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

3

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16

17

18 **Running title:** Detecting invertebrate ecosystem service providers

19

20

21 **Abstract**

22 1. The objective of this study was to assess barcoding of environmental DNA (eDNA) as  
23 a method for monitoring invertebrate ecosystem service providers (IESP) in soil  
24 samples.

25 2. We selected 26 IESP that occur in New Zealand kiwifruit or apple orchards and  
26 produced mitochondrial cytochrome *c* oxidase gene subunit I (COI) and/or 28S  
27 ribosomal DNA sequences for each. Specific barcode primers were designed for each  
28 IESP and tested along with generic barcoding COI primers for their ability to detect  
29 DNA from IESP that had been added to sterilised and unsterilised soil samples.

30 3. While the specific primers accurately detected the IESP in more than 96% of the  
31 samples, the generic COI primers detected only 33% of the IESP added to the  
32 sterilised samples, and none in the unsterilised samples.

33 4. In a field test, we compared metabarcoding with traditional invertebrate trapping  
34 methods to detect the IESP in ten kiwifruit and ten apple orchards. All IESP were

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

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

40

41

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

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46

## 47 **Introduction**

48

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

68

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

90

91    Previous studies have identified a number of IESP in apple and kiwifruit orchards in New  
92    Zealand and the management practices that may affect their populations (Malone *et al.*,  
93    2017b; Todd *et al.*, 2016). The aims for this study were to: (1) develop specific primers for 26  
94    IESP found in New Zealand kiwifruit and/or apple orchards; (2) test the ability of those  
95    specific primers to detect the IESP in soil samples to which the IESP had been added; (3) test  
96    the ability of generic primers for the mitochondrial cytochrome *c* oxidase gene subunit I  
97    (COI) to detect the IESP in soil samples to which the IESP had been added; (4) compare the  
98    ability of traditional invertebrate trapping methods and metabarcoding of eDNA in soil to  
99    detect the IESP in orchards; and (5) detect any differences in IESP populations in relation to  
100   orchard management systems.

101

102

## 103 **Methods**

104

### 105 *Development of IESP-specific primers*

106

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

128

129 Specific primers for each IESP (Table 1) were designed from the COI and/or 28S rDNA  
130 sequences with specificity checked using National Center for Biotechnology Information  
131 (NCBI) primer-BLAST (Ye *et al.*, 2012). The target parameters for primers were 40–60%  
132 GT, T<sub>m</sub> greater than 60°C but within 5°C for a pair of primers, and a product size between  
133 100 and 200 bp, although it was not possible to achieve all target parameters for all primer  
134 pairs. Primer specificity was checked for cross-reactivity against IESP extracts from within  
135 the same invertebrate order and from at least one other order (Table 1) using the PCR  
136 conditions described above apart from the annealing temperatures which are given in Table 1.

137

138 *Detecting IESP in soil samples “augmented” with IESP DNA*

139

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

156

157 The sterilised and unsterilised halves of each sample were further divided into five  
158 subsamples (average weight of 50 g, range 30–70 g) to which were added known weights of  
159 up to six IESP to produce the “augmented” soil samples (Table 2; Figure 1). Each IESP was  
160 added on its own to at least one sterilised subsample, with the 20 IESP found in apple  
161 orchards added only to apple soil, and the 20 IESP found in kiwifruit orchards added only to  
162 kiwifruit soil. The IESP specimens that were added to the soil had been collected during the  
163 previous 6 months and stored in 95% ethanol before being morphologically identified. This  
164 storage medium has been shown to result in high DNA yield from insects (Moreau *et al.*,  
165 2013). Weighed IESP (or fragments thereof) were ground in liquid nitrogen, mixed  
166 thoroughly into the appropriate soil subsample, and stored at -20°C for later DNA extraction.  
167 Equipment that was specific to each IESP was used for handling, grinding and mixing to  
168 avoid cross-contamination.

169

170 DNA was extracted from a 10 g aliquot of each “augmented” soil subsample using DNeasy®  
171 PowerMax® Soil kit (Qiagen, Hilden) following the manufacturer’s instructions, except that  
172 disintegration was 10 minutes at 1250 Hz in a Genogrinder (SPEX SamplePrep, Metucen, NJ,  
173 USA). Extracted DNA was then treated using DNA Clean and Concentrator™ (Zymo  
174 Research, Tustin, CA, USA). Amplification of the DNA was performed twice. Firstly, the  
175 specific IESP primers were used under PCR conditions described above. These primers were  
176 only screened against soils to which the relevant IESP had been added to ensure the primers  
177 could detect the target and to assess the likelihood of detecting false negatives. Secondly, the  
178 Folmer primers were used under PCR conditions described above, except that Platinum Taq  
179 High Fidelity (Invitrogen, Carlsbad, CA, USA) was used, and the products pooled from three  
180 to five PCRs. Folmer primers were chosen for metabarcoding because the COI gene is the  
181 standard barcode for invertebrates, and has been shown to produce beta diversities from  
182 eDNA samples that are strongly correlated with those from traditional biodiversity measures  
183 (Drummond *et al.*, 2015). In addition, the target sequence is long (710-bp; Folmer *et al.*,  
184 1994), potentially enabling us to only detect DNA that had been deposited recently (i.e., by  
185 current IESP populations) and had not had time to degrade. PCR products amplified using the  
186 Folmer primers from each of the five sterilised and five unsterilised subsamples were then  
187 recombined for sequencing, resulting in one sterilised and one unsterilised sample per  
188 orchard.

189

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

203 2015) and other closely related invertebrate taxa available on Genbank. Taxonomy  
204 assignment was performed using a p-confidence threshold of 0.7 (Wang *et al.*, 2007).

205

### 206 *Comparing traditional and metabarcoding methods for detecting IESP in orchards*

207

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

215

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

226

227 To compare the efficiency of the metabarcoding methodology with traditional methods of  
228 invertebrate sampling, five yellow pan traps, five flight-intercept traps, five pitfall traps, and  
229 five yellow sticky traps were placed into the same 100 m<sup>2</sup> area immediately following the  
230 collection of the soil samples from each orchard. Traps were deployed for 6 days. These traps  
231 were selected based on the results of previous surveys of invertebrate taxa in apple and  
232 kiwifruit orchards that showed this combination of traps was the most likely to collect all of  
233 the focal IESP if they were present (Malone *et al.*, 2017a; Todd *et al.*, 2011). Invertebrates  
234 collected in the pan and intercept traps were transferred into containers containing 95%  
235 ethanol, pitfall traps contained monoethylene glycol to preserve captured invertebrates, and



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

239

#### 240 *Statistical Analysis*

241

242 Statistical analyses were carried out using R version 3.5.1 (R Development Core Team,  
243 2018). For the “DNA-augmented” soil samples, the analysis investigated the effect of IESP  
244 identity and weight added to the soil sample, and their interaction, on the ability of the  
245 Folmer primers to detect each IESP. Binomial generalised linear models with a logit link  
246 function using the package mvabund (Wang *et al.*, 2019) were selected for this investigation.  
247 For the samples collected in traps from the ten apple and ten kiwifruit orchards, Poisson  
248 generalised linear mixed models were used to investigate the effects of orchard management  
249 (IFP or organic in apple; IPM or organic in kiwifruit) on the abundance of each IESP. Means  
250 and 95% confidence intervals were obtained with least square means, and post hoc pairwise  
251 comparisons were carried out using the Tukey test.

252

253

## 254 **Results**

255

### 256 *Development of IESP-specific primers*

257

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

267

### 268 *Detecting IESP in soil samples “augmented” with IESP DNA*

269

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

276

277 The sequencing of the PCR products from the combined sterilised soil samples (i.e., one  
278 sample per orchard) using the Folmer primers resulted in an average of 1233 ASVs (range  
279 879 to 1538) per sample. For the combined unsterilised samples, an average of 1313 ASVs  
280 (range 292 to 2675) were produced. Matching these to the sequences for the IESP that had  
281 been added to the sterilised and unsterilised samples resulted in very few detections. Only  
282 33% of the IESP that had been added to the sterilised soil samples were detected, and none of  
283 the added IESP were detected in the unsterilised samples (Table 3). In the sterilised samples,  
284 13 IESP were not detected at all, and for the remaining 13 IESP, detection rates ranged from  
285 7%, for *Arcitalitrus* spp., to 100% for *Lonchoptera bifurcata* (Fallen) and *Tetramorium*  
286 *grassii* Emery. In addition, the Folmer primers detected *Trigonospila brevifacies* (Hardy) in a  
287 sterilised sample to which it had not been added. This may indicate that the sterilisation  
288 procedure was not completely effective at removing all DNA from the soil.

289

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

295

### 296 *Comparing traditional and metabarcoding methods for detecting IESP in orchards*

297

298 Analysis of the sequences produced from the soil samples from each orchard (following  
299 removal of sequences with fewer than 10 reads) identified a total of 34,679 ASVs. Of those,  
300 13,303 ASVs were found only in the kiwifruit orchards, and 19,443 ASVs were found only in  
301 apple orchards, leaving only 1,933 ASVs in common between the two orchard types.  
302 Individual orchards contained 128–5,150 ASVs. Very few of the ASVs could be matched to  
303 the sequences for the focal IESP. Only three IESP were detected: *T. brevifacies* was detected

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

310

311 The morphological analysis of the invertebrates collected in the pan, intercept, pitfall and  
312 sticky traps revealed the presence of all the focal IESP in at least one orchard, and a range of  
313 8 to 17 of the 20 IESP found in each orchard (Figure 2). This contrasts starkly with the  
314 metabarcoding results described above that found very few IESP in the soil collected from  
315 the same location within the orchards. The three IESP that were detected in the soil (i.e., *T.*  
316 *brevifacies*, *Nylanderia* sp(p)., and *A. vulgare*) were also collected in traps from the same  
317 orchards, except for orchards A3 and A6 where *T. brevifacies* was detected in the soil but not  
318 collected in traps.

319

320 The abundances of the IESP varied between orchards, with some species found occasionally  
321 (e.g., the predatory beetle *Thyreocephalus orthodoxus* (Olliff) was found in one apple orchard  
322 and five kiwifruit orchards, with a maximum of seven individuals collected from one  
323 kiwifruit orchard) and others found relatively frequently in all orchards (e.g., between 5 and  
324 209 *Sericoderus* sp. beetles were collected from each of the 20 orchards) (Figure 2). The  
325 Poisson models indicated that that abundances of most of the IESP in the apple orchards were  
326 not affected by orchard management, with equal numbers collected from the IFP and organic  
327 orchards (Table 4). However, four IESP (natural enemies *Aphelinus mali* (Haldeman),  
328 *Nylanderia* sp(p)., *Platygaster demades* Walker, and detritivore *Sericoderus* sp.) were in  
329 greater abundance in IFP orchards than in organic orchards, and four other IESP (natural  
330 enemy *A. albisignatus* and detritivores *A. vulgare*, *Cartodere* spp. and *Ephistemus globulus*  
331 (Paykull)) were captured in greater numbers from the organic orchards (Table 4). In the  
332 kiwifruit orchards, a difference in abundance between the organic and IPM orchards was  
333 detected for 12 of the IESP (Table 5), with nine IESP in greater abundance in the organic  
334 orchards (natural enemies *Anoteropsis hilaris* (L. Koch), *C. exsul*, *Phalangium opilio* L., *T.*  
335 *orthodoxus*, and detritivores *Arcitalitrus* spp., *Atomaria lewisi* Reitter, *Anotylus* sp., *L.*  
336 *bifurcata*, *Sericoderus* sp.), and three in greater abundance in the IPM orchards (natural

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

339

340

## 341 **Discussion**

342

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

353

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

370

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

387

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

403

404

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561

562 **Conflict of Interest**

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

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568

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577

578 **Table 1:** Invertebrate ecosystem service providers (IESP) selected for this project. Species were selected because they were involved in either  
579 decomposition (decomp.) or pest suppression (pest sup.) on apple or kiwifruit orchards or both. At least ten IESP were selected from those that  
580 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  
581 plants). Primers were designed against sequences generated in this study for either 28S ribosomal DNA (28S rDNA) or mitochondrial  
582 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 <sub>A</sub>	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 GACTGTTCGCGATGCTT	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 TGATTATTACCTCCATCATTAACT	EglCOIR TCGGTCAAAATTTATTCCTT	50	B
<i>Anoteropsis hilaris</i> (Ara.)	Both	Pest sup.	Ground	AhiCOIF TCTTCTAGAATAGGTACATAG	AhiCOIR CTAATACAGGTAACGACAACAAC	50	D
<i>Conoderus exsul</i> (Col.)	Both	Pest sup.	Ground	Cex28SF GACACGTTGCTAAACCTAAAG	Cex28SR CGAACGCCTCGCCCATCCT	50	B

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

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

583 Primers are given 5' to 3'; T<sub>A</sub>: temperature used for annealing in PCR; Cross Group: primers for species within a letter group were tested against  
584 all species of that group for cross reactivity; Amph. = Amphipoda; Ara. = Araneae; Col. = Coleoptera; Derm. = Dermaptera; Dipl. = Diplopoda;  
585 Dipt. = Diptera; Hem. = Hemiptera; Hym. = Hymenoptera; Iso. = Isopoda; Neu. = Neuroptera; Opi. = Opiliones.

586

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

589

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

590 \*Maximum weight available for one of the IESPs added to a subsample. <sup>#</sup>Six IESP were  
 591 accidentally added to a subsample from one orchard, but each was added to the subsample at  
 592 approximately 0.02 g.

593

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

IESP	Apple Orchards				Kiwifruit Orchards			
	Detected using specific primers (Tot.Pos.Subs.) <sup>1</sup>		Detected using Folmer primers (Tot.Pos.Comb.) <sup>2</sup>		Detected using specific primers (Tot.Pos.Subs.) <sup>1</sup>		Detected using Folmer primers (Tot.Pos.Comb.) <sup>2</sup>	
	Ster.	Unst.	Ster.	Unst.	Ster.	Unst.	Ster.	Unst.
<i>Aphelinus mali</i>	6 (6)	1 (1)	2 <sup>#</sup> (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 sp(p).</i>	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>Thyreoscephalus 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 <sup>^</sup> (4)	0 (1)	6 (7)	1 (1)	6 (6)	0 (1)
<i>Arcitalitrus spp.</i>	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 spp.</i>	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 sp.</i>	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)

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

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

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

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

604 ^ IESP identified in sample to which it was not added

605

606

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

IESP	Orchard System	Mean	Lower	Upper	Letter of	z.ratio	p.value
			95% CI	95% CI	difference (alpha=0.05)		
<i>Arcitalitrus spp.</i>	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 sp.</i>	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 spp.</i>	IFP	15	11.96	18.81	A	-2.158	0.0309
	Organic	20.8	17.16	25.21	B		
<i>Thyrecephalus 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		

611

612

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

IESP	Orchard System	Mean	Lower	Upper	Letter of difference	z.ratio	p.value
			95% CI	95% CI			
<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>Thyrecephalus 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 sp(p).</i>	IPM	4.6	3.06	6.92	-	0.012	0.9903
	Organic	0	-	-	-		
<i>Tetramorium grasii</i>	IPM	11	8.45	14.33	A	0.488	0.6257
	Organic	10	7.58	13.19	A		
<i>Porcellio 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 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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618

619

620 **Figure Legends**

621

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

624

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

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