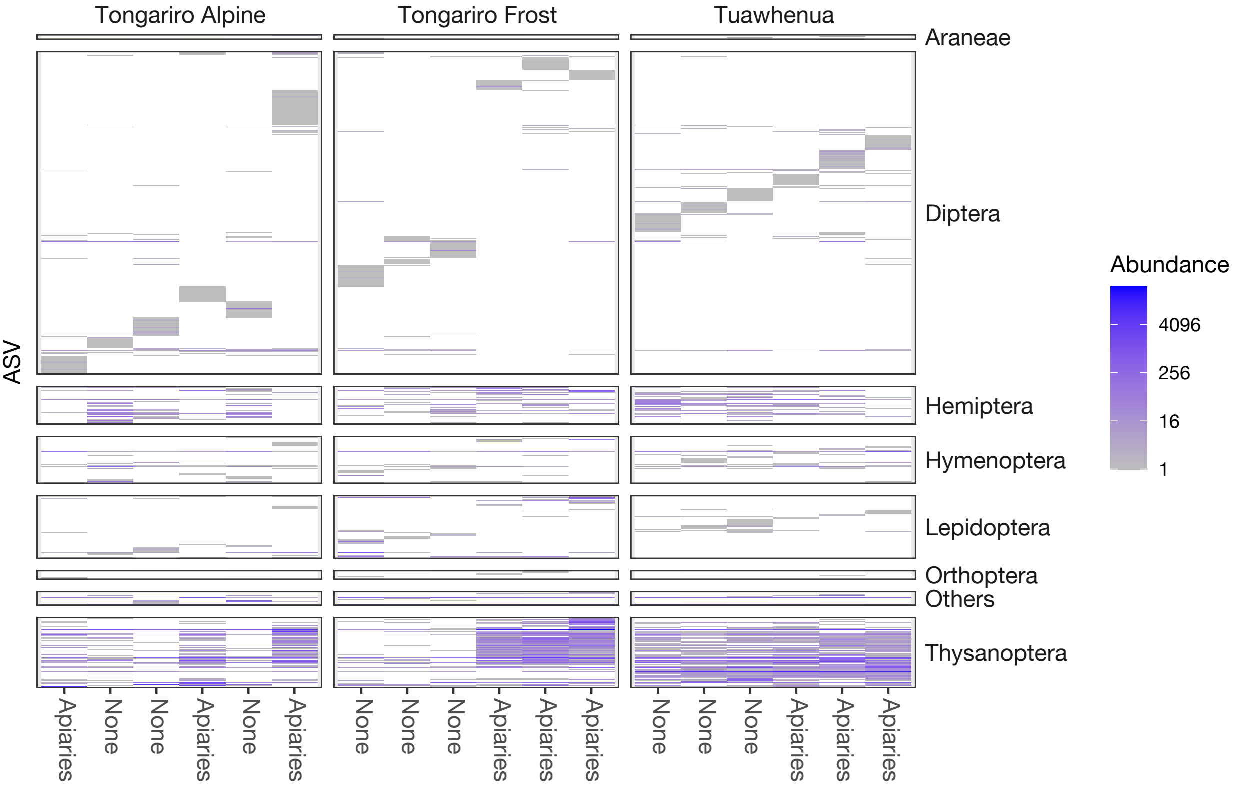

Figure 4. Heatmap showing relative abundances and distributions of 633 invertebrate amplicon sequence variants (ASVs) detected by DNA metabarcoding of 18 bulk flower samples from sites with and without apiaries at three different locations. Samples are ordered on the x-axis according to Bray-Curtis dissimilarities.

Table 2. Results of PERMANOVA tests for differing community structure between sites with and without apiaries, based on amplicon sequence variants (ASVs) detected by DNA metabarcoding of bulk flower samples and of bulk Malaise-trapped invertebrate samples, and Malaise-trapped insects conventionally identified as species of Coleoptera, Diptera, or Hymenoptera.

Distance Metric	Data component	Term	Df	SumOfSqs	R^2	F	Pr(>F)
Bray-Curtis	Bulk flower ASVs	Apiary status	1	0.532	0.152	4.550	<0.001
		Site pair	2	1.057	0.301	4.519	<0.001
		Apiary status:Site pair	2	0.515	0.147	2.203	0.003
		Residual	12	1.403	0.400		
	Bulk invertebrate ASVs	Apiary status	1	0.457	0.080	2.030	0.016
		Site pair	2	1.261	0.220	2.798	<0.001
		Apiary status:Site pair	2	1.318	0.230	2.924	<0.001
		Residual	12	2.703	0.471		
	Conventionally-identified species	Apiary status	1	0.244	0.094	3.153	<0.001
		Site pair	2	0.816	0.313	5.264	<0.001
		Apiary status:Site pair	2	0.615	0.236	3.969	<0.001
		Residual	12	0.930	0.357		
Jaccard	Bulk flower ASVs	Apiary status	1	0.494	0.098	2.025	0.002
		Site pair	2	0.943	0.187	1.934	<0.001
		Apiary status:Site pair	2	0.677	0.134	1.388	0.017
		Residual	12	2.926	0.581		
	Bulk invertebrate ASVs	Apiary status	1	0.412	0.074	1.753	0.031
		Site pair	2	1.132	0.205	2.408	<0.001
		Apiary status:Site pair	2	1.171	0.211	2.489	<0.001
		Residual	12	2.822	0.510		
	Conventionally-identified species	Apiary status	1	0.334	0.088	2.182	0.002
		Site pair	2	0.867	0.228	2.835	<0.001
		Apiary status:Site pair	2	0.770	0.202	2.518	<0.001
		Residual	12	1.835	0.482		

an interactions between apiary status and site pair for Diptera ($F_{1\text{-}2,12}$ = 1.952–3.209, p = 0.008–0.001), and for site pair differences and interactions between apiary status and site pair for Hymenoptera and Lepidoptera ($F_{1\text{-}2,12}$ = 1.437–2.480, p = 0.036–0.001). There was also some evidence for interactions between apiary status and site pair for Orthoptera (Bray-Curtis only), Araneae, and Others ($F_{1\text{-}2,12}$ = 1.403–2.608, p = 0.045–0.008).

PERMANOVA tests for morphologically identified Coleoptera, Diptera, and Hymenoptera species community structure differences found strong evidence of apiary status differences, site pair differences, and interactions between apiary status and site pair, based on both Jaccard distances and Bray-Curtis dissimilarity metrics ($F_{1\text{-}2,12}$ = 2.182–5.264, p = 0.002–0.001; Table 2; Appendix S4d). Very similar results were observed when these three orders were tested separately, although Coleoptera community structure did not differ between sites with and without apiaries.

Discussion

Invertebrate communities detected on flowers

We investigated the effects of honey bee apiaries on invertebrate communities using a combination of DNA metabarcoding of bulk flowers and invertebrates and conventional specimen identification methods. Invertebrate communities detected on bulk flowers showed the clearest apiary-related trends, with the ASV richness of most taxa detected on bulk flower samples clearly lower in apiary sites compared to non-apiary sites. Similarly, NMDS ordinations and heatmaps showing the distributions of ASV relative abundance values among samples based on the bulk flower communities showed clearer apiary-related patterns than those based on bulk invertebrate communities or on conventionally identified species. This might suggest that honey bees consistently disrupt mānuka flower visits and pollination by other invertebrates at different sites, but have more variable effects on wider communities of invertebrates that are not directly involved in pollination.

These observations are consistent with international studies showing honey bee disruption of flower visitation and pollination by native insects (Valido et al. 2019; Page & Williams 2023). Similarly, in New Zealand, Bennik (2009) observed decreasing abundances of large flies (especially tachinids) visiting flowers with increasing honey bee abundances in North Island mānuka shrublands, and also recorded examples of physical disturbance and displacement of large flies by honey bees. Nepia (2020) demonstrated that honey bees may also affect flower-visiting invertebrate communities in North Island submontane *Pterophylla racemosa* (kāmahi) and *Ixerba brexioides* (tāwari) forests, with a negative relationship between flower visitor diversity and honey bee hive density and greater network complexity at low density beehive sites. In contrast, another study showed that floral visitation by New Zealand native bees, a low diversity group of solitary species (currently with 11 species described), is more affected by weather or elevation than by honey bees (Murphy & Robertson 2000).

The abundances of Diptera, Hymenoptera and Lepidoptera ASVs detected on flowers were noticeably lower (typically only a few sequence reads) and more heterogeneous than those of Hemiptera and Thysanoptera. This suggests that the former may represent trace DNA left by transient flower visitors, whereas the latter derive from specimens residing on or in the flowers. More Diptera ASVs were detected on flowers than Hymenoptera or Lepidoptera ASVs, potentially underlining the importance of dipterans in pollination of mānuka. Thysanoptera (commonly known as thrips), was the only taxon with clearly higher mean richness on bulk flowers from apiary sites compared to non-apiary sites, and this was particularly evident for the Tongariro alpine and frost site pairs. These thrip ASVs may be transported among flowers by honey bees in a case of phoresis (Infante et al. 2021). Some thrips contribute to pollination (Eliyahu et al. 2015), while others act as vectors of plant diseases (Riley et al. 2011), raising the possibility that thrip transport by honey bees might influence the health of mānuka plants.

Impacts of honey bees on bulk invertebrate communities

International research indicates that high densities of honey bees can have a negative impact on wild pollinators (Lindström et al. 2016; Torné-Noguera et al. 2016; Mallinger et al. 2017). In this case, similar abundances of native bees such as those in the genus *Leioproctus* were observed in mānuka plots with and without honey bees, indicating that they can compete sufficiently with honey bees at these densities. More broadly, our bulk invertebrate results suggested that honey bees may affect their biodiversity and community composition within mānuka shrublands, but this might vary among habitats. Most invertebrate groups had lower mean specimen abundances in sites with honey bee apiaries compared to those without, but this was statistically significant only for Lepidoptera and the pool of small unidentified specimens. The mean ASV richness of bulk invertebrate orders and species richness of morphologically identified specimens differed little between apiary and non-apiary sites. Instead, mean bulk invertebrate richness trends were often clearer between the different site pairs than between sites with and without apiaries, or there were significant interactions between apiary status and site pair. Our NMDS ordinations and heatmap visualisations of bulk invertebrate ASV abundance and distribution showed further evidence of differing community composition in sites with and without apiaries, but there was a lack of consistency in these differences across the three pairs of sites, in contrast to the bulk flower results.

Together, our data indicate that the responses of bulk invertebrate communities to honey bees varied between the different locations. Each pair of sites represented a different habitat type (i.e. forest clearing, frost flat, and alpine), and aspects of these habitats (e.g. climate-related differences in honey bee activity) may have led to differing effects of honey bees on invertebrate composition and richness. Alternatively, our selection of site pairs may have failed to account for factors that influence native invertebrate community composition within each pair. Certainly, the site pairs were very similar based on vegetation composition and key environmental variables, but more subtle aspects (e.g. minor plant species present alongside mānuka; site history; local microclimate) may influence invertebrate community structure and contribute to the differences we found between site pairs. Any such differences might have affected the magnitude of honey bee impacts on invertebrate communities at each site pair.

Conventional versus DNA-based invertebrate biodiversity

DNA metabarcoding resulted in detection of increased richness of Hymenoptera ASVs and (especially) Diptera ASVs, but lower richness of Coleoptera ASVs, compared to morphologically identified species richness of these groups. The higher ASV richness of Hymenoptera and Diptera can probably be attributed to DNA-based identifications of the pool of small specimens unidentified by conventional means, plus the higher taxonomic resolution represented by ASVs compared to morphologically delineated species. This demonstrates the efficacy of the DNA-based approach for identifying large pools of diverse specimens that are difficult to classify by other means. On the other hand, the lower ASV richness of Coleoptera compared to the morphologically identified species richness of this group might suggest that the DNA metabarcoding protocols used were suboptimal for this group. Primer-to-template mismatches are likely to have skewed the PCR amplification of different taxa, potentially contributing to reduced representation of Coleoptera (Elbrecht & Leese 2015; Pinol et al. 2015). It is also possible that because the specimen pools were highly dominated by Diptera and Hymenoptera, DNA from these orders may have swamped that of the much lower-abundance Coleoptera within the homogenised specimen DNA extracts (Iwaszkiewicz-Eggebrecht et al. 2023), limiting the detection of Coleoptera specimens that were otherwise identified by morphological means. Similarly, the much higher abundance of DNA from the bulk invertebrates compared to flowers may have favoured sequencing of the former over the latter, suggesting that our study may have underestimated the diversity of flower communities.

Conclusions

The strongest evidence of a honey bee effect on invertebrate communities was provided by DNA detected on flowers, suggesting that this may be a valuable approach for future studies of pollination and invertebrate-flower interactions. The presence of honey bee apiaries in mānuka shrublands appears to increase the ASV richness of thrips (Thysanoptera) on and in flowers, reduce mānuka flower visits by other (non-honey bee) insects, and reduce the abundances of specimens of Lepidoptera and of unidentified small insects. Honey bees might also affect the biodiversity and composition of wider invertebrate communities in mānuka shrublands, but these effects appear to be site dependent, influenced by factors such as differences in climate and site productivity, surrounding plant community composition, and historical legacy effects.

Greater replication of site pairs along key environmental gradients is needed to clarify these effects of honey bee apiaries upon native invertebrate communities. Alternatively, identification of non-apiary site pairs with comparable invertebrate communities, followed by the introduction of honey bee hives at one site but not the other, would enable the observation of temporal invertebrate community responses to honey bees.

Acknowledgements

We thank Audrey Edwards and Celia Edwards for field assistance at Ruatāhuna, Makere Biddle for her hospitality while we worked in Ruatāhuna, Puke Timoti for assistance with study site assessment at Ruatāhuna, Robbie Price for creating Figure 1 and providing the climate data in Table 1, Thomas Buckley, Helen O'Leary, Gary Houliston, and two anonymous reviewers for peer review of the draft manuscript. We dedicate this paper to our co-author Stephen Thorpe (1970–2024), who was a generous colleague, an outstanding entomologist, and a friend.

Additional information and declarations

Author contributions: CB and SR conceived the idea; CW, SR, AD and RH designed the research; CW, DT and CB undertook the sample collection; CD carried out molecular laboratory procedures; CW and ST carried out morphological specimen identifications; AD analysed the data; CW, AD and SR wrote the manuscript, with editorial contributions from other authors.

Funding: This research was funded by SSIF funding to Manaaki Whenua - Landcare Research, the Biological Heritage Challenge (Project 1.3) and Department of Conservation, New Zealand.

Data availability: Raw sequence data, processed data outputs, and associated processing and analysis code is available on MWLR Datastore (https://doi.org/10.7931/t2fr-f744)

Ethics: Ethics approval was not required for this research.

Conflicts of interest: The authors declare no conflicts of interest.

References

Beard C 2015. Honeybees (*Apis mellifera*) on public conservation lands: a risk analysis. Wellington, Department of Conservation. 21 p.

Beekman M, Ratnieks FLW 2000. Long-range foraging by the honey-bee, *Apis mellifera* L. Functional Ecology 14(4): 490–496.

Bennik RM 2009. The effects of honeybees (*Apis mellifera*) on the biodiversity of mānuka (*Leptospermum scoparium*) patches. Unpublished MSc thesis thesis. Massey University, Palmerston North.

Callahan BJ, McMurdie PJ, Holmes SP 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal 11(12): 2639–2643.

Chagné D, Montanari S, Kirk C, Mitchell C, Heenan P, Koot E 2023. Single nucleotide polymorphism analysis in *Leptospermum scoparium* (Myrtaceae) supports two highly differentiated endemic species in Aotearoa New Zealand and Australia. Tree Genetics & Genomes 19(4): 31.

Donovan BJ 1980. Interactions between native and introduced bees in New Zealand. New Zealand Journal of Ecology 3: 104–116.

Donovan BJ 2007. Apoidea (Insecta: Hymenoptera). Fauna of New Zealand 57. Lincoln, Manaaki Whenua Press. 295 p.

Dopheide A, Davis C, Nuñez J, Rogers G, Whitehead D, Grelet G-A 2021. Depth-structuring of multi-kingdom soil communities in agricultural pastures. FEMS Microbiology Ecology 97(12): fiab156.

Edgar RC 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. bioRxiv. 074161 p.

Elbrecht V, Leese F 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. PLoS ONE 10(7): e0130324.

Eliyahu D, McCall AC, Lauck M, Trakhtenbrot A, Bronstein JL 2015. Minute pollinators: The role of thrips (Thysanoptera) as pollinators of pointleaf manzanita, *Arctostaphylos pungens* (Ericaceae). Journal of Pollination Ecology 16: 64–71.

El-Senduny FF, Hegazi NM, Abd Elghani GE, Farag MA 2021. Manuka honey, a unique mono-floral honey. A comprehensive review of its bioactives, metabolism, action mechanisms, and therapeutic merits. Food Bioscience 42: 101038.

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(5): 294–299.

Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Brunbjerg AK, Pietroni C, Hansen AJ 2017. Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications 8: 1188.

Geldmann J, González-Varo JP 2018. Conserving honey bees does not help wildlife. Science 359(6374): 392–393.

Hart NH 2016. Monitoring New Zealand's native bees: a collaborative approach using image analysis. Unpublished PhD thesis. Auckland University of Technology, Auckland, New Zealand.

Holdaway RJ, Wood JR, Dickie IA, Orwin KH, Bellingham PJ, Richardson SJ, Lyver POB, Timoti P, Buckley TR 2017. Using DNA metabarcoding to assess New Zealand's terrestrial biodiversity. New Zealand Journal of Ecology 41: 251–262.

Infante F, Ortiz JA, Goldarazena A, Sanchez W 2021. Thrips on honey bees: more than hitchhikers? Ecology 103(1): e03551.

Iwasaki J 2017. Interactions between bee species in relation to floral resources. Unpublished PhD thesis. University of Otago, Dunedin, New Zealand.

Iwaszkiewicz-Eggebrecht E, Granqvist E, Buczek M, Prus M, Kudlicka J, Roslin T, Tack AJM, Andersson AF, Miraldo A, Ronquist F, Łukasik P 2023. Optimizing insect metabarcoding using replicated mock communities. Methods in Ecology and Evolution 14: 1130–1146.

Jackson F 2019. Investigating the potential negative Impacts of managed honey bees (*Apis mellifera*) on native New Zealand plant-pollinator communities. Unpublished MSc thesis. The University of Auckland, Auckland.

Johnson MD, Katz AD, Davis MA, Tetzlaff S, Edlund D, Tomczyk S, Molano-Flores B, Wilder T, Sperry JH 2023. Environmental DNA metabarcoding from flowers reveals arthropod pollinators, plant pests, parasites, and potential predator–prey interactions while revealing more arthropod diversity than camera traps. Environmental DNA 5(3): 551–569.

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez

V, Boehm JT, Machida RJ 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in Zoology 10: 34.

Leray M, Knowlton N, Machida RJ 2022. MIDORI2: A collection of quality controlled, preformatted, and regularly updated reference databases for taxonomic assignment of eukaryotic mitochondrial sequences. Environmental DNA 4(4): 894–907.

Lindström SAM, Herbertsson L, Rundlöf M, Bommarco R, Smith HG 2016. Experimental evidence that honeybees depress wild insect densities in a flowering crop. Proceedings of the Royal Society B: Biological Sciences 283: 20161641.

Makiola A, Dickie IA, Holdaway RJ, Wood JR, Orwin KH, Glare TR 2019. Land use is a determinant of plant pathogen alpha- but not beta-diversity. Molecular Ecology 28: 3786–3798.

Mallinger RE, Gaines-Day HR, Gratton C 2017. Do managed bees have negative effects on wild bees?: A systematic review of the literature. PLOS ONE 12(12): e0189268.

Martin M 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal 17(1): 10–12.

McCarthy JK, Wiser SK, Bellingham PJ, Beresford RM, Campbell RE, Turner R, Richardson SJ 2021. Using spatial models to identify refugia and guide restoration in response to an invasive plant pathogen. Journal of Applied Ecology 58: 192–201.

McKnight DT, Huerlimann R, Bower DS, Schwarzkopf L, Alford RA, Zenger KR 2019. microDecon: A highly accurate read-subtraction tool for the post-sequencing removal of contamination in metabarcoding studies. Environmental DNA 1: 14–25.

McMurdie PJ, Holmes S 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 8(4): e61217.

Ministry for Primary Industries 2020. Apiculture: Ministry for Primary Industries 2020 Apiculture Monitoring Programme. Wellington, Ministry for Primary Industries. 17 p.

Murphy C, Robertson A 2000. Preliminary study of the effects of honey bees (*Apis mellifera*) in Tongariro National Park. Science for Conservation 139. Wellington, Department of Conservation. 18 p.

Nepia RE 2020. Understanding the role and impact of introduced honey bees in a submontane indigenous forest ecosystem. Unpublished PhD thesis. The University of Waikato, Hamilton, New Zealand.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H 2017. vegan: Community ecology package.

Page ML, Williams NM 2023. Honey bee introductions displace native bees and decrease pollination of a native wildflower. Ecology 104(2): e3939.

Pinol J, Mir G, Gomez-Polo P, Agusti N 2015. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. Molecular Ecology Resources 15(4): 819–830.

Prendergast KS, Ollerton J 2022. Impacts of the introduced European honeybee on Australian bee-flower network properties in urban bushland remnants and residential gardens. Austral Ecology 47: 35–53.

Rajaram S, Oono Y 2010. NeatMap–non-clustering heat map alternatives in R. BMC Bioinformatics 11: 45.

Riley DG, Joseph SV, Srinivasan R, Diffie S 2011. Thrips vectors of tospoviruses. Journal of Integrated Pest Management 2(1): I1–I10.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4: e2584.

Thomsen PF, Sigsgaard EE 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecology and Evolution 9: 1665–1679.

Torné-Noguera A, Rodrigo A, Osorio S, Bosch J 2016. Collateral effects of beekeeping: Impacts on pollen-nectar resources and wild bee communities. Basic and Applied Ecology 17(3): 199–209.

Valido A, Rodríguez-Rodríguez MC, Jordano P 2019. Honeybees disrupt the structure and functionality of plant-pollinator networks. Scientific Reports 9(1): 4711.

Wang Y, Naumann U, Wright ST, Warton DI 2012. mvabund– an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3: 471–474.

Wardle P 1991. Vegetation of New Zealand. Cambridge, Cambridge University Press. 672 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(3): 209–227.

Watts C, Dopheide A, Holdaway R, Davis C, Wood J, Thornburrow D, Dickie IA 2019. DNA metabarcoding as a tool for invertebrate community monitoring: a case study comparison with conventional techniques. Austral Entomology 58: 675–686.

Wickham H 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag New York.

Wickham H, François R, Henry L, Müller K 2022. dplyr: A grammar of data manipulation.

Received: 20 March 2025; accepted: 17 October 2025
Editorial board member: David Pattemore

Supplementary material

Additional supporting information may be found in the online version of this article.

Appendix S1. Modified counting technique for small (< 5 mm) Hymenoptera and Diptera.

Appendix S2. Taxonomic composition of specimens, species, and amplicon sequence variants (ASVs).

Appendix S3. Conventionally identified species.

Appendix S4. Results of statistical tests for apiary and site pair effects on biodiversity.

Appendix S5. Apiary and site pair effects on the richness of taxa detected by DNA metabarcoding of bulk invertebrates and bulk flowers.

Appendix S6. Apiary and site pair effects on the abundance and richness of conventionally identified specimens and species.

Appendix S7. Apiary effects on the structure of bulk invertebrate communities.

Appendix S8. Apiary effects on the structure of conventionally identified invertebrate communities.