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Potts, Jessica; Jones, Davey L; Macdonald, Andrew; Ma, Qingxu; Cross, Paul

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Acetamiprid fate in a sandy loam with contrasting soil organic matter contents: A
 comparison of the degradation, sorption and leaching of commercial neonicotinoid
 formulations

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- 5 Jessica Potts^{a,*}, Davey L. Jones^{a,b}, Andrew Macdonald^c, Qingxu Ma^{a,d}, Paul Cross^a
- 6 ^aSchool of Natural Sciences, Bangor University, Bangor, Gwynedd, LL57 2UW, UK
- ⁷ ^bSoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch
- 8 University, Murdoch WA 6105, Australia
- 9 ^cSustainable Agriculture Sciences Department, Rothamsted Research, Harpenden, Herts, AL5
- 10 *2JQ, UK*
- ¹¹ ^d*Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Ministry of*
- 12 Education Key Lab of Environmental Remediation and Ecosystem Health, College of
- 13 Environmental and Resource Sciences, Zhejiang University, Hangzhou, China
- 14
- 15 ^{*}Corresponding author
- 16 *E-mail address*: j.potts@bangor.ac.uk (J. Potts)
- 17
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19 ABSTRACT

The impacts of neonicotinoids have generally focussed on the responses of the pure active 20 ingredient. Using a selection of two commercial formulations and the active ingredient, we ran 21 three laboratory studies using ¹⁴C-labelled acetamiprid to study the leaching, sorption and 22 mineralisation behaviours of the commercially available neonicotinoid formulations compared 23 to the pure active ingredient. We added ¹⁴C-spiked acetamiprid to a sandy loam soil that had 24 25 received long-term additions of farmyard manure at two rates (10 t/ha/yr and 25 t/ha/yr) and mineral fertilisers, as a control. We found significant differences in acetamiprid mineralisation 26 27 across both the SOM and chemical treatments. Sorption was primarily impacted by changes in SOM and any differences in leachate recovery were much less significant across both treatment 28 types. The mineralisation of all pesticide formulations was comparatively slow, with <23% of 29 30 any given chemical/soil organic matter combination being mineralised over the experimental period. The highest mineralisation rates occurred in samples with the highest soil organic 31 matter levels. The results also showed that 82.9 % \pm 1.6 % of the acetamiprid applied was 32 leached from the soil during repeated simulated rainfall events. This combined with the low 33 sorption values, and the low rates of mineralisation, implies that acetamiprid is highly persistent 34 and mobile within sandy soils. As a highly persistent neurotoxin with high invertebrate 35 selectivity, the presence of neonicotinoids in soil presents a high toxicology risk to various 36 37 beneficial soil organisms, including earthworms, as well as being at high risk of transfer to 38 surrounding watercourses.

39

40 *Keywords:* Acetamiprid; Environmental fate; Persistence in soil; Soil management;

41 Environmental risk

42

43 1. Introduction

The last fifty years has seen dramatic declines in insect species richness and population 44 numbers worldwide (Hallmann et al., 2017). Drivers of decline include changes in land-use 45 and management, agricultural intensification and the use of certain agrochemicals and 46 pesticides (Goulson et al., 2015; Lima et al., 2016). Pesticide use has led to many organisms 47 experiencing long-term exposure to a diverse cocktail of synthetic chemicals, with 48 neonicotinoids being strongly linked to insect pollinator decline (Blacquière et al., 2012; 49 50 Rundlöf et al., 2015). Neonicotinoids are a family of systemic agrochemicals, used primarily for the protection of crops from biting and sucking (saprivorous) pests (Tomizawa and Casida, 51 52 2005). Since the global commercialisation of imidacloprid in the early 1990s, neonicotinoids have become one of the most widely used agrochemicals worldwide (Jeschke et al., 2011; 53 Woodcock et al., 2016). As of 2016, neonicotinoids accounted for 24% of all insecticide sales 54 worldwide, with an average market value of \$1.5 billion per year (Woodcock et al., 2016). The 55 major drivers for their increased use were their ease of application and effectiveness at 56 controlling invertebrate pests. 57

Neonicotinoids are acetylcholine antagonists, disrupting the nervous system of 58 invertebrates on contact or through ingestion of treated plant matter (Tomizawa and Casida, 59 2005; Downing and Grimwood, 2017). The mechanism of action of neonicotinoids is primarily 60 attributed to their strong binding to nAChRs of the insect brain, however, they are 61 indiscriminate between target pests and other non-target invertebrates (Tomizawa and Casida, 62 2005; Pisa et al., 2015; Botías et al., 2016). There is growing evidence that many other species 63 are also impacted by neonicotinoids such as various soil invertebrates (Capowiez et al., 2006; 64 Basley and Goulson, 2017; De Lima e Silva et al., 2017; Li et al., 2018). When applied as a 65 66 seed dressing, up to 90% of the neonicotinoid seed coating remains in the soil (Goulson, 2013), and the possibility therefore exists for soil accumulation many times higher than the original 67 concentration applied to the seed (Capowiez and Bérard, 2006; Goulson, 2013; De Lima e Silva 68

et al., 2017). Neonicotinoids are also applied as irrigation additives and foliar sprays by which 69 they can also enter the soil. When originally applied as seed-dressings, the localised area 70 71 surrounding a neonicotinoid-treated seed or plant will often present a much higher level of 72 acute exposure to invertebrates (Girolami et al., 2009), with the compound leaching further through the soil profile over time (Liu et al., 2016; Rodríguez-Liébana et al., 2018). The 73 persistence of these chemicals in soils can pose further ecotoxicological challenges to soil 74 75 organisms (Zaller et al., 2016; Renaud et al., 2018). A variety of impacts on soil fauna as a result of neonicotinoid contact or ingestion have been reported, including behavioural, 76 77 reproductive and changes to community structures (Capowiez et al., 2006; Goulson, 2013; Pisa et al., 2015; Basley and Goulson, 2017). Alterations to the behaviours and reproductive rates 78 and successes in vital ecosystem-engineer species such as earthworms, could imply wider 79 80 changes to ecosystem functions because of neonicotinoid application.

Neonicotinoids are highly water soluble, facilitating their systemic uptake and transport 81 to all crop tissues (Huseth and Groves, 2014). This solubility can lead to leaching and an 82 increase in chemical mobility within the soil profile relative to other insecticides (Kurwadkar 83 et al., 2013; Leiva et al., 2017). Waterlogged and heavy clay-rich soils have been shown to 84 exhibit much higher residue levels due to their restricted mobility (Rexrode et al., 2003; Liu et 85 al., 2015, 2016). The loss of neonicotinoids from a soil is considered biphasic, commencing 86 with a period of rapid loss, followed by a notably slower secondary phase, possibly illustrating 87 88 the sorption of the active substances to available soil particles (Papiernik et al., 2006; Goulson, 2013). The residence time and risk of exposure for neonicotinoids in soils varies greatly, 89 influenced by both changes in environmental conditions and soil characteristics (Karmakar et 90 91 al., 2006; Liu et al., 2016; Castillo Diaz et al., 2017).

In addition to chemical and biological degradation, neonicotinoid availability can be
restricted through sorption to soil particles (Papiernik et al., 2006; Carbo et al., 2007; Banerjee

et al., 2008). The rate and amount of sorption is thought to be dependent on the soil's clay and
organic matter content (Flores-Céspedes et al., 2002; Banerjee et al., 2008; Jin et al., 2016).
Neonicotinoids generally have a high solubility and a relatively low octanol-water partition
coefficient, indicating a hydrophilic nature and a low potential for soil sorption (Murano et al.,
2018). As with other soil processes, leaching appears to be influenced by both the chemical
composition of the neonicotinoid and environmental variables, such as soil properties and
climatic conditions (Jeschke et al., 2011; Liu et al., 2017).

Typically, neonicotinoid studies have focused on the pure active ingredients acting in 101 102 isolation, and as such ecotoxicity tests for the purpose of policy and regulation tend to follow the same methods (Bori et al., 2015). However, such agrochemicals are generally applied in 103 either a carrier matrix or alongside a mixture of surfactants and emulsifiers combined within 104 105 the commercial formulation (Malev et al., 2012; Anderson and Roberts, 1983; Neves et al., 2001). Consequently, studies that focus solely on the pure active ingredient may fail to capture 106 the influence that surfactants and carrier matrices have on the fate and behaviour of 107 neonicotinoids in soil (Bonmatin et al., 2015; Bori et al., 2015). Additionally, neonicotinoids 108 are sometimes applied alongside other agrochemicals (Van der Sluijs et al., 2013; Goulson et 109 al., 2015; Botías et al., 2017). Evaluating the synergistic and additive effects of these 110 agrochemical mixtures is challenging due to the secondary effects on soil biota, as the 111 behaviours and persistence of the active ingredients may be altered when applied in conjunction 112 with other agrochemicals. 113

We hypothesised that (i) increasing soil organic matter (SOM) content would promote acetamiprid sorption and biodegradation, whilst reducing leaching, and (ii) that this effect would be reduced in commercial formulations due to the presence of additional ingredients such as surfactants, adjuvants, and secondary active ingredients.

119 **2. Materials and methods**

120 *2.1. Soil*

The soil used in the experiments was obtained from selected treatments of the long-121 term Woburn Organic Manuring experiment (started in 1964) at Woburn Experimental Farm 122 (Rothamsted Research), Bedfordshire, UK, in June 2018 (Mattingly et al., 1973; Ma et al., 123 2020; Ma et al., 2021). The soil is classified as an Udipsamment (US Soil Taxonomy) or brown 124 125 sand and has a sandy-loam texture (80% sand, 6% silt, and 14% clay) making it susceptible to pesticide leaching (Bromilow et al., 1999). The field was under winter rye (Secale cereale L.) 126 127 at the time of sample collection. Soil samples (0-23 cm; Ahp horizon) were taken using a gouge auger from each replicate plot (n = 4) receiving either a high (25 t ha⁻¹ y⁻¹) or low (10 t ha⁻¹ y⁻¹) 128 ¹) rate of farmyard manure (FYM) or control plots receiving mineral fertilisers only (Ma et al., 129 2020; Macdonald et al., 2018) since 2003. Hereafter, these treatments are referred to as 130 SOM_{high}, SOM_{med} and SOM_{low}. The plots (with the exception of the SOM_{high} treatment) receive 131 annual applications of triple superphosphate (Ca(H₂PO₄)₂ \cdot H₂O, 43-44% P₂O₅ 97.5 kg ha⁻¹ y⁻¹) 132 and sulphate of potash (200 kg ha⁻¹ v⁻¹, 50% K₂O, 45% SO₃). The treatment regimes for the 133 field plots are further described in Table 1. The soil samples collected from these plots were 134 passed through a 5 mm sieve and stored at 4 °C. Properties of the soil from the different 135 treatments are presented in Table 2. Soil moisture was calculated by drying soil at 105 °C for 136 24 h, average soil moisture was determined at 12.9 ± 0.3 %, hereto referred to as field moisture. 137

138

139 2.2. Neonicotinoid products

Two commercially available neonicotinoid products containing acetamiprid (N-[(6chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide) were tested. The first was a
foliar spray product containing acetamiprid (0.05 g l⁻¹) within a mixture of ethanol and 1,2benzisothiazolin-3-one (pH 6.62; Bug Clear Ultra[®], Scotts Miracle-Gro Corp., Marysville, OH;

hereon referred to as AcetCF1; Material Safety Data Sheet, 2008). The second was also a foliar 144 spray containing 0.05 g l^{-1} acetamiprid, but in combination with 0.15 g l^{-1} triticonazole 145 fungicide and a range of emulsifiers and anti-bacterial preservatives (pH 5.22; Rose Clear 146 Ultra[®], Scotts Miracle-Gro Corp.; from here on in referred to as *Acet*_{CF2}; (Material Safety Data 147 Sheet, 2010). A third solution of pure acetamiprid (0.05 g l⁻¹; Sigma-Aldrich) was used to 148 account for any differences caused by any additional ingredients such as surfactants, adjuvants, 149 150 emulsifiers, and fungicides present in the commercial mixtures (hereon referred to as AcetPure; Table 3). 151

152

153 *2.3. Acetamiprid mineralisation in soil*

154 To determine acetamiprid degradation, 5 g of field moist soil from each SOM treatment were placed in individual sterile 50 cm³ polypropylene centrifuge tubes and spiked with one of 155 the three ¹⁴C-labelled acetamiprid formulations (Acet_{CF1}, Acet_{CF2} or Acet_{Pure}). ¹⁴C-labelled 156 acetamiprid [pyridyl-2,6-¹⁴C; 1850 MBq mmol⁻¹] was purchased from the Institute of Isotopes 157 Co. Ltd., Hungary. This was spiked into the two commercial formulations and into a pure 158 solution of acetamiprid. An aliquot of each spiked pesticide (0.5 ml, 0.05 g acetamiprid l^{-1} ; 159 0.83 kBq sample⁻¹) was then applied to the soil surface. A 1 M NaOH trap (1 ml) was then 160 suspended above the soil surface to capture any released ${}^{14}CO_2$ and the tubes were sealed. The 161 NaOH traps were replaced periodically over 60 d (minimum of twice a week). Samples were 162 incubated at 20 °C \pm 1 °C. The amount of ¹⁴CO₂ within the traps was determined using 163 Optiphase HiSafe 3 liquid scintillation fluid (PerkinElmer Inc., Waltham, MA) and analysed 164 on a Wallac 1404 liquid scintillation counter (PerkinElmer Inc.) with automated quench 165 correction. After 60 d, the soil was extracted with ethanol (20 ml; 30 min, 200 rev min⁻¹) to 166 determine the amount of available pesticide residue remaining in the soil. The extracts were 167

then centrifuged (18,000 g, 5 min) and the amount of ${}^{14}C$ in the supernatant determined by liquid scintillation fluid as described above.

170 2.4. Acetamiprid sorption in soil

To quantify acetamiprid sorption to the solid phase, 2 g of field moist soil from each 171 SOM treatment was placed into individual 20 cm³ polypropylene tubes. Subsequently, 10 ml 172 of each ¹⁴C-labelled acetamiprid formulation (*Acet*_{CF1}, *Acet*_{CF2} or *Acet*_{Pure}; 0.05 g acetamiprid 173 1⁻¹; 37 kBq sample⁻¹) was added to the tubes. The soil suspensions were then shaken (200 rev 174 min⁻¹) for either 1, 2, 4, 24, 120 or 192 h. The soil suspensions were then centrifuged (18,000 175 g, 5 min) and the amount of acetamiprid remaining in solution determined by liquid scintillation 176 counting as described above. The amount of sorption to the solid phase was calculated by 177 difference. 178

As well as measuring the proportion of pesticide adsorbed to the soil, endpoint K_d partition values were also calculated for each treatment combination using the following formulae:

182
$$Cs = \frac{V(Ci-Ce)}{ms}$$
(1)

183 Calculating the concentration $(g g^{-1})$ in the solid phase (Cs), where *V* is the volume of 184 water (ml), *Ci* is the initial concentration of chemical $(g ml^{-1})$, *Ce* is the equilibrium 185 concentration $(g ml^{-1})$, and *ms* is the starting mass of soil (g).

186 K_d (g ml⁻¹) is then defined as the following-

187
$$K_d = \frac{Ls}{Ce}$$
(2)

188 K_d partition values, also known as the adsorption-desorption distribution coefficient, 189 assist in our understand of a compound's mobility in the environment and how it distributes 190 between the soils and solution phase.

191 2.5. Acetamiprid leaching from soil

To determine the effects of SOM on acetamiprid leaching, field moist soil from each 192 treatment (20 g) was placed into individual 20 cm³ polypropylene syringe barrels ($\phi = 1.5$ cm) 193 to achieve a bulk density of 1.3 g cm^{-3} . The base of the syringe barrels was covered with a disk 194 of glass microfiber filter paper (Whatman GF/C) to prevent soil loss. An aliquot of each ¹⁴C-195 labelled acetamiprid formulation (0.5 ml, 0.05 g acetamiprid l⁻¹, 0.83 kBq sample⁻¹) was then 196 applied to the soil surface and left to equilibrate for 1 h. The average total pore volume of the 197 198 test samples was calculated via saturation, averaging 7.5 ml. Subsequently, the soil columns were leached by adding 7.5 ml of artificial rainwater to the soil surface (Jones and Edwards, 199 200 1993). This was repeated 6 times (i.e., 6 pore volumes), waiting for each pore volume to percolate through before applying the next. Polypropylene vials were placed underneath each 201 soil column to collect the leachate. The amount of ¹⁴C-acetamiprid in the leachate was 202 203 determined by liquid scintillation counting as described above.

204 2.6. Data analysis

205 All laboratory studies used a fully factorial experimental design, allowing for every combination of SOM level (n = 3) and pesticide formulation (n = 3) to be tested. All 206 combinations were replicated four times, consistent with the replicated block design of the field 207 experiment (Mattingly et al., 1973). The data for this study was analysed using the ANOVA 208 packages in JASP (JASP Team (2020). JASP (Version 0.14.1) [Computer software]). For the 209 purpose of this analyses, and due to the production of extreme outlying results, a single 210 replicate from the Acet_{CF1}:SOM_{low} treatment combination was removed for the mineralisation 211 study. 212

213

214 **3. Results**

215 *3.1. Acetamiprid mineralisation in soil*

The highest level of mineralisation was found in the $Acet_{CF1}$ formulation under the SOM_{high} treatment with 21.1 ± 0.81 % of the acetamiprid mineralised after the 60-day study period. Conversely, the $Acet_{CF2}$ formulation produced the lowest level of overall mineralisation across the study, with only 8.0 ± 0.83 % of the acetamiprid mineralised under the SOM_{low} treatment. Across all treatments, an average of 14.6 ± 0.71% of the applied acetamiprid was mineralised after 60 d of incubation.

222 We analysed the results from across the three chemical treatments and found that they were significantly affecting the mineralisation of the test compound ($F_{(2,26)} = 31.1, P < 0.001$). 223 The results from the ¹⁴C- mineralisation study shows that all three formulations had a 224 significantly different mineralisation behaviours to each other, with AcetCF2 consistently 225 producing lower levels and rates of degradation throughout the study (Holm post-hoc 226 comparisons- $Acet_{CF2}$: $Acet_{CF1} P < 0.001$, $Acet_{CF2}$: $Acet_{Pure} P < 0.001$, $Acet_{CF1}$: $Acet_{Pure} P = 0.016$). 227 All three chemical treatments displayed the same pattern across the SOM treatments, with the 228 highest rate and level of mineralisation found under the highest SOM treatment level and the 229 lowest found under the lowest SOM treatment level (Fig. 1). 230

SOM level was also shown to produce significantly different results across the 231 treatment levels, with the higher SOM levels producing higher total cumulative mineralisation 232 values ($F_{(2,26)} = 15.87$, P < 0.001), suggesting a higher rate of pesticide degradation (Mean \pm 233 SEM; SOM_{low}, 11.6 \pm 1.2%, SOM_{med}, 14.5 \pm 1.0%, SOM_{high}, 17.3 \pm 1.0%). The Acet_{CF1} 234 chemical formulation had the highest cumulative mineralisation across all three SOM levels 235 $(17.9 \pm 1.0\%)$, while Acet_{CF2} had the lowest rates of mineralisation after 60 d ($11.4 \pm 1.0\%$) 236 (Holm post-hoc analysis P < 0.001) (Fig. 1). The interaction between the two treatment regimes 237 were also found to produce significant results ($F_{(4,26)} = 3.148, P = 0.031$). 238

239

240 *3.2. Acetamiprid sorption to soil*

The highest levels of sorption $(15.9 \pm 0.78 \%)$ by the end of the study were found in 241 AcetPure under the SOM_{high} treatment. Under the SOM_{low} treatment the lowest levels of sorption 242 were also found in the Acet_{Pure} chemical solution, with only 9.3 ± 1.17 % of the acetamiprid 243 being adsorbed by the end of the study period (Fig. 2). Both experimental treatments, the 244 chemical mixture and the SOM level significantly affected the sorption behaviour of 245 acetamiprid within the agricultural soils (formulation: $F_{(2,27)} = 9.590$, P < 0.001, SOM: $F_{(2,27)} =$ 246 247 159.0, P < 0.001). The interaction between the two treatments also had a significant influence on the sorption behaviour ($F_{(4,27)} = 11.725$, P = 0.031). Significant differences in sorption 248 249 behaviour occurred under each of the SOM treatments, with Acet_{CF2} behaving significantly differently to the other two formulations ($Acet_{CF2}$: $Acet_{CF1} P < 0.001$, $Acet_{CF2}$: $Acet_{Pure} P =$ 250 0.013; Fig. 2). 251

When solely analysing the endpoint data, the chemical treatment no longer exhibited significant differences in total sorption with an average of 12.3% \pm 0.45% being adsorbed to the soil after 192 h. However, the SOM levels still had a significant influence on the sorption of acetamiprid (SOM: F_(2,27) = 65.273, *P* < 0.001), with the SOM_{high} treatments having a higher final proportion adsorbed to the soil relative to the other treatments (Mean \pm SEM; SOM_{high} = 14.8 \pm 0.7%, SOM_{med} = 12.0% \pm 0.5%, SOM_{low} = 10.1% \pm 0.4%; Fig. 2). The corresponding *K_d* values for each treatment are presented in Table S1.

259

260 *3.3. Acetamiprid leaching from soil*

The lowest level of acetamiprid recovery was found for the $Acet_{Pure}$ and SOM_{med} combination with 75.4 ± 5.0 % of the acetamiprid detected by the end of the study. The highest levels of recovery were for the $Acet_{CF2}$ formulation under the SOM_{low} treatment with 92.8 ± 0.96 % of the applied acetamiprid recovered. Across all treatments, an average of 82.9% ± 1.6% of the applied acetamiprid was recovered in the leachate throughout the experiment. When comparing the endpoint data for this study, there were no significant differences in the percentage recovery of acetamiprid between the different chemical mixtures used (Fig. 3).

268 Previous research has shown that an increase in SOM can reduce the leaching of organic pollutants (Bollag et al., 1992), due to an increase in sorption and therefore less being available 269 for leaching. Our results support this, as SOM had a significant impact on the level of 270 acetamiprid recovered within the leachate ($F_{(2,27)} = 3.516$, P = 0.044). We also found that the 271 272 chemical mixture and the interaction between the chemical and SOM treatments did not have a significant effect on the leachability and behaviour of the neonicotinoid compound (Chemical 273 274 formulation: $F_{(2,27)} = 2.439$, P = 0.106, Chemical formulation: SOM: $F_{(4,27)} = 0.969$, P = 0.440) (Fig. 3). 275

276

277 **4. Discussion**

278 4.1. Soil organic matter treatment effects

279 4.1.1. Acetamiprid mineralisation in soil

The differences in chemical fate across the varying levels of SOM could most likely be 280 attributed to the increasing microbial biomass found to accompany the soils with increased 281 SOM. Long term manure applications have previously been shown to be a primary driver for 282 regulating and altering microbial communities within agricultural soils (Lin et al., 2019). 283 Selected plots on the Woburn Organic Manuring Experiment (Rothamsted Research, UK), 284 have received different manure applications since the experiment started in 1964 (Mattingly et 285 al., 1973). The different treatments have resulted in significantly different levels of SOM and 286 microbial biomass in each of the three test soils; these changes in microbial biomass are further 287 reflected by an increase in basal respiration rate in soils treated with FYM in comparison to the 288 mineral fertiliser treatment (Table 2). These increases and changes in microbial biomass and 289 activity could be linked to the increases in acetamiprid degradation in the FYM treated soils 290

(SOM_{high} and SOM_{med}). Though despite the increases in microbial biomass across these soils
(Table 2), previous work, using the same study soils, has shown that the structure of the
microbial communities remain similar, irrespective of SOM level (Ma et al., 2020), indicating
that it is the growth in community rather than any changes in composition that are having an
effect.

Within our ¹⁴C mineralisation experiment we are accounting for total C mineralisation, 296 297 rather than just the metabolism of the original compound into a secondary or tertiary metabolite. This means that whilst we were able to track how much of the original compound 298 299 had been degraded, we were unable to quantify the levels of other intermediate metabolites generated. Whilst known to be produced in much lower quantities, some metabolites have 300 similar and sometimes higher invertebrate toxicities than the original neonicotinoid compound 301 302 (Suchail et al., 2001). The compounds will also degrade and metabolise differently under different soil conditions (aerobic/anaerobic), and the metabolites will also vary depending on 303 the biological communities in the soil (Liu et al., 2011, 2015; Rana et al., 2015; Zhang et al., 304 2018a). 305

The degradation of neonicotinoid compounds has previously also been investigated 306 under chemically sterilised conditions. Zhang et al. (2018b) demonstrated that, in the absence 307 of microbial activity, neonicotinoids can be hydrolysed via amino and cyano hydrolysis. The 308 rates of these mechanisms were strongly influenced by the pH and cation exchange capacity of 309 310 the tested soils, with the rate of hydrolysis increasing as the solution becomes alkaline (Liu et al., 2006; Zhang et al., 2018b). The soils used within this experiment all had similar pH levels 311 (7.1-7.2) (Table 2), suggesting that pH level had, in this case, little influence over the fate of 312 the applied formulations. The formulations themselves were found to have substantially 313 different pH levels (Table 3). However, when accounting for the absolute amount of chemical 314

applied and soils innate buffering capacity these differences are unlikely to have beenresponsible for any noticeable differences.

317 *4.1.2. Acetamiprid sorption to soil*

The fate of agrochemicals in soil are affected by various physical and chemical properties, although generally sorption is viewed as being one of the most important controlling processes (Pietrzak et al., 2020). The level of sorption exhibited by a chemical will impact all other processes within the soil such as the chemical's persistence, and biological or chemical degradation, as well as a pesticides' migration pathway (Pietrzak et al., 2020).

The high water-solubility and low adsorption potential of neonicotinoid pesticides 323 makes them highly susceptible to loss into the wider environment (Leiva et al., 2015; Morrissey 324 et al., 2015). K_d values, also known as the adsorption-desorption distribution coefficient, assist 325 in our understanding of a compound's mobility in the environment and how it distributes 326 between the solid and solution phase. Throughout the literature, acetamiprid is generally noted 327 as exhibiting K_d partition values as below 1.0 g ml⁻¹ (Carbo et al., 2007; Dankyi et al., 2018; 328 Murano et al., 2018). Studies by Carbo et al. (2007) present evidence suggesting that K_d values 329 increase with soil horizons of increasing depth; associating soil sorption not only to the 330 chemical differences of the applied formulations but also to the physicochemical attributes of 331 the soil itself. Since our experiment tested soil collected from the top 0-23 cm (Ahp horizon) 332 our values support those collected in previous studies using soils from similar depths (Carbo et 333 al., 2007; Murano et al., 2018). 334

The level of adsorption across different soils can often be attributed to the organic matter content (Flores-Céspedes et al., 2002; Banerjee et al., 2008; Jin et al., 2016; Mörtl et al., 2016). Our results support these previous findings, as with the higher levels of FYM application we found higher levels of sorption. Recent studies have shown that the addition of humic substances to the soils, can alter the sorption of certain compounds to the soil mineral phase

(Murano et al., 2018). Murano et al. (2018) were able to show that the sorption of the 340 neonicotinoid acetamiprid was reduced through the addition of humic or fulvic acids. This 341 342 reduction in sorption has been attributed to the hydrophobic interactions between humic/fulvic acid and humin, where the dissociated carboxyl and phenolic groups have reoriented to face 343 the soil solution (Murano et al., 2018). Alternatively, work by Jin et al. (2016) investigated the 344 345 sorption of the neonicotinoid imidacloprid, found that the addition of organic bio-amendments, 346 such as biochar from straw and manure increased the sorption capacity of the soil mixtures. These two studies indicate that, whilst organic matter can alter the transport and mobility of 347 348 pesticides, only certain organic materials can be used to stabilise soils contaminated with neonicotinoids. 349

350 *4.1.3. Acetamiprid leaching from soil*

The increases in SOM investigated within our study have shown significant changes to 351 the level of acetamiprid recovered in the leachate, with the highest level of recovery found in 352 353 the lowest SOM treatments. These results correspond well to findings from Bollag et al. (1992), who found that the increases in SOM level often reduced the amounts of chemical available 354 for leaching. The further differences in behaviour exhibited by the different chemical mixtures 355 could be due to the presence or absence of the additional surfactants added during manufacture. 356 These additional chemicals are thought not to affect the designed action of the active ingredient, 357 358 but in this study, it appears they may be involved in either an interaction with the active ingredient and/or the surrounding soil matrix, and as such are causing unexpected changes to 359 acetamiprid fate in soil. 360

The ability of neonicotinoid pesticides to enter agricultural runoff and groundwater systems further extends the reach of their insecticide toxicity, increasing the risk to aquatic and marine invertebrates and other organisms (Morrissey et al., 2015). To date, there has been little research reporting the leaching behaviour of acetamiprid. However, when compared to other neonicotinoid compounds our results fall within the expected range. Gupta et al. (2008),
reported thiamethoxam leachate recovery rates of between 66-79%, with zero detectable
residue left within the soil. When combined with other research findings we conclude that all
currently registered neonicotinoid compounds have a high leaching potential (Gupta et al.,
2008; Liu et al., 2016; Wettstein et al., 2016; Pietrzak et al., 2020).

The methods of agricultural pesticide application are also considered major 370 371 contributors to neonicotinoid leachability and rate of degradation (Wettstein et al., 2016). Compounds that are applied either directly to the soil or plant, such as soil drenches, irrigation 372 373 additives or foliar sprays, may be more susceptible to run-off and leaching as they are readily incorporated into the aqueous phase of the soil. Whilst, topically applied compounds (foliar 374 sprays) were significantly more vulnerable to entering soil if applied directly before rainfall 375 376 events (Anderson et al., 2015), it has been suggested that point source applications, such as seed dressings, could have a higher leaching potential when compared to diffuse (spray) 377 applications (Wettstein et al., 2016). 378

Previous studies have also shown that various soil properties, such as structure and 379 texture, can also have significant impacts on the persistence of agrochemicals such as 380 neonicotinoids (Bollag, Myers and Minard, 1992; Castillo Diaz et al., 2017; Murano et al., 381 2018; Rodríguez-Liébana, Mingorance and Peña, 2018; Pietrzak et al., 2020). The need to fully 382 understand previous land management strategies and practices, as well as underlying historic 383 384 soil contamination, are of paramount importance to better understand how these chemicals behave in the soil. In addition, many of these studies do not consider secondary and tertiary 385 metabolites, and consequently published field and laboratory half-lives may underestimate 386 long-term persistence of the possible breakdown products. 387

388 *4.2. Chemical formulation differences*

The differences in the behaviours of the tested chemicals could most likely be attributed 389 to the presence of additives (including surfactants and adjuvants), and other secondary active 390 ingredients, such as those present in $Acet_{CF2}$. These differences could suggest a chemical 391 interaction either between the active ingredients or the additional ingredients (including 392 surfactants, adjuvants, and emulsifiers) featured within the commercial products. From the 393 394 information available there appears to be little difference in the carrier formulations for the two 395 commercial products, therefore these results possibly highlight a microbial preference to the secondary active ingredients (triticonazole) present in *Acet*_{CF2}, therefore resulting in a slower 396 397 rate of mineralisation and lower final percentage degradation end point. Whilst this study restricted itself to comparing two commercial products, a study by Van der Sluijs et al. (2013) 398 has suggested a synergistic relationship between neonicotinoids and azole-based fungicides, 399 400 influencing both their environmental persistence but also their ecotoxicity. This combination of agrochemicals also impacts the decomposition of plant matter by influencing soil organisms 401 (Zaller et al., 2016). It is uncommon for a single agrochemical to be applied in isolation as a 402 pure ingredient. As well as additional active ingredients, many commercial pesticides are 403 premixed with a combination of additional ingredients such as surfactants, preservatives (e.g., 404 benzisothiazolinone), adjuvants, and other additives such as geraniol. These assist in ease of 405 application or for aesthetic or perfumed purposes and can alter the natural behaviour of the 406 pure ingredient. These changes can occur through a chemical relationship (i.e., synergistic, or 407 408 additive) or through assisting with the physical dispersion of the pesticides.

The rate and extent of sorption exhibited by a chemical are controlled by the physicochemical properties of the chemical itself and also by environmental factors such as pH, temperature, soil type and organic matter persistence and amount (Bollag et al., 1992; Stefanakis et al., 2014). In our case the chemical treatment with the highest pH (*Acet*_{CF1}) exhibited the highest levels of mineralisation (Fig. 1), whilst the most acidic chemical treatment

414 (*Acet*_{CF2}) had the lowest mineralisation end point (Fig. 1) and highest percentage leachate
415 recovery (Fig. 3).

416 When analysing the sorption results from this study there were no significant differences between our commercial formulation with just one active ingredient (Acet_{CF1}) and 417 the sorption of the pure acetamiprid (Acet_{pure}), however, the commercial formulation with two 418 active ingredients (Acet_{CF2}) had significantly different sorption behaviour from the other two 419 420 test chemicals; indicating a possible interaction between the active ingredients. We attribute this to the blocking of sorption sites by triticonazole which is known to readily bind to soil 421 422 (Beigel and Pietro, 1999), therefore leaving fewer active sorption sites for the remaining acetamiprid. In this case the addition of additional ingredients (including secondary active 423 ingredients, surfactants and adjuvants) alone did not appear to significantly impact the sorption 424 of our test chemicals. This is also consistent with the results for triticonazole which also showed 425 no impact of surfactant on its sorption to the soil's solid phase (Beigel and Barriuso, 2020). 426

As well as altering the physical and chemical behaviours of the pesticide action, the 427 addition of surfactants and secondary active ingredients can have impacts on their interactions 428 with the microbial communities within the soils (Pescatore et al., 2020), which in turn can alter 429 the chemical's environmental persistence and resilience. In the case of Acet_{CF1}, the presence of 430 additional ingredients within the commercial formulation produced a high level of soluble C 431 (Table 3). Acet_{CF1} underwent the highest level of biodegradation across all three SOM 432 treatments. We therefore hypothesise that the extra C provided by the additional ingredients 433 encouraged additional microbial growth within the soils, thus increasing the rate of microbial 434 degradation (Fig. 1). This is supported by studies on other pesticides, where the degradation of 435 trifluralin was reduced when in the presence of surfactants (Mata-Sandoval et al., 2001). 436 However, with AcetCF2, despite an increase in TOC compared to the pure acetamiprid the 437 opposite results were found (Fig. 1). In this case it is possible that the additional active 438

ingredient, triticonazole, was more attractive or more easily utilised, than the acetamiprid;
ultimately resulting in a lower degradation of the acetamiprid. Alternatively, the anti-fungal
effect of triticonazole, and the antibacterial properties of certain additives, may have reduced
the components of the microbial community responsible for acetamiprid transformation in soil.

443

444 5. Conclusions

445 Our findings show that the chemical carrier matrix and surfactants used in commercial formulations have a significant impact on the behaviour of acetamiprid when applied to 446 447 agricultural soil. These findings imply that these additional ingredients, including secondary active ingredients, surfactants, and other additives may have adverse effects on the microbial 448 communities influencing rates of mineralisation and altering degradation pathways. We also 449 theorise that in the presence of additional ingredients, the additives could be being 450 preferentially adsorbed on to the soil and targeted by microbial communities, reducing their 451 interaction with acetamiprid. We also found that the addition of farmyard manure also affected 452 the sorption of acetamiprid, and mineralisation and leaching when measured in combination 453 with the differences in chemical formulation. 454

These findings are of particular interest when considering that many pesticide policies 455 and regulations are supported by experiments focussing solely on the behaviour of the pure 456 active ingredient. In the case of our pure active ingredient treatment (Acet_{Pure}), it often yielded 457 significantly different results than those produced by the commercial formulations. These 458 findings were particularly obvious when assessing the rate of mineralisation, with Acetpure 459 continually falling in between the two commercial formulations. These differences imply that 460 the experimental practice of using the active ingredient in isolation could provide unrealistic 461 results, and thus may misrepresent the rate of persistence and associated risks to soil biota. This 462

is especially pertinent when considering the ecotoxicological risk from contact or ingestion ofcontaminated material to various beneficial organisms, including pollinators and earthworms.

There are major knowledge gaps as to how neonicotinoids interact when applied in combination with other agrochemicals. Further work is required to improve our understanding of the major chemical interactions and evaluate the risks that these may pose. Overall, there is a need to better understand how land management (including gardening habits and farming practices) can influence the persistence and toxicity of neonicotinoid insecticides.

470

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482

483 **CRediT authorship contribution statement**

D.L.J, P.C., J.P. and A.M. conceived the study. J.P. and Q.M. performed the field sampling
and soil characterisation. J.P. undertook the radiotracer studies and data analysis and wrote
the first draft of the manuscript. All authors contributed to revisions of the manuscript and
approved the final version of the manuscript.

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| 489 | Conflict of interest |
| 490 | The authors declare no competing interests. |
| 491 | |
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746 **Figure legends**

- 747 Fig. 1. Cumulative mineralisation (by relative percentage) of acetamiprid in the three chemical
- formulations in soils with different soil organic matter contents (SOM_{high}, SOM_{med}, SOM_{low}).

749 Values represent means \pm SEM (n = 4, except Acet_{CF1}:SOM_{low} where n = 3).

- **Fig. 2.** Sorption of acetamiprid in the three chemical formulations in soils with different soil organic
- 751 matter contents (SOM_{high}, SOM_{med}, SOM_{low}). Measured by relative percentage adsorbed within
- the soil. Values represent means \pm SEM (n = 4).
- 753 Fig. 3. Cumulative percentage of acetamiprid in three different formulations recovered in leachate
- from soils with different soil organic matter contents (SOM_{high}, SOM_{med}, SOM_{low}). Values

represent means \pm SEM (n = 4).