Detecting invertebrate ecosystem service providers in orchards: traditional methods versus barcoding of environmental DNA in soil

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Running title: Detecting invertebrate ecosystem service providers

Abstract

1. The objective of this study was to assess barcoding of environmental DNA (eDNA) as a method for monitoring invertebrate ecosystem service providers (IESP) in soil samples.
2. We selected 26 IESP that occur in New Zealand kiwifruit or apple orchards and produced mitochondrial cytochrome *c* oxidase gene subunit I (COI) and/or 28S ribosomal DNA sequences for each. Specific barcode primers were designed for each IESP and tested along with generic barcoding COI primers for their ability to detect DNA from IESP that had been added to sterilised and unsterilised soil samples.
3. While the specific primers accurately detected the IESP in more than 96% of the samples, the generic COI primers detected only 33% of the IESP added to the sterilised samples, and none in the unsterilised samples.
4. In a field test, we compared metabarcoding with traditional invertebrate trapping methods to detect the IESP in ten kiwifruit and ten apple orchards. All IESP were

collected in traps in at least one orchard, however very few were identified by metabarcoding of soil eDNA.

5. While the specific primers can be used as a tool for monitoring IESP in soil samples, methodological improvements are needed before metabarcoding of soil eDNA can be used to monitor these taxa.

Keywords: species-specific primers, metabarcoding, environmental DNA, decomposition, natural enemies

Introduction

Invertebrate ecosystem service providers (IESP) are integral to the sustainable management of agro-ecosystems (Saunders, 2018). The services provided by invertebrates include pollination, pest suppression and decomposition (Beynon *et al.*, 2015; Cross *et al.*, 2015; Minarro *et al.*, 2018), and are estimated to be worth billions of dollars per year to land managers worldwide (Losey & Vaughan, 2006; Sandhu *et al.*, 2008). However, management practices, such as the application of agrichemicals, can interrupt services through negative effects on IESP populations (Atwood *et al.*, 2018; Chagnon *et al.*, 2015), potentially resulting in increased production costs (e.g., through needing to control secondary pest outbreaks: Gallardo *et al.*, 2016). Employing mitigation techniques to restore or protect populations and services (e.g., by adding protective shelters, alley-cropping, or ground-covers to increase populations of natural enemies and decomposers: Ashraf *et al.*, 2018; Horton *et al.*, 2002; Shields *et al.*, 2016) would consequently be highly beneficial. However, the invertebrate species providing the services often remain unidentified and unmonitored, at least partially because current invertebrate monitoring methods are slow and time consuming. For example, it may take many months to morphologically identify all individual invertebrates collected in a few traps placed in an orchard for a single week (Todd *et al.*, 2011). Interruptions to services are, therefore, usually discovered too late (e.g., when the secondary pest outbreak occurs) and land managers are required to implement emergency measures, such as applying additional agrichemicals, rather than mitigation techniques.

Barcoding and metabarcoding of environmental DNA (eDNA) in soil samples (e.g., Decaens *et al.*, 2016; Taberlet *et al.*, 2012) can produce information on invertebrate populations more quickly, and without removing viable individuals from the system, compared with traditional monitoring methods (Oliverio *et al.*, 2018; Yang *et al.*, 2014). This technology could be used, therefore, to detect changes in IESP populations in time for land managers to employ mitigation techniques to restore or protect those populations. This hypothesis is based on work that has shown that invertebrates contribute free DNA molecules in the form of secretions, eggs, faeces and decomposing bodies to the environment, and that this eDNA is detectable in soil (Bohmann *et al.*, 2014). In water samples, these molecules are harder to detect when the species' population is small, and easier if the population increases (Bohmann *et al.*, 2014). If this is also the case for soil samples, then it may be possible to use changes in the detectability of IESP populations to warn land managers of potential changes in ecosystem services provided by those populations. However, since it is not possible to extract DNA from all the soil in an agro-ecosystem, subsamples must be taken, and these may not contain DNA from all taxa present in that ecosystem. This subsampling error plus the differential deterioration of DNA from different sources, the influence of capture and extraction protocols on DNA yield, and the tendency of PCR primers to bind to some sequences more readily than others, may mean that some species may not be detected even when they are abundant in the environment (Deiner *et al.*, 2015, 2017). Thus, comparing the results of barcoding and traditional sampling methods (Deiner *et al.*, 2017) is a useful first step for testing this method as a tool for monitoring IESP populations.

Previous studies have identified a number of IESP in apple and kiwifruit orchards in New Zealand and the management practices that may affect their populations (Malone *et al.*, 2017b; Todd *et al.*, 2016). The aims for this study were to: (1) develop specific primers for 26 IESP found in New Zealand kiwifruit and/or apple orchards; (2) test the ability of those specific primers to detect the IESP in soil samples to which the IESP had been added; (3) test the ability of generic primers for the mitochondrial cytochrome *c* oxidase gene subunit I (COI) to detect the IESP in soil samples to which the IESP had been added; (4) compare the ability of traditional invertebrate trapping methods and metabarcoding of eDNA in soil to detect the IESP in orchards; and (5) detect any differences in IESP populations in relation to orchard management systems.

Methods

Development of IESP-specific primers

Focal IESP were selected from lists of taxa previously collected in New Zealand apple and kiwifruit orchards (Malone *et al.*, 2017a; Todd *et al.*, 2011). The 26 selected taxa were either natural enemies of orchard pests or involved in decomposition processes (Table 1). Most of the IESPs primarily occur on or under the soil surface, with seven taxa that spend very little time in these habitats also included (Table 1). Specimens of each IESP were collected and identified using morphological taxonomic keys (e.g., Berry, 1997; Eyles & Schuh, 2003; Herman, 1970). DNA was extracted from these specimens using the *prepGEM*® insect kit (ZyGem, Southampton, UK) following the manufacturer's instructions. COI and/or 28S ribosomal DNA (28S rDNA) sequences were amplified from these extracts by PCR using KAPA2G Robust (Kapa Biosystems, Wilmington, MA, USA) with buffer A. The PCR cycle used was 94°C for 5 minutes, 40 cycles of 94°C for 30 seconds, 44°C (COI) or 49°C (28S rDNA) for 30 seconds, and 72°C for 45 seconds, with a final extension phase of 72°C for 10 minutes. The primers used for COI PCRs were LCOI490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994), hereafter referred to as "Folmer primers". For 28S rDNA PCRs, primers 500F (5'-CTTTGAAGAGAGAGTTCAAGAG-3') and 501R (5'-TCGGAAGGAACCAGCTACTA-3') (Nadler *et al.*, 2000), targeting the D2/D3 region, were used. PCR amplicons were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA), and Sanger sequenced in both directions. Primer design and genetic data manipulation were performed using Geneious R10.0.3 (<https://www.geneious.com>).

Specific primers for each IESP (Table 1) were designed from the COI and/or 28S rDNA sequences with specificity checked using National Center for Biotechnology Information (NCBI) primer-BLAST (Ye *et al.*, 2012). The target parameters for primers were 40–60% GT, T_m greater than 60°C but within 5°C for a pair of primers, and a product size between 100 and 200 bp, although it was not possible to achieve all target parameters for all primer pairs. Primer specificity was checked for cross-reactivity against IESP extracts from within the same invertebrate order and from at least one other order (Table 1) using the PCR conditions described above apart from the annealing temperatures which are given in Table 1.

Detecting IESP in soil samples “augmented” with IESP DNA

Approximately 1 L of soil was collected from eight kiwifruit orchards (Bay of Plenty, New Zealand) and eight apple orchards (Hawke’s Bay, New Zealand), in September 2016 (Figure 1). Soil was collected haphazardly from within a 500 m² area in each orchard, using multiple soil cores 8 cm in diameter and 2 cm deep, bulked to form one sample (in a 1 L beaker) per orchard and frozen at -20°C. In May 2017 each sample was defrosted, sieved to 2 mm, and divided in half. To test the ability of the primers to detect the IESP in the presence and absence of other DNA, one half of each sample was sterilised through receiving a total exposure of 73–74 kGy gamma radiation at the MSD Animal Health Laboratory in Wellington, New Zealand (www.msd-animal-health.co.nz). The pH of the samples ranged from 5.1 to 6.6, the acidity of which is likely to promote the binding of extracellular DNA to the soil surface (Young *et al.*, 2014). Consequently, each sterilised sample was inoculated with 50 g of potting mix. We hypothesised that the potting mix was likely to contain bacterial DNA but very little invertebrate DNA because the amount of time the mix had been sealed in its bag allowed for bacterial degradation of extant invertebrate DNA: the bacterial DNA would be available to bind to the soil during DNA extraction and, thus, reduce the loss of the IESP DNA through surface absorption during extraction.

The sterilised and unsterilised halves of each sample were further divided into five subsamples (average weight of 50 g, range 30–70 g) to which were added known weights of up to six IESP to produce the “augmented” soil samples (Table 2; Figure 1). Each IESP was added on its own to at least one sterilised subsample, with the 20 IESP found in apple orchards added only to apple soil, and the 20 IESP found in kiwifruit orchards added only to kiwifruit soil. The IESP specimens that were added to the soil had been collected during the previous 6 months and stored in 95% ethanol before being morphologically identified. This storage medium has been shown to result in high DNA yield from insects (Moreau *et al.*, 2013). Weighed IESP (or fragments thereof) were ground in liquid nitrogen, mixed thoroughly into the appropriate soil subsample, and stored at -20°C for later DNA extraction. Equipment that was specific to each IESP was used for handling, grinding and mixing to avoid cross-contamination.

DNA was extracted from a 10 g aliquot of each “augmented” soil subsample using DNeasy® PowerMax® Soil kit (Qiagen, Hilden) following the manufacturer’s instructions, except that disintegration was 10 minutes at 1250 Hz in a Genogrinder (SPEX SamplePrep, Metuchen, NJ, USA). Extracted DNA was then treated using DNA Clean and Concentrator™ (Zymo Research, Tustin, CA, USA). Amplification of the DNA was performed twice. Firstly, the specific IESP primers were used under PCR conditions described above. These primers were only screened against soils to which the relevant IESP had been added to ensure the primers could detect the target and to assess the likelihood of detecting false negatives. Secondly, the Folmer primers were used under PCR conditions described above, except that Platinum Taq High Fidelity (Invitrogen, Carlsbad, CA, USA) was used, and the products pooled from three to five PCRs. Folmer primers were chosen for metabarcoding because the COI gene is the standard barcode for invertebrates, and has been shown to produce beta diversities from eDNA samples that are strongly correlated with those from traditional biodiversity measures (Drummond *et al.*, 2015). In addition, the target sequence is long (710-bp; Folmer *et al.*, 1994), potentially enabling us to only detect DNA that had been deposited recently (i.e., by current IESP populations) and had not had time to degrade. PCR products amplified using the Folmer primers from each of the five sterilised and five unsterilised subsamples were then recombined for sequencing, resulting in one sterilised and one unsterilised sample per orchard.

The 32 samples produced using Folmer primers were sent to the Australian Genome Research Facility (AGRF, www.agrf.org.au) where barcoded Nextera transposon libraries were generated and the resulting libraries sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, USA) generating 300 bp paired end fragments (V3 chemistry). Sequences obtained from AGRF were assessed for quality using Fast QC v1.91 and analysed using the Qiime2 v2018.2 workflow (Bolyen *et al.*, 2018). Briefly, samples were error-corrected and assigned to Amplicon Sequence Variants (ASVs) using DADA2 (Callahan *et al.*, 2016), the phylogeny of ASVs and alpha and beta diversity of samples was assessed, and ASVs were assigned to a taxonomic group. Taxonomic assignment was conducted within Qiime2 using the scikit-learn Python library (<https://scikit-learn.org/stable/index.html>), using custom sequence databases. Custom databases were constructed from COI sequences for each IESP (either obtained during this project or from Genbank) as well as a customised library of almost 2000 COI sequences for New Zealand invertebrates (e.g., from Drummond *et al.*,

2015) and other closely related invertebrate taxa available on Genbank. Taxonomy assignment was performed using a p-confidence threshold of 0.7 (Wang *et al.*, 2007).

Comparing traditional and metabarcoding methods for detecting IESP in orchards

Ten soil cores (8 cm diameter × 2 cm deep) were collected haphazardly from within a 100 m² area in each of ten kiwifruit orchards (Bay of Plenty, New Zealand) and ten apple orchards (Hawke's Bay, New Zealand), during February and March 2017 (Figure 1). Five of the kiwifruit orchards (K1–K5) were managed using an integrated pest management system (IPM), and the remaining five (K6–K10) were under organic management, whereas five of the apple orchards (A1–A5) were managed using an integrated fruit production system (IFP), with five (A6–A10) under organic management.

The ten soil cores were combined into a single sample per orchard, sieved to 2 mm, and stored at -20°C for later DNA extraction. DNA was initially extracted from two 10 g aliquots from each sample, but if the DNA quantity seemed low (i.e., below 10 ng µL⁻¹), a further two aliquots were extracted. DNA extracts for each orchard were combined and treated using DNA Clean and Concentrator^{TM} (Zymo Research, Irvine, CA, USA). PCRs with the Folmer primers were conducted as described above, and PCR products (one sample per orchard) were sent to AGRF for sequencing. The resulting sequences were analysed for presence of the focal IESP sequences using the Qiime2 v2018.6 workflow as described above. Full details of the bioinformatics workflows can be viewed on request at <https://github.com/PlantandFoodResearch/bioinf-eDNA-ESP>.

To compare the efficiency of the metabarcoding methodology with traditional methods of invertebrate sampling, five yellow pan traps, five flight-intercept traps, five pitfall traps, and five yellow sticky traps were placed into the same 100 m² area immediately following the collection of the soil samples from each orchard. Traps were deployed for 6 days. These traps were selected based on the results of previous surveys of invertebrate taxa in apple and kiwifruit orchards that showed this combination of traps was the most likely to collect all of the focal IESP if they were present (Malone *et al.*, 2017a; Todd *et al.*, 2011). Invertebrates collected in the pan and intercept traps were transferred into containers containing 95% ethanol, pitfall traps contained monoethylene glycol to preserve captured invertebrates, and

sticky traps were stored at -20°C until captured invertebrates could be identified. Conventional morphological identification methods were used to determine the abundance of each of the focal IESP in each sample.

Statistical Analysis

Statistical analyses were carried out using R version 3.5.1 (R Development Core Team, 2018). For the “DNA-augmented” soil samples, the analysis investigated the effect of IESP identity and weight added to the soil sample, and their interaction, on the ability of the Folmer primers to detect each IESP. Binomial generalised linear models with a logit link function using the package mvabund (Wang *et al.*, 2019) were selected for this investigation. For the samples collected in traps from the ten apple and ten kiwifruit orchards, Poisson generalised linear mixed models were used to investigate the effects of orchard management (IFP or organic in apple; IPM or organic in kiwifruit) on the abundance of each IESP. Means and 95% confidence intervals were obtained with least square means, and post hoc pairwise comparisons were carried out using the Tukey test.

Results

Development of IESP-specific primers

COI and/or 28S rDNA sequences were produced for each of the 26 IESP selected for this study (COI GenBank MK736030–47, 28S GenBank MK748223–40), and specific primers for each taxon were successfully developed from these sequences (Table 1). It was not possible to obtain COI sequences for *Conoderus exsul* (Sharp), *Anthomyia punctipennis* Weidemann and *Akamptogonus novarae* (Humbert & Saussure), and COI sequences for several of the other IESP were difficult to obtain with the Folmer primers. Consequently, 28S rDNA sequences and primers for these sequences were developed for several IESP (Table 1). There was no correlation between the taxonomic identity of the IESP and the ease of obtaining a barcode for that IESP.

Detecting IESP in soil samples “augmented” with IESP DNA

The IESP-specific primers detected the IESP in 96% of the sterilised and 100% of the unsterilised soil subsamples to which each IESP was added (Table 3), with primers for COI and 28S working equally well. The only species the specific primers failed to detect was *Ausejanus albisignatus* (Knight), which was only added to a single sterile soil subsample because of a lack of specimens. The detection rates for the other taxon-specific primers were greater than 90%, except for those for *A. punctipennis* at 75%.

The sequencing of the PCR products from the combined sterilised soil samples (i.e., one sample per orchard) using the Folmer primers resulted in an average of 1233 ASVs (range 879 to 1538) per sample. For the combined unsterilised samples, an average of 1313 ASVs (range 292 to 2675) were produced. Matching these to the sequences for the IESP that had been added to the sterilised and unsterilised samples resulted in very few detections. Only 33% of the IESP that had been added to the sterilised soil samples were detected, and none of the added IESP were detected in the unsterilised samples (Table 3). In the sterilised samples, 13 IESP were not detected at all, and for the remaining 13 IESP, detection rates ranged from 7%, for *Arcitalitrus* spp., to 100% for *Lonchoptera bifurcata* (Fallen) and *Tetramorium grassii* Emery. In addition, the Folmer primers detected *Trigonospila brevifacies* (Hardy) in a sterilised sample to which it had not been added. This may indicate that the sterilisation procedure was not completely effective at removing all DNA from the soil.

Further analysis detected an interaction effect between the identity of the IESP and the weight of the IESP added to the sterilised soil on the detection of the IESP using the Folmer primers in both the apple ($P_{\text{taxa:weight}} = 0.03$) and kiwifruit ($P_{\text{taxa:weight}} = 0.05$) orchards. Consequently, there does not appear to be a direct relationship between the detectability of each IESP and the amount of DNA in the soil.

Comparing traditional and metabarcoding methods for detecting IESP in orchards

Analysis of the sequences produced from the soil samples from each orchard (following removal of sequences with fewer than 10 reads) identified a total of 34,679 ASVs. Of those, 13,303 ASVs were found only in the kiwifruit orchards, and 19,443 ASVs were found only in apple orchards, leaving only 1,933 ASVs in common between the two orchard types. Individual orchards contained 128–5,150 ASVs. Very few of the ASVs could be matched to the sequences for the focal IESP. Only three IESP were detected: *T. brevifacies* was detected

in orchards A3, A6 and K8; *Nylanderia* sp(p). was detected in orchards A2 and A6; *Armadillidium vulgare* (Latreille) was detected in orchard A6. Additionally, some of the ASVs matched one other species in the customised COI library: *Carpophilus davidsoni* Dobson (a beetle that was not included in the list of IESP) was detected in orchard A4. Some of the remaining ASVs were similar enough to be classified with a group of dipteran sequences or Arthropoda sequences, but the majority were unassigned.

The morphological analysis of the invertebrates collected in the pan, intercept, pitfall and sticky traps revealed the presence of all the focal IESP in at least one orchard, and a range of 8 to 17 of the 20 IESP found in each orchard (Figure 2). This contrasts starkly with the metabarcoding results described above that found very few IESP in the soil collected from the same location within the orchards. The three IESP that were detected in the soil (i.e., *T. brevifacies*, *Nylanderia* sp(p)., and *A. vulgare*) were also collected in traps from the same orchards, except for orchards A3 and A6 where *T. brevifacies* was detected in the soil but not collected in traps.

The abundances of the IESP varied between orchards, with some species found occasionally (e.g., the predatory beetle *Thyreocephalus orthodoxus* (Olliff) was found in one apple orchard and five kiwifruit orchards, with a maximum of seven individuals collected from one kiwifruit orchard) and others found relatively frequently in all orchards (e.g., between 5 and 209 *Sericoderus* sp. beetles were collected from each of the 20 orchards) (Figure 2). The Poisson models indicated that that abundances of most of the IESP in the apple orchards were not affected by orchard management, with equal numbers collected from the IFP and organic orchards (Table 4). However, four IESP (natural enemies *Aphelinus mali* (Haldeman), *Nylanderia* sp(p)., *Platygaster demades* Walker, and detritivore *Sericoderus* sp.) were in greater abundance in IFP orchards than in organic orchards, and four other IESP (natural enemy *A. albisignatus* and detritivores *A. vulgare*, *Cartodere* spp. and *Ephistemus globulus* (Paykull)) were captured in greater numbers from the organic orchards (Table 4). In the kiwifruit orchards, a difference in abundance between the organic and IPM orchards was detected for 12 of the IESP (Table 5), with nine IESP in greater abundance in the organic orchards (natural enemies *Anoteropsis hilaris* (L. Koch), *C. exsul*, *Phalangium opilio* L., *T. orthodoxus*, and detritivores *Arcitalitrus* spp., *Atomaria lewisi* Reitter, *Anotylus* sp., *L. bifurcata*, *Sericoderus* sp.), and three in greater abundance in the IPM orchards (natural

enemies *Micromus tasmaniae* (Walker), *Monomorium antarcticum* (F. Smith), and *T. brevifacies*).

Discussion

The results of this study show that the development of specific primers for IESP may be a useful way to monitor these beneficial invertebrates using eDNA in soil samples. Detection probabilities for the primers developed for most of the 26 focal taxa were greater than 90% in sterilised soil samples, and 100% in unsterilised samples to which the DNA of the taxa had been added. These taxon-specific primers could be used to monitor these IESP in future studies, and to potentially detect changes in the ecosystem services they provide, without needing to remove viable individuals from the system. Use of these primers is reasonably inexpensive, especially when compared with the cost of metabarcoding, and PCR results give an immediate result regarding the presence (or absence) of the IESP. The next step will be to test these primers with soil samples taken directly from orchards.

In contrast, the sequences produced using the Folmer primers did not match the sequences of most of the IESP, even in the sterilised soil samples. This may have resulted from preferential amplification of other DNA in the samples by these primers, or because the PCR conditions were not favourable for amplifying the IESP DNA. Whatever the reason, these results suggest that the Folmer primers are not appropriate for monitoring these IESP in orchard soil samples. In addition, the finding that it was difficult, if not impossible, to obtain COI sequences from the IESP DNA extracts using the Folmer primers also indicates that these primers are not adequate for detecting these IESP. There are a number of other primers that may be better alternatives. While the COI gene is the traditional barcode sequence for invertebrates, recent studies have shown that ribosomal 18S (Horton *et al.*, 2017) or 16S rDNA (Clarke *et al.*, 2014) genes may be more reliable sequences for detecting invertebrates. Even with the COI barcode, the best primers for detecting different taxa can vary because of sequence mismatches in the target annealing position (Geller *et al.*, 2013). Consequently, primers that are better able to detect the COI sequences for New Zealand's terrestrial invertebrates, potentially those developed by Geller *et al.* (2013) and Rennstam Rubbmark *et al.* (2018), are needed.

The interaction effect of IESP identity and the weight of the IESP added to the soil samples on the detectability of those taxa indicate that increasing the amount of DNA present in the soil does not increase detectability for all taxa. This is consistent with other studies that have shown that the Folmer primers have sequence biases (Clarke *et al.*, 2014; Pinol *et al.*, 2015), and are, therefore, more likely to detect some taxa than others. This is backed up by the finding that the IESP that were detected in the soil collected from the ten apple and ten kiwifruit orchards (i.e., *T. brevifacies*, *Nylanderia* sp(p). and *A. vulgare*) were not the most abundant IESP collected in the traps in the orchards in which they were detected. The detection of *T. brevifacies* in the soil of three orchards using the Folmer primers does at least indicate that metabarcoding of eDNA in soil can be used to detect taxa that are present but that do not live primarily in the soil or on the soil surface. *Trigonospila brevifacies* is a tachinid parasitoid of Lepidoptera and, therefore, in the larval stage occurs within lepidopteran hosts that feed on plant material, and the adult stage disperses through flight (Munro, 1998). Thus, if more consistent primers can be produced for metabarcoding of invertebrate eDNA in soil samples, then it may be possible to use this method to monitor both ground-dwelling and plant-dwelling taxa.

Finally, differences in the abundances of IESP in orchards with different management systems was not unexpected given the results of earlier surveys of the invertebrate communities in apple and kiwifruit orchards (Malone *et al.*, 2017a; Todd *et al.*, 2011). For instance, greater abundances of *A. albisignatus*, *A. vulgare* and *E. globulus* in organic apple orchards, and greater abundances of *A. mali* and *Nylanderia* spp. in the IFP apple orchards were found in both this study and that by Malone *et al.* (2017a). In the kiwifruit orchards, nine IESP (four natural enemies and five detritivores) were collected in greater abundances from the organic orchards, whereas three natural enemies were collected in greater abundances from the IPM orchards. This adds to the finding of greater taxonomic richness in the organic orchards by Todd *et al.* (2011), although in that study there was no indication of differences in detritivore communities between the two orchard types. Further work is needed to determine if these differences translate into functional differences in the ecosystem services provided by these taxa. Initial investigations suggest that the difference in natural enemy taxa between organic and IPM kiwifruit orchards does not translate into a difference in parasitism rates of leafroller pests (Todd *et al.*, 2018).

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Conflict of Interest

All contributing authors declare that they have no conflicting interests with the research described in this manuscript.

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Table 1: Invertebrate ecosystem service providers (IESP) selected for this project. Species were selected because they were involved in either decomposition (decomp.) or pest suppression (pest sup.) on apple or kiwifruit orchards or both. At least ten IESP were selected from those that spend most of their life cycle in soil and/or leaf litter (ground), and at least five were selected from those that occur primarily above ground (on plants). Primers were designed against sequences generated in this study for either 28S ribosomal DNA (28S rDNA) or mitochondrial cytochrome *c* oxidase gene subunit 1 (COI), except those for *Forficula auricularia* where a Genbank sequence was used.

IESP	Orchard	Service	Primary habitat	Forward primer	Reverse primer	T _A	Cross Group
<i>Aphelinus mali</i> (Hym.)	Apple	Pest sup.	On plants	Ama28SF GCTGTCGCTGCGGTATAA	Ama28SR GGCCAATACCGTTCAATTA	50	A
<i>Ausejanus albisignatus</i> (Hem.)	Apple	Pest sup.	On plants	Aal28SF GTGGTAGTGGAGTTGCAGAG	Aal28SR GTGCAAGCACGTCGAA	54	B
<i>Platygaster demades</i> (Hym.)	Apple	Pest sup.	On plants	Pde28SF GACTGTTGCGGATGCTT	Pde28SR ATCTTTCGGGTCCCAAC	55	A
<i>Anthomyia punctipennis</i> (Dipt.)	Apple	Decomp.	Ground	Apu28SF ATGCTAGAATTTCTGCTTCG	Apu28SR GGTGATACTGCCAGCTTAAA	45	C
<i>Armadillidium vulgare</i> (Iso.)	Apple	Decomp.	Ground	Avu28SF CCCCACTAGATGGGTCA	Avu28SR GAGACCGGGACACGAA	55	D
<i>Ephistemus globulus</i> (Col.)	Apple	Decomp.	Ground	EglCOIF TGATTATTACCTCCATCATTAAC	EglCOIR TCGGTCAAAATTTATTCCTT	50	B
<i>Anoteropsis hilaris</i> (Ara.)	Both	Pest sup.	Ground	AhiCOIF TCTTCTAGAATAGGTCACATAG	AhiCOIR CTAATACAGGTAACGACAACAAC	50	D
<i>Conoderus exsul</i> (Col.)	Both	Pest sup.	Ground	Cex28SF GACACGTTGCTAAACCTAAAG	Cex28SR CGAACGCCTCGCCCATCCT	50	B

<i>Forficula auricularia</i> (Derm.)	Both	Pest sup.	Ground, on plants	Fau28SF CGTTATCAAGAGATGTTATG	Fau28SR CAGATTTTCGGATTCTCCC	50	C
<i>Micromus tasmaniae</i> (Neu.)	Both	Pest sup.	On plants	Mta28SF GCGTAATGAAAGTAAATGGTT	Mta28SR TGCGACTCTTATTCATTCA	50	A
<i>Nylanderia sp(p).</i> (Hym.)	Both	Pest sup.	Ground	NtaCOIF CTGACTACTCCCCCTTCTATTTTC	NtaCOIR GCCCCTGCTAATACAGGTAATG	55	A
<i>Phalangium opilio</i> (Opi.)	Both	Pest sup.	Ground, on plants	Pop28SF GCCGAATAAACCATGGTGTTTAAAGC	Pop28SR CGGGACTTGCGAATGAGAGGTC	50	D
<i>Thyreocephalus</i> <i>orthodoxus</i> (Col.)	Both	Pest sup.	Ground	Tor28SF CGAGTGGCGGTGAT	Tor28SR GGTCCGACGGAGGAT	50	B
<i>Trigonospila brevifacies</i> (Dipt.)	Both	Pest sup.	On plants	TbrCOIF AGATTCTGATTACTTCCACCA	TbrCOIR AAAATAGTTAAATCTACTGAAGGA	54	C
<i>Arcitalitrus spp.</i> (Amph.)	Both	Decomp.	Ground	Arspp28SF TGGGAGGTGCGCAAG	Arspp28SR GGTAGGAGAGCTTCAACACA	50	D
<i>Atomaria lewisi</i> (Col.)	Both	Decomp.	Ground	Ale28SF GCGACGCGTGCAT	Ale28SR CCGCAAAGCGAGCA	45	B
<i>Cartodere spp.</i> (Col.)	Both	Decomp.	Ground	Caspp28SF GACCAAGGAGTCTAGCATGT	Caspp28SR GACCGCCGTATTAGGAA	55	B
<i>Lonchoptera bifurcata</i> (Dipt.)	Both	Decomp.	Ground	LbiCOIF GGAGCACCAGACATAGCATTCCC	LbiCOIR CTCCAGCATGAGCAATTCCAGAG	50	C
<i>Porcellio scaber</i> (Iso.)	Both	Decomp.	Ground	Psc28SF GCGGAACGAAAGTGATT	Psc28SR GCGCCGTCCACATATTA	50	D
<i>Sericoderus sp.</i> (Col.)	Both	Decomp.	Ground	Sespp28SF CAACATTAGTTTGCGTTCAA	Sespp28SR CGCCTTAGGTTTAATCAAT	45	B

<i>Meteorus pulchricornis</i> (Hym.)	Kiwifruit	Pest sup.	On plants	MpuCOIF GGTGTGGTAGATTTTAGG	MpuCOIR CAGCTCCTATAATCGAAGAAGCC	55	A
<i>Monomorium antarcticum</i> (Hym.)	Kiwifruit	Pest sup.	Ground	Man28SF GAGTCATTGGGACTTGACA	Man28SR GATGCTCGTGGCTTCATA	55	A
<i>Scymnus loewii</i> (Col.)	Kiwifruit	Pest sup.	On plants	SloCOIF CGCGAGTCATTGGGATAA	SloCOIR TCGCAATGAGAATGAGACG	55	B
<i>Tetramorium grassii</i> (Hym.)	Kiwifruit	Pest sup.	Ground	TgrCOIF AGATTTTGACTTTTACCTCCA	TgrCOIR AAGATTGATAAGTCGATAGAAGGT	50	A
<i>Akamptogonus novarae</i> (Dipl.)	Kiwifruit	Decomp.	Ground	Ano28SF GTCCAGTCTGATCGCCTCGCTTAG	Ano28SR GGACTTCCACCAGAGTTTC	50	D
<i>Anotylus sp.</i> (Col.)	Kiwifruit	Decomp.	Ground	AnsppCOIF TTTAGAAGAATTGTTGAAAGT	AnsppCOIR AGAAGAGATTCTGCTAAAT	55	B

Primers are given 5' to 3'; T_A: temperature used for annealing in PCR; Cross Group: primers for species within a letter group were tested against all species of that group for cross reactivity; Amph. = Amphipoda; Ara. = Araneae; Col. = Coleoptera; Derm. = Dermaptera; Dipl. = Diplopoda; Dipt. = Diptera; Hem. = Hemiptera; Hym. = Hymenoptera; Iso. = Isopoda; Neu. = Neuroptera; Opi. = Opiliones.

Table 2: Quantity of invertebrate ecosystem service providers (IESP), or parts thereof, added to sterilised and unsterilised soil samples.

Sterilised subsample no.	No. of IESP added	Target weight (g)	Actual weight of each IESP added to soil	Unsterilised subsample no.	No. of IESP added	Target weight (g)	Actual weight of each IESP added to soil
1	1	0.1	0.059*–0.13	1	1	0.1	0.101–0.113
2	1	0.01	0.006*–0.013	2	1	0.01	0.008*–0.013
3	3	0.033	0.033–0.049	3	1	0.033	0.033–0.037
4	5 [#]	0.02	0.020–0.034	4	1	0.02	0.020–0.026
5	5	0.002	0.002–0.004	5	1	0.002	0.002–0.003

*Maximum weight available for one of the IESPs added to a subsample. [#]Six IESP were accidentally added to a subsample from one orchard, but each was added to the subsample at approximately 0.02 g.

Table 3: Detection of invertebrate ecosystem service providers (IESP) added to sterilised (Ster.) and unsterilised (Unst.) soil subsamples obtained from eight apple and eight kiwifruit orchards.

IESP	Apple Orchards				Kiwifruit Orchards			
	Detected using specific primers (Tot.Pos.Subs.) ¹		Detected using Folmer primers (Tot.Pos.Comb.) ²		Detected using specific primers (Tot.Pos.Subs.) ¹		Detected using Folmer primers (Tot.Pos.Comb.) ²	
	Ster.	Unst.	Ster.	Unst.	Ster.	Unst.	Ster.	Unst.
<i>Aphelinus mali</i>	6 (6)	1 (1)	2 [#] (6)	0 (1)				
<i>Ausejanus albisignatus</i>	0 (1)	*	0 (1)	*				
<i>Platygaster demades</i>	1 (1)	*	0 (1)	*				
<i>Anthomyia punctipennis</i>	3 (4)	*	4 (4)	*				
<i>Armadillidium vulgare</i>	12 (13)	4 (4)	2 (7)	0 (4)				
<i>Ephistemus globulus</i>	1 (1)	*	1 (1)	*				
<i>Anoteropsis hilaris</i>	11 (11)	4 (4)	6 (7)	0 (4)	8 (8)	2 (2)	3 (7)	0 (2)
<i>Conoderus exsul</i>	10 (10)	4 (4)	0 (7)	0 (4)	7 (7)	2 (2)	0 (5)	0 (2)
<i>Forficula auricularia</i>	7 (8)	4 (4)	0 (6)	0 (4)	8 (9)	3 (3)	0 (6)	0 (3)
<i>Micromus tasmaniae</i>	1 (1)	*	0 (1)	*	*	*	*	*
<i>Nylanderia</i> sp(p).	6 (6)	3 (3)	1 (6)	0 (3)	6 (6)	2 (2)	2 (5)	0 (2)
<i>Phalangium opilio</i>	10 (10)	3 (3)	3 (7)	0 (3)	8 (9)	4 (4)	2 (6)	0 (4)
<i>Thyreocephalus orthodoxus</i>	7 (8)	4 (4)	0 (7)	0 (4)	9 (9)	2 (2)	0 (7)	0 (2)
<i>Trigonospila brevifacies</i>	4 (4)	1 (1)	5 [^] (4)	0 (1)	6 (7)	1 (1)	6 (6)	0 (1)
<i>Arcitalitrus</i> spp.	9 (9)	4 (4)	1 (8)	0 (4)	7 (7)	2 (2)	0 (6)	0 (2)
<i>Atomaria lewisi</i>	1 (1)	*	0 (1)	*	*	*	*	*
<i>Cartodere</i> spp.	7 (7)	3 (3)	0 (6)	0 (3)	5 (5)	2 (2)	0 (5)	0 (2)
<i>Lonchoptera bifurcata</i>	3 (3)	1 (1)	3 (3)	0 (1)	4 (4)	*	3 (3)	*
<i>Porcellio scaber</i>	8 (9)	3 (3)	6 (7)	0 (3)	8 (8)	3 (3)	6 (7)	0 (3)
<i>Sericoderus</i> sp.	7 (7)	1 (1)	0 (7)	0 (1)	5 (5)	2 (2)	0 (5)	0 (2)
<i>Meteorus pulchricornis</i>					8 (8)	1 (1)	5 (6)	0 (1)
<i>Monomorium antarcticum</i>					2 (2)	4 (4)	0 (1)	0 (4)

<i>Scymnus loewii</i>	7 (7)	1 (1)	0 (6)	0 (1)
<i>Tetramorium grassii</i>	4 (4)	3 (3)	3 (3)	0 (3)
<i>Akamptogonus novarae</i>	10 (10)	4 (4)	0 (7)	0 (4)
<i>Anotylus sp.</i>	6 (6)	2 (2)	0 (5)	0 (2)

¹Specific primers designed for each IESP (see Table 1) were tested for their ability to detect the IESP in each soil subsample to which it had been added (Tot.Pos.Subs.).

²Folmer primers were used to produce COI sequences that were then matched to sequences for each IESP. Tot.Pos.Comb. = total number of combined samples to which each IESP had been added.

* indicates where there were not enough specimens of the IESP to add to soil samples

these sequences matched the sequence for *Aphelinus abdominalis* but not *A. mali*

^ IESP identified in sample to which it was not added

Table 4: Comparison of invertebrate ecosystem service provider (IESP) abundances in ten apple orchards under two different management systems: five organic and five integrated fruit production (IFP) orchards were sampled. Mean abundances and 95% confidence intervals (CI) have been back-transformed. Each IESP was modelled separately.

IESP	Orchard System	Mean	Lower 95% CI	Upper 95% CI	Letter of difference (alpha=0.05)	z.ratio	p.value
<i>Arcitalitrus</i> spp.	IFP	0	-	-	-	0	1
	Organic	0	-	-	-		
<i>Anoteropsis hilaris</i>	IFP	1.6	0.8	3.2	-	0.005	0.9963
	Organic	0	-	-	-		
<i>Sericoderus</i> sp.	IFP	86.2	78.43	94.73	A	10.79	<.0001
	Organic	31.6	27.04	36.93	B		
<i>Atomaria lewisi</i>	IFP	0.6	0.19	1.86	A	-0.377	0.7064
	Organic	0.8	0.3	2.13	A		
<i>Ephistemus globulus</i>	IFP	0.2	0.03	1.42	A	-2.975	0.0029
	Organic	4.2	2.74	6.44	B		
<i>Conoderus exsul</i>	IFP	6.8	4.86	9.52	A	1.883	0.0597
	Organic	4	2.58	6.2	A		
<i>Cartodere</i> spp.	IFP	15	11.96	18.81	A	-2.158	0.0309
	Organic	20.8	17.16	25.21	B		
<i>Thyreocephalus orthodoxus</i>	IFP	0	-	-	-	-0.003	0.9979
	Organic	0.2	0.03	1.42	-		
<i>Forficula auricularia</i>	IFP	0	-	-	-	-0.003	0.9978
	Organic	0.4	0.1	1.6	-		
<i>Anthomyia punctipennis</i>	IFP	1.2	0.54	2.67	A	-0.989	0.3226
	Organic	2	1.08	3.72	A		
<i>Lonchoptera bifurcata</i>	IFP	1.8	0.94	3.46	A	1.924	0.0543
	Organic	0.4	0.1	1.6	A		
<i>Trigonospila brevifacies</i>	IFP	0.2	0.03	1.42	A	0	1
	Organic	0.2	0.03	1.42	A		
<i>Ausejanus albisignatus</i>	IFP	0.8	0.3	2.13	A	-2.721	0.0065

	Organic	3.6	2.27	5.71	B		
<i>Aphelinus mali</i>	IFP	236.2	223.11	250.06	A	22.921	<.0001
	Organic	43.6	38.18	49.79	B		
<i>Nylanderia sp(p).</i>	IFP	46	40.42	52.35	A	10.315	<.0001
	Organic	7.4	5.36	10.21	B		
<i>Platygaster demades</i>	IFP	5	3.38	7.4	A	2.805	0.005
	Organic	1.6	0.8	3.2	B		
<i>Armadillidium vulgare</i>	IFP	4.6	3.06	6.92	A	-2.219	0.0265
	Organic	8.2	6.04	11.14	B		
<i>Porcellio scaber</i>	IFP	0	-	-	-	-0.012	0.9902
	Organic	6	4.2	8.58	-		
<i>Micromus tasmaniae</i>	IFP	1	0.42	2.4	A	-0.301	0.7633
	Organic	1.2	0.54	2.67	A		
<i>Phalangium opilio</i>	IFP	2.4	1.36	4.23	A	0.652	0.5141
	Organic	1.8	0.94	3.46	A		

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Table 5: Comparison of invertebrate ecosystem service provider (IESP) abundances in ten kiwifruit orchards under two different management systems: five organic and five integrated pest management (IPM) orchards were sampled. Mean abundances and 95% confidence intervals (CI) have been back-transformed. Each IESP was modelled separately.

IESP	Orchard System	Mean	Lower 95% CI	Upper 95% CI	Letter of difference (alpha=0.05)	z.ratio	p.value
<i>Arcitalitrus spp.</i>	IPM	0.2	0.03	1.42	A	-5.373	<.0001
	Organic	43.6	38.18	49.79	B		
<i>Anoteropsis hilaris</i>	IPM	2.2	1.22	3.97	A	-2.846	0.0044
	Organic	6	4.2	8.58	B		
<i>Scymnus loewii</i>	IPM	0.2	0.03	1.42	A	-1.24	0.215
	Organic	0.8	0.3	2.13	A		
<i>Sericoderus sp.</i>	IPM	18.4	15	22.57	A	-7.388	<.0001
	Organic	45.8	40.24	52.13	B		
<i>Atomaria lewisi</i>	IPM	0.4	0.1	1.6	A	-2.464	0.0137
	Organic	2.6	1.51	4.48	B		
<i>Conoderus exsul</i>	IPM	4.2	2.74	6.44	A	-3.833	0.0001
	Organic	11.2	8.62	14.55	B		
<i>Cartodere spp.</i>	IPM	35.4	30.55	41.02	A	-1.48	0.1388
	Organic	41.2	35.94	47.23	A		
<i>Thyreocephalus orthodoxus</i>	IPM	0.4	0.1	1.6	A	-2.078	0.0377
	Organic	2	1.08	3.72	B		
<i>Anotylus sp.</i>	IPM	4.4	2.9	6.68	A	-2.457	0.014
	Organic	8.4	6.21	11.37	B		
<i>Forficula auricularia</i>	IPM	0.2	0.03	1.42	-	0.003	0.9979
	Organic	0	-	-	-		
<i>Akamptogonus novarae</i>	IPM	0	-	-	-	-0.003	0.9978
	Organic	0.4	0.1	1.6	-		
<i>Lonchoptera bifurcata</i>	IPM	2.6	1.51	4.48	A	-2.041	0.0413
	Organic	5.2	3.54	7.64	B		
<i>Trigonospila brevifacies</i>	IPM	3.2	1.96	5.22	A	2.27	0.0232
	Organic	1	0.42	2.4	B		

<i>Meteorus</i>	IPM	1.6	0.8	3.2	A	0	1
<i>pulchricornis</i>	Organic	1.6	0.8	3.2	A		
<i>Monomorium</i>	IPM	11.4	8.79	14.78	A	5.077	<.0001
<i>antarcticum</i>	Organic	2	1.08	3.72	B		
<i>Nylanderia</i> sp(p).	IPM	4.6	3.06	6.92	-	0.012	0.9903
	Organic	0	-	-	-		
<i>Tetramorium</i> <i>grasii</i>	IPM	11	8.45	14.33	A	0.488	0.6257
	Organic	10	7.58	13.19	A		
<i>Porcellio</i> <i>scaber</i>	IPM	1	0.42	2.4	A	1.469	0.1418
	Organic	0.2	0.03	1.42	A		
<i>Micromus</i>	IPM	10.6	8.1	13.87	A	3.714	0.0002
<i>tasmaniae</i>	Organic	4	2.58	6.2	B		
<i>Phalangium</i> <i>opilio</i>	IPM	22.4	18.61	26.96	A	-4.96	<.0001
	Organic	40.2	35.01	46.16	B		

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Figure Legends

Figure 1: Sample collection and processing methods used in this study. IESP = invertebrate ecosystem service providers.

Figure 2: Relative abundance of invertebrate ecosystem service providers (IESP) in (a) ten apple orchards and (b) ten kiwifruit orchards. Orchards K1 – K5 were under integrated pest management; A1 – A5 under an integrated fruit production system; and the remaining kiwifruit and apple orchards were under organic management. IESP were collected from each orchard using pan, intercept, pitfall and sticky traps. Note that some of the IESP were specific to either apple or kiwifruit orchards (see Table 1 for more details).