

1 Title: Neonicotinoid insecticide residues in New Zealand maize paddock soil
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15 Abstract: Neonicotinoid are the most commonly used class of insecticides. Between 2005 and
16 2010 neonicotinoid use in the USA and UK more than doubled. Anecdotal evidence suggests
17 similar trends exist in New Zealand, where neonicotinoid seed coatings are now often applied
18 prophylactically in contravention of the principles of Integrated Pest Management. This
19 widespread use of neonicotinoid insecticides is controversial due to a lack of understanding
20 about their persistence in the environment and the long-term consequences of their use. We
21 present a novel, simple, low-cost method for the extraction and quantification of five
22 neonicotinoids from soil with a detection limit $<1 \text{ ng g}^{-1}$. We have applied this method to soil
23 collected from maize paddocks in New Zealand and found clothianidin and imidacloprid in 48
24 out of 50 samples. Neonicotinoid concentrations ranged from 0.5 to 9.4 $\text{ng g wet weight}^{-1}$
25 imidacloprid and 2.1 to 26.7 $\text{ng g wet weight}^{-1}$ clothianidin. These concentrations are likely
26 to be hazardous to non-target organisms exposed to them. This is the first study to report the
27 prevalence of neonicotinoid residues in New Zealand's environment.

28 Keywords: sustainable agriculture; integrated pest management; beneficial insects;
29 ecotoxicology; pesticide; ecotoxicology, pesticides, emerging pollutants, soil ecotoxicology,
30 persistent compounds

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33

34 **Introduction**

35 Neonicotinoids are the most commonly used type of insecticide (Douglas and Tooker 2015).
36 Where neonicotinoid use is documented—for example in the United States of America and
37 the United Kingdom—both the mass of active ingredient applied and the diversity of
38 applications continue to increase (DEFRA 2014, Douglas and Tooker 2015). Recent research
39 shows these compounds are more persistent in soil than previously understood (Goulson
40 2013, de Perre *et al.* 2015). Very low concentrations of neonicotinoid residues in plants, soil,
41 and groundwater are associated with reductions in the diversity and abundance of non-target
42 insects and insectivorous birds (Goulson 2013, Van Dijk *et al.* 2013, Hallmann *et al.* 2014).
43 Direct, mechanistic links between environmentally relevant concentrations of neonicotinoids
44 and population-level effects upon non-target organisms are now being established (Laycock
45 *et al.* 2012, Whitehorn *et al.* 2012, Pisa *et al.* 2015). In 2013 the European Commission placed
46 restrictions on the use of three neonicotinoids following assessments carried out by the
47 European Food Safety Authority. Due to the controversy around this ubiquitous class of
48 insecticides, it is important to continue to investigate the consequences of their large-scale
49 use.

50 The ultimate environmental fate of neonicotinoid residues has not been established and the
51 threat they pose to non-target species is not well understood (Goulson 2013). Neonicotinoids
52 are most commonly applied as a coating onto planted seeds, where they then disperse into
53 the soil. Their persistence in soil is highly variable with reported half-lives of up to seven years
54 (Goulson 2013, Jones *et al.* 2014). The small size of neonicotinoid molecules and their polarity
55 makes them systemic, facilitating their uptake into plants' roots and dispersal throughout their
56 tissue where they act against biting, chewing and boring insect pests. These properties limit
57 their bioaccumulation in food chains; however, these properties also allow them to dissolve in
58 groundwater and mobilise, resulting in their presence in soils, water, and organisms distinct
59 from their site of application (eg. Main *et al.* 2015). Sur & Stork (2003) reported that 80-98%
60 of imidacloprid seed treatment was not taken up by the target plant. This material will leach
61 through the soil in surface and groundwater flows instead, contaminating plants, soil,
62 waterways and wetlands distinct from their site of application (Bonmatin *et al.* 2015, de Perre
63 *et al.* 2015, Main *et al.* 2015). So the fate of neonicotinoids in the environment can be
64 categorised as either persisting *in situ*, broken down, or exported. Neonicotinoids breakdown
65 quickly when exposed to sunlight and they can be metabolised by plants and animals (Sur
66 and Stork 2003, Suchail *et al.* 2004). Export is a relative process depending on the scale in
67 question, but results from either biological processes (uptake by mobile organisms or
68 biological transport systems) or physical ones, via dissolution and by the mobilisation of
69 sediment or biological material to which residues are adsorbed. Residues dispersed in this

70 manner to field margins can be taken up by wild plants at concentrations similar to those
71 present in the crop (Botías *et al.* 2015). How far neonicotinoid residues can disperse and for
72 how long they can persist is not known.

73 Many different animals inhabit agricultural ecosystems and provide ecosystem services that
74 contribute to crop productivity such as pollination, pest predation, soil engineering, and nutrient
75 cycling. For example, the presence of moderate numbers of soil Collembola have been shown
76 to increase plant productivity (Harris and Boerner 1990). The mechanisms underlying these
77 effects are complex and may involve interactions between invertebrates, vertebrates, fungi
78 bacteria and plants. As a result of exposure to neonicotinoid residues insects can suffer
79 impaired reproductive performance, impaired foraging or defensive behaviour, loss of prey or
80 hosts, and death (Kunkel *et al.* 2001, Pisa *et al.* 2015). This will impact ecosystem services
81 provided by beneficial insects as well as those provided by any commensal, mutualistic or
82 symbiotic partners with implications for the productivity of the agricultural system.

83 Assessment of the risks associated with use of a pesticide is contingent upon understanding
84 the prevalence, persistence, and availability of that compound in the environment. Residues
85 of the neonicotinoid imidacloprid in arable soil at the end of a growing season have been
86 reported to be in the range of one to 100 ng g⁻¹ (Bonmatin *et al.* 2005, Krupke *et al.* 2012,
87 Goulson 2013, Jones *et al.* 2014, Botías *et al.* 2015, Schaafsma *et al.* 2015). The New Zealand
88 Environmental Protection Agency [NZEPA] has established an Environmental Exposure Limit
89 for imidacloprid in soil of 1 µg per kg dry weight. However, no monitoring programs appear to
90 have been implemented and we are unaware of any research published on the distribution,
91 persistence, and fate of neonicotinoid insecticides applied in New Zealand.

92 The current standard for the quantitative analysis of organic biocide residues involves solvent
93 extraction from environmental or biological samples followed by separation by liquid- or gas-
94 chromatography and detection by tandem mass spectrometry (eg. Payá *et al.* 2007). Many
95 such methods are time-consuming and costly to apply at scale. We have developed a novel,
96 simple, low-cost extraction technique for five neonicotinoid residues in arable soils.
97 Imidacloprid, clothianidin, thiacloprid, and thiamethoxam are the most common neonicotinoid
98 seed treatments used in the United Kingdom [UK] and United States of America [USA]
99 (DEFRA 2014, Douglas and Tooker 2015). Acetamiprid is the least used and is not currently
100 licensed for use in New Zealand [NZ]. We report here the results of a pilot study to test where
101 we have applied the method to measured concentrations of neonicotinoid insecticides in soil
102 samples collected from New Zealand maize paddocks prior to planting, when the lowest
103 concentrations of neonicotinoid residues can be expected.

104

105 **Methodology and materials**

106 *Reagents and analytical standards*

107 Optima LC-MS grade acetonitrile and analytical reagent grade ethyl acetate, boric acid,
108 sodium chloride and magnesium sulphate heptahydrate were obtained from Thermo Fisher
109 (Thermo Fisher Scientific, New Zealand). Mass-spectrometry grade formic acid and analytical
110 standards of thiamethoxam, clothianidin, imidacloprid, and imidacloprid-d4 were obtained from
111 Sigma Aldrich (Sigma Aldrich, New Zealand). Ultrapure water was obtained from a Purite
112 Select Fusion system (Total Lab Systems, New Zealand).

113

114 *Extraction of neonicotinoid residues from soil*

115 Approximately 1.5 g of wet soil was placed in a 15 mL polypropylene centrifuge tube and
116 spiked with 10 μL of 20 mg L^{-1} imidacloprid-d4 in 50% acetonitrile. Then, 5 mL of ultrapure
117 water was added and the sample was vortexed thoroughly to mix and disperse the soil before
118 2 mL of ethyl acetate was added and the mixture was vortexed again. Finally, 2 g of salt
119 mixture (eight parts $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, two parts NaCl and three parts H_3BO_3) was added and the
120 tube vortexed thoroughly to allow it to dissolve. Extracts were incubated at room temperature
121 for 15 minutes with regular vortexing before being centrifuged at 4,000 RCF for 5 minutes at
122 room temperature. A 1.4 mL volume of the upper, organic layer was removed and placed in a
123 2 mL microcentrifuge tube with 0.4 mL of 1% formic acid in ultrapure water. This mixture was
124 vortexed briefly before the ethyl acetate layer was evaporated *in situ* using a centrifugal
125 concentrator (Centrivap Console, Labconco, USA). The remaining aqueous solution was
126 centrifuged at 10,000 RCF for 5 minutes at 4 °C (Z216MK microcentrifuge, Hermle
127 Labortechnik, Germany) before a 100 μL volume was transferred to a low volume glass insert
128 inside an amber 1.8 mL autosampler vial and capped for injection to LC-MS/MS.

129

130 *Liquid chromatography with tandem mass spectrometry – LC-MS/MS*

131 Neonicotinoids were quantified using an Agilent 1260 Series liquid chromatograph comprising
132 a G1311C quaternary pump, G1329B thermostatted autosampler and a G1330B
133 thermostatted column compartment (Agilent Technologies, Santa Clara, USA). Mobile phase
134 A was 0.1% formic acid in ultrapure water, mobile phase B was 0.1% formic acid in acetonitrile,
135 the injection volume was 5 μL and the column, a ZORBAX Rapid Resolution HT SB-C18
136 measuring 2.1x30 mm, with 1.8 μm diameter packing material, was maintained at 30 °C. The
137 chromatographic gradient started at 5% B, ramped to 33% B at 3 minutes, 80% B at 4 minutes,
138 held at 80% B for 0.2 minutes and then returned to 5% B at 5 minutes. The total run time was
139 10.5 minutes.

140 Neonicotinoids were quantified with an Agilent 6420 triple quadrupole mass spectrometer
141 fitted with an Agilent Multimode Ionisation source operating in positive electrospray mode and

142 using Multiple Reaction Monitoring [MRM]. MRM transitions were established using Agilent
143 MassHunter Optimiser software and are presented in Supplementary Data Table 1.

144

Supplementary Data Table 1: Multiple Reaction Monitoring (MRM) transitions for LC-MS/MS of neonicotinoid pesticides. The dwell time for each MRM was 100ms and the cell accelerator voltage was 7.

neonicotinoid	MRM transition	fragmentor voltage	collison energy
thiamethoxam	292.0 → 211.1	100	14
clothianidin	250.0 → 169.1	100	14
imidacloprid	256.1 → 209.1	123	18
imidacloprid-d4	260.1 → 213.1	91	10
thiacloprid	253.0 → 126.0	122	22
acetamiprid	223.1 → 126.0	91	10

145

146

147 *Instrument Detection Limits and extraction validation*

148 Five 1.5 g samples of arable soil that showed no trace of neonicotinoid contamination were
149 spiked with a 10 μ L volume of a 5 mg L⁻¹ solution of the six targets in acetonitrile and shaken
150 for 60 seconds. These were then extracted and analysed as described above. Instrument
151 Detection Limits [IDL] were calculated by the method given in Wells et al. (Wells *et al.* 2011)
152 in accordance with US Guidelines Establishing Test Procedures for the Analysis of Pollutants
153 (United States Government Code of Federal Regulations, title 40, sec 1.136, appendix B).

154

155 *Field Sampling, Locations and Processing*

156 A total of 45 soil samples were collected from nine maize paddocks around the Waikato, East
157 Cape, and Bay of Plenty regions of New Zealand's North Island. The location of each paddock
158 and the seed treatment, where it could be established, is shown in Table 1. Paddocks had
159 been planted with maize in late Spring 2014 (Sept to Nov), harvested in Autumn 2015 (April
160 to June) and left fallow for the winter. Paddocks were sampled on the 28th and 29th of
161 September, 2015. Five replicate soil samples were taken from each paddock using a clean,
162 stainless steel trowel to a depth of 100 mm, placed into a zip-lock bag and shaken to
163 homogenise the contents. Samples were collected every 10 metres along a transect from the
164 corner of the paddock towards the centre, starting from 10 metres in to the paddock. Samples
165 were immediately refrigerated at 4°C until analysis.

166

167 *Soil water and organic matter content*

168 Approximately one gram of homogenised, wet soil was weighed into a foil boat and
 169 lyophilised for 24 hours to obtain the dry weight. The foil boat was then placed in a muffle
 170 furnace and heated to 590°C for two hours to obtain the ash weight of the soil. The organic
 171 content of the soil was calculated by subtraction of the ash weight from the dry weight.

172

Table 1: Location of nine maize paddocks in New Zealand's North Island sampled for soil neonicotinoid residue analysis. Known neonicotinoid seed treatments are stated, where known.

site	town	coordinates	seed treatment
A	Matamata	-37.799719, 175.773603	Bayer Poncho (clothianidin)
B	Awakeri	-37.995229, 176.901423	Bayer Poncho (clothianidin)
C	Poroporo	-37.997580, 176.955328	Bayer Poncho (clothianidin)
D	Te Teko	-38.074122, 176.818585	unknown
E	Poroporo	-38.001260, 176.926664	unknown
F	Whakatane	-37.951891, 176.950098	unknown
G	Te Puke	-37.760998, 176.296918	Bayer Poncho (clothianidin)
H	Te Puke	-37.760832, 176.295859	Bayer Poncho (clothianidin)
I	Te Karaka	-38.473873, 177.882189	Bayer Poncho (clothianidin)

173

174

175 **Results**176 *Method validation*

177 Instrument Detection Limits ranged from 0.201 ng g⁻¹ for imidacloprid to 0.516 ng g⁻¹ for
 178 thiamethoxam. Recoveries (mean ±SD) for the six targets spiked into uncontaminated soil
 179 were consistent and ranged from 85.3% ±2.4 for thiamethoxam to 110.2% ±5.4 for
 180 acetamiprid (Table 2).

181

Table 2: Recovery and Instrument Detection Limits [IDL] for six neonicotinoid insecticide residues in soil using the method reported here.

neonicotinoid	mean % recovery (standard deviation)	IDL (ng g wet weight ⁻¹)
acetamiprid	110.2 (5.4)	0.096
clothianidin	103.0 (13.5)	0.413
imidacloprid	109.9 (19.4)	0.250
imidacloprid-D4	106.0 (1.7)	0.246
thiacloprid	93.9 (7.6)	0.153
thiamethoxam	85.3 (2.4)	0.208

182

183 *Neonicotinoid residues in maize paddock soil samples*

184 Of the five neonicotinoids targeted for quantification with this method, we detected only two—
 185 clothianidin and imidacloprid—in the maize paddock soil samples. However, we detected
 186 these two neonicotinoids in almost every sample analysed at concentrations up to 109.3 ng g⁻¹
 187 ¹ clothianidin at site I and 13.7 ng g⁻¹ imidacloprid at site F. Imidacloprid concentration was
 188 below the IDL in just two samples: one at site G and one at site I. Imidacloprid concentrations
 189 (mean ±SD) varied from 0.5 ±0.5 ng g⁻¹ at site I to 9.4 ±3.1 ng g⁻¹ imidacloprid at site E.
 190 Clothianidin concentrations ranged from 2.1 ±2.4 ng g⁻¹ at site E to 26.7 ±46.5 ng g⁻¹ at site I.
 191 The highest concentration for total neonicotinoids was also at site I with 27.3 ±46.26 ng g⁻¹.
 192 The mean concentration across all sites was 8.16 ±16.78 ng g⁻¹ clothianidin, 5.06 ±3.73 ng g⁻¹
 193 ¹ imidacloprid and 13.22 ±8.12 ng g⁻¹ for total neonicotinoids. These results are displayed in
 194 Figure 1.

195

196 *Soil water content and organic matter content*

197 Soil water content (mean ±SD) was 32.2 ±8.0 % and organic content was 12.3 ±4.7 %. Linear
 198 models revealed no significant relationships between neonicotinoid concentrations and soil
 199 water or organic content (statistics not shown). There was a significant linear relationship
 200 between soil water and organic matter content, shown in Figure 2.

201

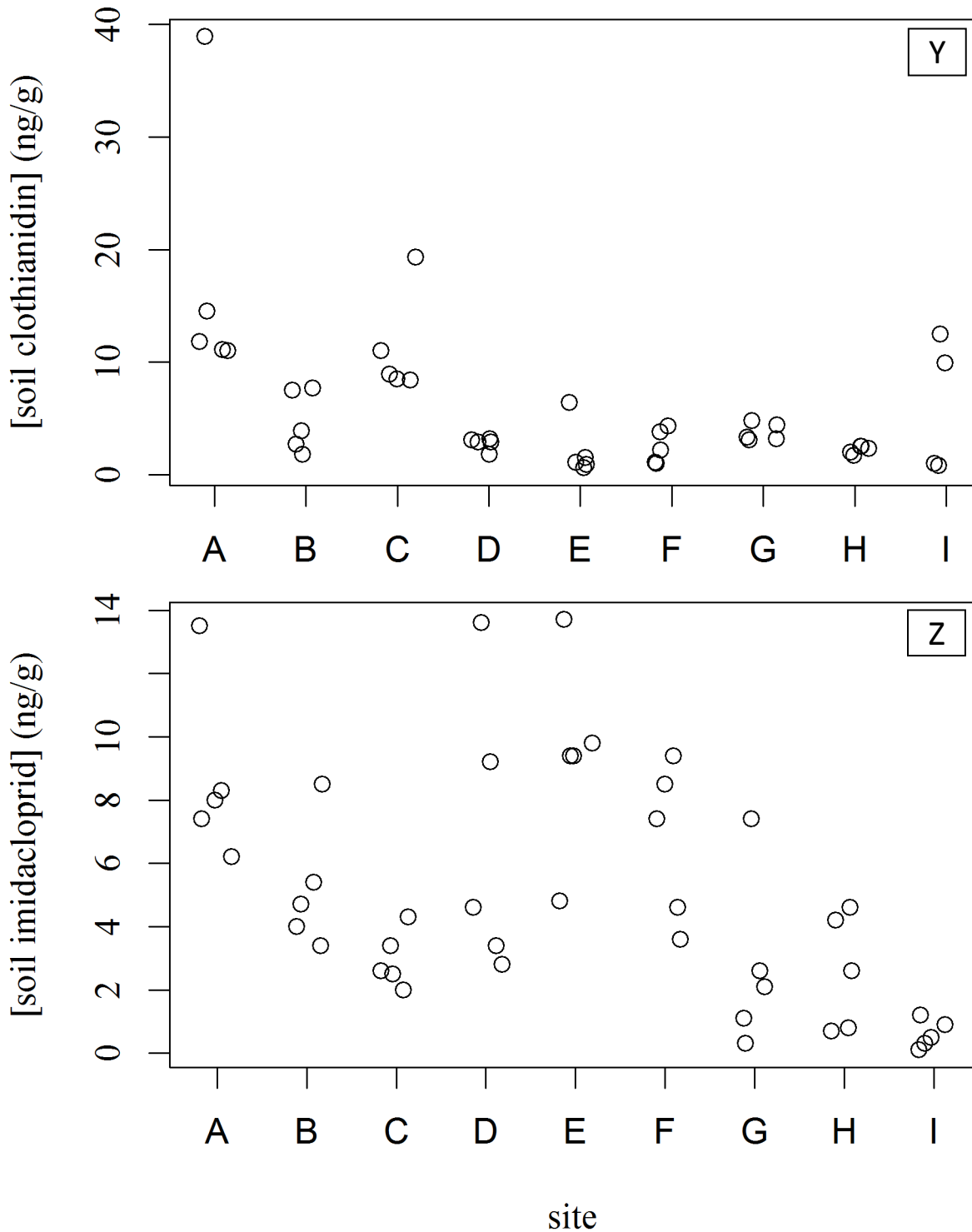
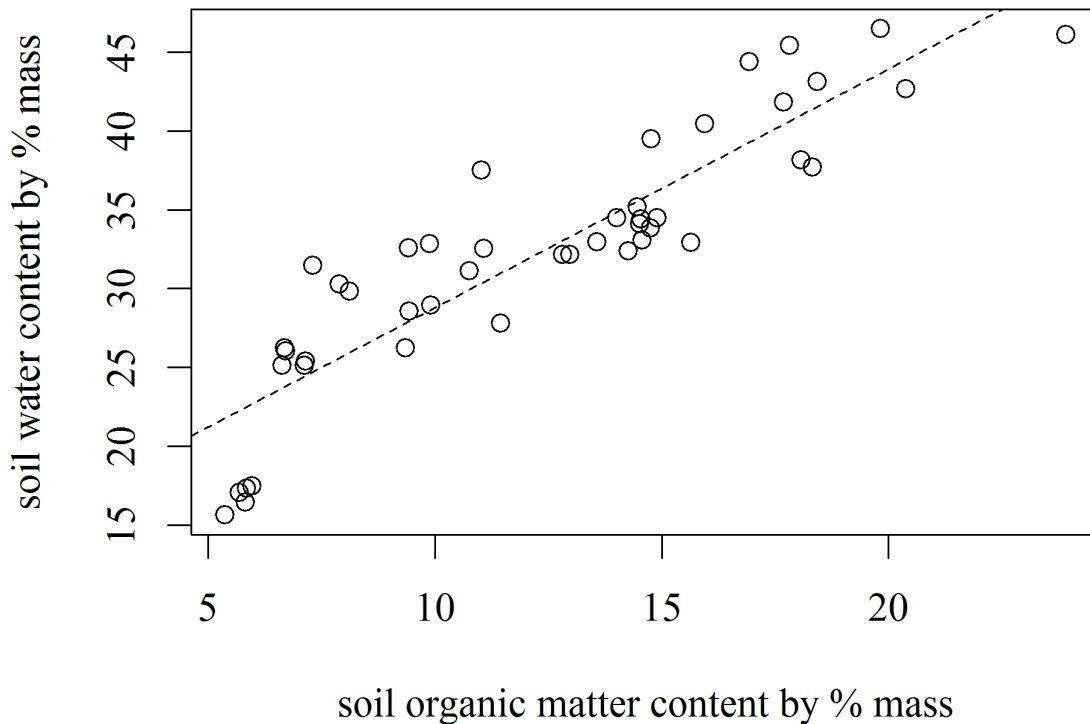


Figure 1: Concentrations of clothianidin (Y) and imidacloprid (Z), in ng g^{-1} wet weight of soil from nine maize paddocks in New Zealand's North Island. To better visualise the distribution of the data one outlying data point at 109.3 ng g^{-1} for site I has been excluded from plot Y.



209

210 Figure 2: Plot of soil water content and organic matter content for all of the samples
211 analysed. The dotted line was fitted using a linear model (water content = 1.5142 × organic
212 content + 13.6481, $r^2 = 0.8047$, $F_{1,43} = 182.4$, $p < 0.001$).

213

214

215 **Discussion**

216 The widespread use of neonicotinoid insecticides is controversial due to a lack of
217 understanding about their persistence in the environment and the long-term consequences of
218 their use. It is therefore important to monitor their prevalence and effects. We present a novel,
219 simple, low-cost method for the extraction of five neonicotinoids from soil with a detection limit
220 $<1 \text{ ng g}^{-1}$. We have applied this method to soil collected from maize paddocks in New Zealand
221 and found clothianidin and imidacloprid in 48 out of 50 samples. Neonicotinoid concentrations
222 ranged from 0.5 to 9.4 $\text{ng g wet weight}^{-1}$ imidacloprid and 2.1 to 26.7 $\text{ng g wet weight}^{-1}$
223 clothianidin. This is the first study to report the prevalence of neonicotinoid residues in New
224 Zealand's environment.

225 The concentration of neonicotinoids found here compare well with reported neonicotinoid
226 residues in arable soil (Bonmatin *et al.* 2003, Krupke *et al.* 2012, Jones *et al.* 2014, Botías *et*
227 *al.* 2015, Schaafsma *et al.* 2015). The New Zealand Environmental Protection Agency
228 [NZEPA] has set an Environmental Exposure Limit [EEL] for imidacloprid in soil of 1 ng g dry

229 weight⁻¹. We have found imidacloprid concentrations that exceed that value by as much as 14
230 times at eight out of nine sites sampled. The NZEPA has set no EEL for clothianidin in soil,
231 however, clothianidin concentrations exceeded the EEL for imidacloprid at all nine sites and
232 clothianidin appears to be equally as toxic to insects as imidacloprid (Pisa *et al.* 2015,
233 Cavallaro *et al.* 2017). Therefore it appears that potentially hazardous concentrations of
234 neonicotinoid residues persist at all of the sites sampled.

235 As our samples were collected immediately prior to planting of new seed, they represent the
236 lowest concentrations of neonicotinoid residues to be found throughout the year. It is not
237 surprising that clothianidin concentrations exceeded imidacloprid as all of the paddocks we
238 could establish seed treatment histories for had received the former. Clothianidin is the most
239 commonly applied neonicotinoid seed treatment in the USA and UK (DEFRA 2014, Douglas
240 and Tooker 2015). Residues are likely to accumulate from successive years of planting, which
241 could be why we have found multiple neonicotinoids in almost all of our soil samples. This
242 suggests that most of the imidacloprid residues we have measured are sourced from seed
243 treatment applications nearly two years earlier. Other possible explanations for multiple
244 residues are that some of the residues detected may have leached from seed coating
245 applications in adjacent paddocks or that they originate from other types of application, such
246 as foliar sprays. Although we did not detect thiamethoxam, this neonicotinoid decomposes or
247 is metabolised to form clothianidin. Acetamiprid was also not detected, but is not currently
248 licensed for use in any New Zealand products. Clothianidin is reported to have a higher
249 capacity for leaching through soils and so this may indicate that residues we have measured
250 here have leached from elsewhere, although given the known application histories this seems
251 unlikely (Bonmatin *et al.* 2015). The retention and persistence of neonicotinoid residues is
252 influenced by soil characteristics, with higher organic matter contents being associated with
253 greater retention (Bonmatin *et al.* 2015). However, we found no relationship between the
254 organic matter content of soils and the concentrations of imidacloprid or clothianidin residues.
255 This could be a result of insufficient replication at each site or a consequence of the differential
256 application of neonicotinoids across sites. Although we were able to obtain neonicotinoid
257 application histories for several sites, we could not obtain them for sites D, E and F and
258 therefore their treatment history remains unknown. However, the concentrations of residues
259 found here suggest that it is likely that neonicotinoids were applied.

260 On the assumption of a normal planting rate for New Zealand of 90,000 seeds ha⁻¹ (Stone *et*
261 *al.* 2000), maize coated with Bayer's Gaucho seed treatment according to the manufacturer's
262 guidelines carries 452 µg imidacloprid per seed. That represents an application rate of 41
263 grams of active ingredient per hectare. This accords with the findings of Jones *et al.* (2014),
264 who reported application rates on wheat, sugarbeet and canola of 10-100 g Ha⁻¹. If the

265 insecticide is evenly dispersed in the top 20 cm of soil it will result in a mean concentration of
266 20.5 $\mu\text{g L}^{-1}$. Concentrations we have measured are approximately 50% of that estimate,
267 indicating that neonicotinoids are highly persistent in New Zealand maize paddock soil.

268 Because the seed coated with neonicotinoids represents a point source, their distribution in
269 undisturbed soil might be patchy. While we took care to homogenise soil samples, it is possible
270 that our subsampling incorporated plant matter derived from the original seed or soil particles
271 that were proximate to the seed. This could explain the high concentrations of imidacloprid
272 and clothianidin found in some samples, one of which exceeded our estimate for the initial
273 mean concentration. Further analysis is needed to assess whether these indicate variation in
274 the application rate or the soil conditions influencing neonicotinoid persistence in those
275 samples.

276 Some studies have detailed the hazards posed by residues from neonicotinoid seed
277 treatments to non-target species (Krupke *et al.* 2012, Goulson 2013, Bonmatin *et al.* 2015,
278 Botías *et al.* 2015, Pisa *et al.* 2015). Botías *et al.* (2015) demonstrated that neonicotinoid
279 residues from seed coatings applied to canola can be measured in the soil beyond the margins
280 of the field, at concentrations similar to those reported here. Beyond the margins, they are
281 taken up by wild plants and transferred to the pollen and nectar at concentrations higher than
282 those found in the flowers of the crop itself (Botías *et al.* 2016). This represents a significant
283 threat to honeybees foraging in the area as wildflower pollen constituted the majority of the
284 pollen they returned to the hive (Botías *et al.* 2015). The concentrations of soil neonicotinoid
285 residues measured here are similar to those measured by Botías *et al.* (2015). If the same
286 mechanisms are at work in the margins of the paddocks sampled here then imidacloprid and
287 clothianidin residues available to bees and other pollinators may be high enough throughout
288 the year to compromise a number of sublethal endpoints including navigation, communication,
289 and reproduction (Henry *et al.* 2012, Laycock *et al.* 2012, Whitehorn *et al.* 2012, Botías *et al.*
290 2016).

291 It is not clear how long-term neonicotinoid use is affecting the productivity of arable soil
292 ecosystems in New Zealand or elsewhere. Populations of New Zealand maize pest species,
293 such as the Australian soldier fly, *Inopus rubriceps*, and cosmopolitan armyworm, *Mythimna*
294 *separata*, have been alleged to spike as a result of the removal of natural predators and
295 parasites through the application of insecticides (Chapman 1984). Soil engineers, such as
296 earthworms and microarthropods, such as Collembola, are major service providers in arable
297 ecosystems, enhancing soil productivity by mobilising nutrients through their diet of organic
298 detritus and increasing microbial activity and soil porosity. Earthworms are unlikely to
299 experience acute toxicity from neonicotinoid residues either at the concentrations that we have

300 estimated are present immediately after seed sowing or that have been reported in the
301 literature (Pisa *et al.* 2015). However, little data exists regarding the hazard to earthworms of
302 chronic exposure to these toxicants and little information on chronic toxicity of neonicotinoids
303 to microarthropods (Dilling *et al.* 2009, Pisa *et al.* 2015). Several species of insect associated
304 with New Zealand maize crops are known to parasitise or predate upon major maize pests.
305 The parasitic wasp, *Apanteles rubricus*, and metallic green rove beetles, *Thyreocephalus spp.*,
306 parasitise or predate upon many of the major New Zealand pest species (Early 1984). These,
307 and other beneficial species, will be exposed to neonicotinoids either through their hosts and
308 prey or through contact with contaminated soil and plant material (eg. Kunkel *et al.* 2001). For
309 example, it has been demonstrated that thiamethoxam can be harmlessly accumulated in the
310 tissue of slugs at concentrations that are lethal to arthropod predators (Douglas *et al.* 2015).

311 It is established that productivity gains from prophylactic application of pesticides will
312 eventually be outweighed by losses associated with the effects upon ecosystem service
313 provision and the development of resistance (Heckel 2012). The concentrations of
314 neonicotinoid residues we have measured are symptomatic of this. Animals that habitually
315 ingest or burrow through soil, sediment, or tissue cannot avoid exposure to pervasive
316 toxicants, such as neonicotinoids (Pook *et al.* 2009). Chronic exposure to sublethal
317 concentrations of a toxicant are an evolutionary pressure that selects for resistive mechanisms
318 (Orr 1998). Resistance to imidacloprid has already been documented in the USA in Colorado
319 potato beetle, *Leptinotarsa decemlineata*, across Southeast Asia in the brown planthopper
320 *Nilaparvata lugens*, and in Australian green peach aphids, *Myzus persicae* (Alyokhin *et al.*
321 2007, de Little *et al.* 2016, Garrood *et al.* 2016). The latter species is found throughout New
322 Zealand and is an economically important pest on many crops. However, there is no empirical
323 data on the resistance of this or any other New Zealand pest, predator or parasite to
324 neonicotinoids.

325 Finally, the novel extraction method deployed here is effective and enables sensitive analysis
326 of environmentally relevant concentrations of neonicotinoid residues in arable soil. The
327 process is simpler than many other soil extraction methods (eg. Botías *et al.* 2015) with only
328 one extraction step, requires no clean-up using the costly dSPE materials that some
329 commonly used methods require, and uses a single concentration step. The final sample
330 matrix is aqueous and can be injected directly to reverse-phase liquid chromatography. We
331 have injected volumes of 25 μ L without observing matrix effects (data not shown) with
332 implications for improving the sensitivity further.

333

334

335 **Conclusions**

336 This is the first study to report quantities of neonicotinoid residues in New Zealand's
337 environment. We have found that these residues persist in maize paddock soil throughout the
338 year at concentrations that are likely to be hazardous to non-target invertebrates. They either
339 persist from year to year and/or are mobile enough to disperse from paddock to paddock to
340 create multi-residue hazards. Significant knowledge gaps exist in our understanding of the
341 effects of long-term prophylactic application of these compounds. Soil residues of
342 neonicotinoid insecticides should be considered emerging contaminants and the following
343 knowledge gaps should be addressed as a matter of priority:

- 344 ▪ Are soil neonicotinoid residues a direct threat to non-target species, such as pollinators
345 and other beneficial insects?
- 346 ▪ What are the indirect impact of neonicotinoid residues upon the productivity and
347 ecosystem service provision of the soil community?
- 348 ▪ Are current neonicotinoid use patterns likely to accelerate the evolution of resistance
349 to neonicotinoids in pest species?

350

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