

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

4

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17

18

## 19 **ABSTRACT**

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

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

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

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

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

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

94 et al., 2008). The rate and amount of sorption is thought to be dependent on the soil's clay and  
95 organic matter content (Flores-Céspedes et al., 2002; Banerjee et al., 2008; Jin et al., 2016).  
96 Neonicotinoids generally have a high solubility and a relatively low octanol-water partition  
97 coefficient, indicating a hydrophilic nature and a low potential for soil sorption (Murano et al.,  
98 2018). As with other soil processes, leaching appears to be influenced by both the chemical  
99 composition of the neonicotinoid and environmental variables, such as soil properties and  
100 climatic conditions (Jeschke et al., 2011; Liu et al., 2017).

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

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

118

## 119 2. Materials and methods

### 120 2.1. Soil

121 The soil used in the experiments was obtained from selected treatments of the long-  
122 term Woburn Organic Manuring experiment (started in 1964) at Woburn Experimental Farm  
123 (Rothamsted Research), Bedfordshire, UK, in June 2018 (Mattingly et al., 1973; Ma et al.,  
124 2020; Ma et al., 2021). The soil is classified as an Udipsamment (US Soil Taxonomy) or brown  
125 sand and has a sandy-loam texture (80% sand, 6% silt, and 14% clay) making it susceptible to  
126 pesticide leaching (Bromilow et al., 1999). The field was under winter rye (*Secale cereale* L.)  
127 at the time of sample collection. Soil samples (0-23 cm; Ahp horizon) were taken using a gouge  
128 auger from each replicate plot ( $n = 4$ ) receiving either a high ( $25 \text{ t ha}^{-1} \text{ y}^{-1}$ ) or low ( $10 \text{ t ha}^{-1} \text{ y}^{-1}$ )  
129 rate of farmyard manure (FYM) or control plots receiving mineral fertilisers only (Ma et al.,  
130 2020; Macdonald et al., 2018) since 2003. Hereafter, these treatments are referred to as  
131  $\text{SOM}_{\text{high}}$ ,  $\text{SOM}_{\text{med}}$  and  $\text{SOM}_{\text{low}}$ . The plots (with the exception of the  $\text{SOM}_{\text{high}}$  treatment) receive  
132 annual applications of triple superphosphate ( $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ , 43-44%  $\text{P}_2\text{O}_5$   $97.5 \text{ kg ha}^{-1} \text{ y}^{-1}$ )  
133 and sulphate of potash ( $200 \text{ kg ha}^{-1} \text{ y}^{-1}$ , 50%  $\text{K}_2\text{O}$ , 45%  $\text{SO}_3$ ). The treatment regimes for the  
134 field plots are further described in Table 1. The soil samples collected from these plots were  
135 passed through a 5 mm sieve and stored at  $4 \text{ }^\circ\text{C}$ . Properties of the soil from the different  
136 treatments are presented in Table 2. Soil moisture was calculated by drying soil at  $105 \text{ }^\circ\text{C}$  for  
137 24 h, average soil moisture was determined at  $12.9 \pm 0.3 \%$ , hereto referred to as field moisture.

138

### 139 2.2. Neonicotinoid products

140 Two commercially available neonicotinoid products containing acetamiprid (N-[(6-  
141 chloropyridin-3-yl)methyl]-N'-cyano-N-methylethanimidamide) were tested. The first was a  
142 foliar spray product containing acetamiprid ( $0.05 \text{ g l}^{-1}$ ) within a mixture of ethanol and 1,2-  
143 benzisothiazolin-3-one (pH 6.62; Bug Clear Ultra<sup>®</sup>, Scotts Miracle-Gro Corp., Marysville, OH;

144 hereon referred to as *Acet*<sub>CF1</sub>; Material Safety Data Sheet, 2008). The second was also a foliar  
145 spray containing 0.05 g l<sup>-1</sup> acetamiprid, but in combination with 0.15 g l<sup>-1</sup> triticonazole  
146 fungicide and a range of emulsifiers and anti-bacterial preservatives (pH 5.22; Rose Clear  
147 Ultra<sup>®</sup>, Scotts Miracle-Gro Corp.; from here on in referred to as *Acet*<sub>CF2</sub>; (Material Safety Data  
148 Sheet, 2010). A third solution of pure acetamiprid (0.05 g l<sup>-1</sup>; Sigma-Aldrich) was used to  
149 account for any differences caused by any additional ingredients such as surfactants, adjuvants,  
150 emulsifiers, and fungicides present in the commercial mixtures (hereon referred to as *Acet*<sub>Pure</sub>;  
151 Table 3).

152

### 153 2.3. Acetamiprid mineralisation in soil

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



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

#### 170 2.4. Acetamiprid sorption in soil

171 To quantify acetamiprid sorption to the solid phase, 2 g of field moist soil from each  
172 SOM treatment was placed into individual 20 cm<sup>3</sup> polypropylene tubes. Subsequently, 10 ml  
173 of each  $^{14}\text{C}$ -labelled acetamiprid formulation (*Acet*<sub>CF1</sub>, *Acet*<sub>CF2</sub> or *Acet*<sub>Pure</sub>; 0.05 g acetamiprid  
174 l<sup>-1</sup>; 37 kBq sample<sup>-1</sup>) was added to the tubes. The soil suspensions were then shaken (200 rev  
175 min<sup>-1</sup>) for either 1, 2, 4, 24, 120 or 192 h. The soil suspensions were then centrifuged (18,000  
176 g, 5 min) and the amount of acetamiprid remaining in solution determined by liquid scintillation  
177 counting as described above. The amount of sorption to the solid phase was calculated by  
178 difference.

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

$$182 \quad C_s = \frac{V(C_i - C_e)}{m_s} \quad (1)$$

183 Calculating the concentration (g g<sup>-1</sup>) in the solid phase ( $C_s$ ), where  $V$  is the volume of  
184 water (ml),  $C_i$  is the initial concentration of chemical (g ml<sup>-1</sup>),  $C_e$  is the equilibrium  
185 concentration (g ml<sup>-1</sup>), and  $m_s$  is the starting mass of soil (g).

186  $K_d$  (g ml<sup>-1</sup>) is then defined as the following-

$$187 \quad K_d = C_s / C_e \quad (2)$$

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

192 To determine the effects of SOM on acetamiprid leaching, field moist soil from each  
193 treatment (20 g) was placed into individual 20 cm<sup>3</sup> polypropylene syringe barrels ( $\phi = 1.5$  cm)  
194 to achieve a bulk density of 1.3 g cm<sup>-3</sup>. The base of the syringe barrels was covered with a disk  
195 of glass microfiber filter paper (Whatman GF/C) to prevent soil loss. An aliquot of each <sup>14</sup>C-  
196 labelled acetamiprid formulation (0.5 ml, 0.05 g acetamiprid l<sup>-1</sup>, 0.83 kBq sample<sup>-1</sup>) was then  
197 applied to the soil surface and left to equilibrate for 1 h. The average total pore volume of the  
198 test samples was calculated via saturation, averaging 7.5 ml. Subsequently, the soil columns  
199 were leached by adding 7.5 ml of artificial rainwater to the soil surface (Jones and Edwards,  
200 1993). This was repeated 6 times (i.e., 6 pore volumes), waiting for each pore volume to  
201 percolate through before applying the next. Polypropylene vials were placed underneath each  
202 soil column to collect the leachate. The amount of <sup>14</sup>C-acetamiprid in the leachate was  
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  
206 combination of SOM level ( $n = 3$ ) and pesticide formulation ( $n = 3$ ) to be tested. All  
207 combinations were replicated four times, consistent with the replicated block design of the field  
208 experiment (Mattingly et al., 1973). The data for this study was analysed using the ANOVA  
209 packages in JASP (JASP Team (2020). JASP (Version 0.14.1) [Computer software]). For the  
210 purpose of this analyses, and due to the production of extreme outlying results, a single  
211 replicate from the *Acet*<sub>CF1</sub>:*SOM*<sub>low</sub> treatment combination was removed for the mineralisation  
212 study.

213

## 214 3. Results

### 215 3.1. Acetamiprid mineralisation in soil

216 The highest level of mineralisation was found in the *Acet*<sub>CF1</sub> formulation under the  
217 SOM<sub>high</sub> treatment with  $21.1 \pm 0.81$  % of the acetamiprid mineralised after the 60-day study  
218 period. Conversely, the *Acet*<sub>CF2</sub> formulation produced the lowest level of overall mineralisation  
219 across the study, with only  $8.0 \pm 0.83$  % of the acetamiprid mineralised under the SOM<sub>low</sub>  
220 treatment. Across all treatments, an average of  $14.6 \pm 0.71$ % of the applied acetamiprid was  
221 mineralised after 60 d of incubation.

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

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

239

240 *3.2. Acetamiprid sorption to soil*

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

252 When solely analysing the endpoint data, the chemical treatment no longer exhibited  
253 significant differences in total sorption with an average of  $12.3\% \pm 0.45\%$  being adsorbed to  
254 the soil after 192 h. However, the SOM levels still had a significant influence on the sorption  
255 of acetamiprid (SOM:  $F_{(2,27)} = 65.273$ ,  $P < 0.001$ ), with the SOM<sub>high</sub> treatments having a higher  
256 final proportion adsorbed to the soil relative to the other treatments (Mean  $\pm$  SEM; SOM<sub>high</sub> =  
257  $14.8 \pm 0.7\%$ , SOM<sub>med</sub> =  $12.0\% \pm 0.5\%$ , SOM<sub>low</sub> =  $10.1\% \pm 0.4\%$ ; Fig. 2). The corresponding  
258  $K_d$  values for each treatment are presented in Table S1.

259

### 260 3.3. Acetamiprid leaching from soil

261 The lowest level of acetamiprid recovery was found for the *Acet*<sub>Pure</sub> and SOM<sub>med</sub>  
262 combination with  $75.4 \pm 5.0$  % of the acetamiprid detected by the end of the study. The highest  
263 levels of recovery were for the *Acet*<sub>CF2</sub> formulation under the SOM<sub>low</sub> treatment with  $92.8 \pm$   
264  $0.96$  % of the applied acetamiprid recovered. Across all treatments, an average of  $82.9\% \pm$   
265  $1.6\%$  of the applied acetamiprid was recovered in the leachate throughout the experiment.

266 When comparing the endpoint data for this study, there were no significant differences in the  
267 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  
269 pollutants (Bollag et al., 1992), due to an increase in sorption and therefore less being available  
270 for leaching. Our results support this, as SOM had a significant impact on the level of  
271 acetamiprid recovered within the leachate ( $F_{(2,27)} = 3.516$ ,  $P = 0.044$ ). We also found that the  
272 chemical mixture and the interaction between the chemical and SOM treatments did not have  
273 a significant effect on the leachability and behaviour of the neonicotinoid compound (Chemical  
274 formulation:  $F_{(2,27)} = 2.439$ ,  $P = 0.106$ , Chemical formulation:SOM:  $F_{(4,27)} = 0.969$ ,  $P = 0.440$ )  
275 (Fig. 3).

276

## 277 **4. Discussion**

### 278 *4.1. Soil organic matter treatment effects*

#### 279 *4.1.1. Acetamiprid mineralisation in soil*

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

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

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

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

315 applied and soils innate buffering capacity these differences are unlikely to have been  
316 responsible for any noticeable differences.

#### 317 *4.1.2. Acetamiprid sorption to soil*

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

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

335 The level of adsorption across different soils can often be attributed to the organic  
336 matter content (Flores-Céspedes et al., 2002; Banerjee et al., 2008; Jin et al., 2016; Mörtl et al.,  
337 2016). Our results support these previous findings, as with the higher levels of FYM application  
338 we found higher levels of sorption. Recent studies have shown that the addition of humic  
339 substances to the soils, can alter the sorption of certain compounds to the soil mineral phase

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

#### 350 *4.1.3. Acetamiprid leaching from soil*

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

361 The ability of neonicotinoid pesticides to enter agricultural runoff and groundwater  
362 systems further extends the reach of their insecticide toxicity, increasing the risk to aquatic and  
363 marine invertebrates and other organisms (Morrissey et al., 2015). To date, there has been little  
364 research reporting the leaching behaviour of acetamiprid. However, when compared to other



365 neonicotinoid compounds our results fall within the expected range. Gupta et al. (2008),  
366 reported thiamethoxam leachate recovery rates of between 66-79%, with zero detectable  
367 residue left within the soil. When combined with other research findings we conclude that all  
368 currently registered neonicotinoid compounds have a high leaching potential (Gupta et al.,  
369 2008; Liu et al., 2016; Wettstein et al., 2016; Pietrzak et al., 2020).

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

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

#### 388 *4.2. Chemical formulation differences*

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

409           The rate and extent of sorption exhibited by a chemical are controlled by the physico-  
410 chemical properties of the chemical itself and also by environmental factors such as pH,  
411 temperature, soil type and organic matter persistence and amount (Bollag et al., 1992;  
412 Stefanakis et al., 2014). In our case the chemical treatment with the highest pH (*Acet*<sub>CF1</sub>)  
413 exhibited the highest levels of mineralisation (Fig. 1), whilst the most acidic chemical treatment

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

427 As well as altering the physical and chemical behaviours of the pesticide action, the  
428 addition of surfactants and secondary active ingredients can have impacts on their interactions  
429 with the microbial communities within the soils (Pescatore et al., 2020), which in turn can alter  
430 the chemical's environmental persistence and resilience. In the case of *Acet*<sub>CF1</sub>, the presence of  
431 additional ingredients within the commercial formulation produced a high level of soluble C  
432 (Table 3). *Acet*<sub>CF1</sub> underwent the highest level of biodegradation across all three SOM  
433 treatments. We therefore hypothesise that the extra C provided by the additional ingredients  
434 encouraged additional microbial growth within the soils, thus increasing the rate of microbial  
435 degradation (Fig. 1). This is supported by studies on other pesticides, where the degradation of  
436 trifluralin was reduced when in the presence of surfactants (Mata-Sandoval et al., 2001).  
437 However, with *Acet*<sub>CF2</sub>, despite an increase in TOC compared to the pure acetamiprid the  
438 opposite results were found (Fig. 1). In this case it is possible that the additional active

439 ingredient, triticonazole, was more attractive or more easily utilised, than the acetamiprid;  
440 ultimately resulting in a lower degradation of the acetamiprid. Alternatively, the anti-fungal  
441 effect of triticonazole, and the antibacterial properties of certain additives, may have reduced  
442 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  
446 formulations have a significant impact on the behaviour of acetamiprid when applied to  
447 agricultural soil. These findings imply that these additional ingredients, including secondary  
448 active ingredients, surfactants, and other additives may have adverse effects on the microbial  
449 communities influencing rates of mineralisation and altering degradation pathways. We also  
450 theorise that in the presence of additional ingredients, the additives could be being  
451 preferentially adsorbed on to the soil and targeted by microbial communities, reducing their  
452 interaction with acetamiprid. We also found that the addition of farmyard manure also affected  
453 the sorption of acetamiprid, and mineralisation and leaching when measured in combination  
454 with the differences in chemical formulation.

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

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

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

470

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482

#### 483 **CRedit authorship contribution statement**

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

488

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  
748 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  $n = 3$ ).

750 **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  
752 the soil. Values represent means  $\pm$  SEM ( $n = 4$ ).

753 **Fig. 3.** Cumulative percentage of acetamiprid in three different formulations recovered in leachate  
754 from soils with different soil organic matter contents ( $SOM_{high}$ ,  $SOM_{med}$ ,  $SOM_{low}$ ). Values  
755 represent means  $\pm$  SEM ( $n = 4$ ).

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