

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

Correspondence: Aditi Dubey
4112 Plant Sciences Building
University of Maryland
College Park 20742
email: aditid@umd.edu

Evaluating the ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation

Aditi Dubey, Margaret T. Lewis, Galen P. Dively, Kelly A. Hamby

Department of Entomology, University of Maryland, College Park 20742

31 **Abstract**

32 1. While many studies have investigated non-target impacts of neonicotinoid seed treatments
33 (NSTs), they usually take place within a single crop and focus on specific pest or beneficial
34 arthropod taxa.

35 2. We compared the impacts of three seed treatments to an untreated control: imidacloprid +
36 fungicide products, thiamethoxam + fungicide products, and fungicide products alone in a three-
37 year crop rotation of full-season soybean, winter wheat, double-cropped soybean and maize.
38 Specifically, we quantified neonicotinoid residues in the soil and in weedy winter annual flower
39 buds and examined treatment impacts on soil and foliar arthropod communities, and on plant
40 growth and yield.

41 3. Trace amounts of insecticide were found in winter annual flowers of one species in one site
42 year, which did not correspond with our treatments. Although low levels of insecticide residues
43 were present in the soil, residues were not persistent. Residues were highest in the final year of
44 the study, suggesting some accumulation.

45 4. We observed variable impacts of NSTs on the arthropod community; principle response curve
46 analysis, diversity and evenness values exhibited occasional community disturbances, and
47 treatments impacted the abundance of various taxa. Overall, imidacloprid had a greater effect
48 than thiamethoxam, with the fungicide only treatment also occasionally impacting communities
49 and individual taxa.

50 5. Pest pressure was low throughout the study, and although pest numbers were reduced by the
51 insecticides no corresponding increases in yield were observed. However, the fungicide products
52 contributed to higher yields in wheat.

53 6. *Synthesis and applications.* Pesticide seed treatments can disturb arthropod communities, even
54 when environmental persistence and active ingredient concentrations are low. The foliar

55 community in wheat and maize exhibited a trend of increasing disturbance throughout the
56 sampling period, suggesting that recovery from the impacts of NSTs is not always rapid. Our
57 study is among the first to demonstrate that seed applied fungicides alone can disrupt arthropod
58 communities in agroecosystems and highlights the need for further investigation into the impacts
59 of seed applied fungicides.

60 **Key words**

61 Fungicides; Imidacloprid; *Glycine max* L. Merr.; Neonicotinoid residues; Thiamethoxam;
62 *Triticum* spp.; *Zea mays* L.;

63 **1. Introduction**

64 Declines in arthropod biomass have been documented at multiple locations and are likely linked
65 to habitat loss, climate change, and agrochemical pollutants (Hallmann et al., 2014; Lister &
66 Garcia, 2018). Since their introduction in the 1990s, neonicotinoid insecticides have become the
67 most used insecticide class worldwide, gaining popularity due to their low vertebrate toxicity,
68 systemic nature and versatility of application methods (Nauen, Jeschke, & Copping, 2008).
69 Neonicotinoids are especially popular as seed treatments (NSTs); by 2011, NSTs were used in
70 79-100% of maize *Zea mays* L. and 34-44% of soybean *Glycine max* L. Merr. planted in the
71 USA (Douglas & Tooker, 2015). When neonicotinoids are applied as NSTs, less than 20% of the
72 active ingredients are taken up by the plant (Alford & Krupke, 2017; Sur & Stork, 2003), instead
73 largely remaining in the soil, where their environmental fate is not fully understood. The half-
74 lives of neonicotinoids in soil vary considerably and they may persist and accumulate for
75 multiple years post planting (Bonmatin et al., 2015). Due to their water solubility, neonicotinoids
76 can also leach into groundwater and run-off into waterbodies; neonicotinoid residues are

77 frequently detected at levels above ecological thresholds in waterbodies that are adjacent to or
78 receive runoff from crop lands (Morrissey et al., 2015). In addition, neonicotinoids may also
79 contaminate non-crop plants. Several studies have found neonicotinoid residues in plants
80 growing near treated fields, but it is difficult to determine whether the active ingredients were
81 taken up from the soil or deposited aerially (Botías et al., 2015; Pecenka & Lundgren, 2015;
82 Stewart et al., 2014). Due to the widespread use, environmental persistence, and mobility of the
83 active ingredients from NSTs, they are common pesticide pollutants.

84 NSTs pollution can negatively impact many non-target organisms. Although NSTs
85 require relatively low active ingredient concentrations and can reduce non-target exposure due to
86 pesticide drift, they have similar impacts on non-target arthropod abundance as soil and foliar
87 pyrethroid applications (Douglas & Tooker, 2016). Beneficial natural enemies may be exposed
88 to NST active ingredients indirectly by consuming herbivores or directly, either through physical
89 contact or by feeding on plant material or nectar (Gontijo, Moscardini, Michaud, & Carvalho,
90 2015; Khani, Ahmadi, & Ghadamyari, 2012; Moscardini, Gontijo, Michaud, & Carvalho, 2014;
91 Moser & Obrycki, 2009; Papachristos & Milonas, 2008; Seagraves & Lundgren, 2012). For
92 example, the presence of neonicotinoids in the soil can suppress predatory ground beetles
93 (Coleoptera: Carabidae) through direct contact with active ingredients (Pisa et al., 2015; Simon-
94 Delso et al., 2015), or by ingestion of contaminated prey (Douglas, Rohr, & Tooker, 2015).
95 Work characterizing the impact of neonicotinoids typically focuses on specific pest or beneficial
96 taxa; however, the interconnected arthropod community should also be evaluated as whole.
97 Increased taxon diversity and evenness is associated with reduced pest pressure (Lundgren &
98 Fausti, 2015); therefore, community-level impacts of NSTs could disrupt natural pest control. In
99 maize, clothianidin treated seed disturbed the overall arthropod community after planting, with

100 several beneficial predators decreasing in abundance (Disque, Hamby, Dubey, Taylor, & Dively,
101 2018). Neonicotinoids can also negatively impact pollinators (Godfray et al., 2014). Because
102 pollinators often rely on non-crop floral resources, uptake by non-crop plants may be an
103 important route of exposure (Botias, David, Hill, & Goulson, 2016). Given the risks associated
104 with NST pollution, the use of NSTs in multiple crops, their potential long-term environmental
105 persistence, and their effects on arthropod communities must all be taken into consideration
106 when evaluating non-target impacts.

107 In addition to the many risks associated with NSTs, they often provide limited benefits.
108 Active ingredients from NSTs generally remain bioactive in plant tissue for 3-4 weeks post
109 planting, so they only provide protection against early season soil and seedling pests (Alford &
110 Krupke, 2017; Myers & Hill, 2014). Because pest pressure is often not monitored prior to
111 planting, NSTs are frequently used prophylactically, and growers may not recoup the cost of
112 treatment unless significant early season pest pressure occurs (Cox, Cherney, & Shields, 2007;
113 Myers & Hill, 2014; Wilde et al., 2007). The economic benefits of NSTs vary greatly based on
114 region and cropping system and must be evaluated on a case by case basis.

115 In this study, we evaluated the impacts of repeated use of two popular NSTs [Gaucho 600
116 (imidacloprid), and Cruiser 5FS (thiamethoxam)] during a three-year grain crop rotation
117 common to the mid-Atlantic United States: full-season soybean, winter wheat, double-cropped
118 soybean and maize. Because commercial NSTs always include fungicides in addition to
119 insecticides, we included a fungicide only treatment as well as an untreated control in order to
120 isolate the impacts of the fungicides from those of the insecticides. To the best of our knowledge,
121 this is among the first studies to quantify the impacts of seed applied fungicides on the arthropod
122 community. The location and concentration of pesticide active ingredients drive non-target

123 effects; therefore, we quantified the persistence of neonicotinoids in the soil and determined
124 whether weedy winter annual flowers uptake residues. We hypothesized that higher levels of
125 neonicotinoid residues would be present in the soil later in the study due to accumulation from
126 multiple crops. Our second object was to evaluate the impacts of pesticide seed treatments on the
127 overall arthropod community and on key arthropod taxa. We anticipated the strongest impacts on
128 the soil community, given the potential for persistence of active ingredients in the soil, and the
129 short activity period in plant tissue. We expected community disturbance early on with recovery
130 during each cropping cycle as observed previously in maize (Disque et al., 2018), but
131 hypothesized that disturbance in the soil community would increase over the course of the study
132 due to potential cumulative impacts of repeated NST use. We also hypothesized that the
133 fungicide only treatment could also impact the arthropod community, due to direct toxicity of
134 seed-applied fungicides towards arthropods (MDA, 2012) or indirect alteration of crop fungal
135 communities. Our final objective was to measure the economic value of the treatments in terms
136 of plant growth metrics and yield, to determine whether the environmental risks of NSTs are
137 justified by economic benefits in mid-Atlantic grain production. We did not expect the
138 insecticide treatment to significantly improve yield because Maryland tends to have low pressure
139 from pests targeted by NSTs; however, neonicotinoids can stimulate plant growth in the absence
140 of pest pressure (Simon-Delso et al., 2015), which could improve growth parameters and yield.

141 **2. Materials & Methods**

142 The study was conducted at the Wye Research and Education Center in Queenstown, MD, USA
143 (38°54'02.80" N 76°08'22.06" W) and the Central Maryland Research and Education Center in
144 Beltsville, MD, USA (39°01'08.11" N 76°49'25.10" W) and compared treatments over a three

145 year rotation of four crops at each site. The four treatments were untreated seeds (control),
146 fungicide products alone, fungicide products + imidacloprid insecticide (Gaucho 600; Bayer
147 Crop Science), and fungicide products + thiamethoxam insecticide (Cruiser[®] 5FS; Syngenta).
148 Full-season soybean was planted in spring 2015, winter wheat in fall 2015, double-cropped
149 soybean in summer 2016, and maize in spring 2017. At each site, four replicate plots of each
150 treatment measuring 9.1m x 15.2m were arranged in a Latin square (Fig. S1). The plot rows were
151 separated by rows of untreated grain that provided space for the planter to turn. Plot columns
152 were separated by three-foot bare strips to delimit plots and facilitate sampling. To determine
153 cumulative effects of repeated treatments, each treatment replicate was planted in the same
154 location for each crop in the rotation. Standard agronomic practices were followed throughout;
155 no foliar fungicides or insecticides were applied. The field at Beltsville was previously planted
156 with untreated soybean, and at Queenstown with neonicotinoid seed treated maize. The seeding
157 rate, variety, and active ingredient rate for each treatment and crop are listed in Tables S1-S2.
158 Due to differences in seeding and application rates, the amount of active ingredient per acre
159 varied slightly between soybean and maize, with wheat concentrations almost double that of the
160 other crops.

161 *2.1 Residue analysis*

162 In spring 2016 and 2017, we collected flower buds from winter annual plants growing within the
163 experimental plots for neonicotinoid residue analysis. Winter annual species were chosen based
164 on abundance and attractiveness to pollinators. In 2016, common henbit *Lamium amplexicaule*
165 L. was collected at Beltsville and common chickweed *Stellaria media* L. Vill. at Queenstown. In
166 2017, we collected common chickweed at Queenstown and both species at Beltsville. Soil was

167 collected for residue analysis before and shortly after soybean and maize were planted in 2015
168 and 2017, and in March 2016, while wheat was dormant.

169 Residue samples were sent to the USDA National Science Laboratory (Gastonia, NC,
170 USA) for analysis in 2019, where they were tested for imidacloprid, thiamethoxam, and
171 clothianidin, another popular neonicotinoid that is also a breakdown product of thiamethoxam
172 (Simon-Delso et al. 2015). Further details about material collection and residue analysis are
173 included in section 1.1 of the supporting information.

174 *2.2 Arthropod sampling*

175 Throughout the study, the epigeal and soil invertebrate community was measured using sticky
176 cards (3 subsamples per plot), pitfall traps (3 subsamples per plot) and surface litter extractions
177 (4 subsamples pooled into two Berlese funnel extractions per plot). Samples were collected three
178 times during each growing season, and pitfall trap and litter samples were also collected before
179 planting in 2017 maize. In soybean, arthropod abundance in the plant canopy was measured by
180 sweep netting, where 15 sweeps were taken in a straight line through the center of each plot once
181 per season. Samples from one 2015 sweep net imidacloprid replicate at Beltsville and one 2016
182 sticky card double-cropped soybean sampling date at Queenstown were misplaced prior to
183 processing. We also conducted visual inspections of plants to quantify pest and beneficial
184 arthropods in all crops. The sampling timeline (Tables S3-S6) and further details can be found in
185 section 1.2 of the supporting materials.

186 *2.3 Crop sampling*

187 We measured the impact of NSTs on plant growth by recording stand density and plant height in
188 all crops. In wheat, we also counted the number of tillers and measured the Normalized
189 Difference Vegetative Index (NDVI), which can be used to indirectly measure crop biomass

190 (Erdle, Mistele, & Schmidhalter, 2011). We also measured yield at the time of harvest. Details
191 for each crop are included in section 1.3 of the supporting information.

192 *2.4 Statistical analysis*

193 For arthropod sampling, taxa were identified to family in most cases, and adults and immatures
194 were combined for all taxa. Insects from the following orders that could not be identified to
195 family were excluded from all analyses: Coleoptera, Diptera, Hemiptera, Hymenoptera and
196 Lepidoptera. After averaging subsamples within each replicate, Shannon Diversity Index (H) and
197 taxa evenness [$H/\log(n)$ ratio] were calculated for sticky cards, pitfall traps, litter and sweep nets
198 from each crop using CANOCO 5 (Microcomputer Power, Ithaca, NY, USA).

199 To characterize the impact of treatment over time, arthropod community composition was
200 analyzed in CANOCO 5 using Principal Response Curve Analysis (PRC) for pitfall traps, litter
201 extraction and sticky card data for each crop (Disque et al. 2018). Briefly, PRC multivariate
202 analysis is based on Redundancy Analysis (Van den Brink & Ter Braak J. F., 1999), with
203 adjustments for the change in community response over time. In our study, total abundances for
204 each taxon were averaged over subsamples within a replicate plot for each site prior to analysis.
205 Taxa where the sum of individuals across sampling dates and sites for a crop was less than one
206 were excluded from the PRC. For each crop and sample type, the date*treatment interaction term
207 was used as an explanatory variable, and date and the site*replicate interaction were used as
208 covariates to restrict data shuffling. Canonical coefficients were generated for each date and
209 plotted over time to evaluate the community response to the treatments relative to the untreated
210 control; the control is plotted along the horizontal axis (representing time), with the magnitude
211 (represented by canonical coefficients plotted on the vertical axis) and shape of curves
212 representing the deviation of treatments from the control. The analysis also generates taxon-

213 specific weights for the individual taxa that exhibit the strongest effects; taxa with high positive
214 weights are more likely to follow the pattern depicted in the PRC, while taxa with high negative
215 weights exhibit an opposite response. A Monte-Carlo permutation procedure with N=499 was
216 used to test the null hypothesis that the canonical coefficients of the treatment response equaled
217 zero for all sampling times, and to calculate a Pseudo-F statistic (Disque et al., 2018). Because
218 sweep net samples were conducted on a single date, captures were analyzed using RDA (Van
219 den Brink & Ter Braak J. F., 1999). Ants (Hymenoptera: Formicidae) were excluded from PRC,
220 diversity and evenness for sticky cards, pitfall traps and litter due to their highly clumped
221 distribution on the ground, but were included in RDA, diversity and evenness for sweep net
222 sampling.

223 Analysis of variance (Proc Mixed, SAS 9.4, SAS Institute Inc., Cary, NC, USA) was
224 used to evaluate treatment effects for Shannon Index, evenness, stand count, plant height, NDVI,
225 tiller count, yield and abundances of key taxa. For each crop and sample type, treatment was
226 included as an explanatory variable, and site, row and column were random blocking factors,
227 with row and column nested within site. For data collected on multiple dates, date and
228 date*treatment interactions were also included as fixed effects. The date*treatment interaction
229 was dropped when not significant. Before analysis, residual plots and the Shapiro-Wilk's W test
230 were performed to examine data normality and homogeneity of variances, and appropriate
231 transformations or variance groupings were applied as needed. When $P < 0.10$ for the treatment
232 effect, contrasts were used to compare the fungicide, imidacloprid and thiamethoxam treatments
233 to the control.

234

235

236

237 **3. Results**

238 *3.1 Residue analysis*

239 *3.1.1 Winter annual flowers*

240 The detection level was 10 ppb for imidacloprid, 5 ppb for thiamethoxam and 30 ppb for
241 clothianidin in flowers. In 2016, neonicotinoid residues were not found in any samples. In 2017,
242 trace amounts (<10ppb) of imidacloprid were found in five of the chickweed samples from
243 Beltsville, specifically two control samples and one from each of the other treatments. Detections
244 did not occur in a spatial pattern.

245 *3.1.2 Soil*

246 In soil the detection level was 5ppb for imidacloprid, 10ppb for thiamethoxam and 15ppb for
247 clothianidin. Before planting in 2015, low levels (≤ 10 ppb) of imidacloprid were present in
248 several replicates at Beltsville and no residues were present at Queenstown (Table S7). Similar
249 levels were detected after treated soybean was planted, and trace amounts of thiamethoxam and
250 clothianidin were found in one thiamethoxam and one imidacloprid treated replicate. Only one
251 thiamethoxam replicate contained detectable residues at Queenstown. In 2016, during wheat
252 dormancy, 7ppb of imidacloprid was found in the imidacloprid treated plots from Beltsville, with
253 trace amounts in the other plots from Beltsville and the imidacloprid plots from Queenstown.
254 Before maize was planted in 2017, low levels of imidacloprid were present in both imidacloprid
255 sample replicates, and one control and thiamethoxam sample replicate at Beltsville. After maize
256 was planted, imidacloprid was detected across multiple treatments at Beltsville, and in the
257 imidacloprid treated plots at Queenstown, with higher levels (≥ 10 ppb) present in the
258 imidacloprid treated plots at both sites. Thiamethoxam was detected in both thiamethoxam

259 replicates (15-16ppb) at Queenstown, and thiamethoxam (17ppb) and clothianidin (23ppb) were
260 found in one thiamethoxam replicate from Beltsville.

261 *3.2 Arthropod sampling*

262 The date by treatment interaction was not significant for arthropod and crop analyses. Therefore,
263 the interaction term was removed from the model and we present information solely for the
264 treatment effect.

265 *3.2.1 Effects of seed treatments on the overall community over time*

266 *3.2.1.1 Diversity and evenness*

267 *Pitfall traps:* Activity density of arthropods collected using pitfall traps was measured during
268 three one-week periods. After averaging subsamples and removing unidentifiable individuals and
269 ants (Formicidae), we collected 53,359 individuals across sites and crops (Table S8). Shannon
270 diversity indices were not impacted for any crop: 2015 soybean ($F_{3,77}=0.43$, $P=0.729$), 2015-
271 2016 wheat ($F_{3,77}=0.95$, $P=0.420$), 2016 double cropped soybean ($F_{3,77}=0.75$, $P=0.528$), pre-
272 planting 2017 maize ($F_{3,15}=1.23$, $P=0.333$), post-planting 2017 maize ($F_{3,77}=0.79$, $P=0.502$).
273 Similarly, evenness was also not impacted: 2015 soybean ($F_{3,77}=0.30$, $P=0.828$), 2015-2016
274 wheat ($F_{3,77}=0.78$, $P=0.509$), 2016 double cropped soybean ($F_{3,77}=0.29$, $P=0.831$), pre-planting
275 2017 maize ($F_{3,15}=0.57$, $P=0.640$), post-planting 2017 maize ($F_{3,77}=0.60$, $P=0.617$).

276 *Litter extraction:* Impacts on arthropod abundance were measured by litter extraction three times
277 in each crop. After averaging subsamples, a total of 69,332 individuals were identified from
278 across crops and sites (Table S9). Shannon diversity indices for litter communities were
279 generally similar across treatments in the first two years: 2015 soybean ($F_{3,77}=0.14$, $P=0.937$),
280 2015-2016 wheat ($F_{3,77}=2.83$, $P=0.044$, no contrast differences), and 2016 double cropped

281 soybean ($F_{3,77}=0.67$, $P=0.571$). However, in 2017 maize both insecticide treatments reduced
282 diversity ($F_{3,77}=3.90$, $P=0.031$) relative to the control pre-planting (Table 1). Post-planting
283 ($F_{3,77}=3.97$, $P=0.011$), only imidacloprid reduced diversity. Litter community evenness was also
284 not impacted until the last year of the study: 2015 soybean ($F_{3,77}=0.25$, $P=0.863$), 2015-2016
285 wheat ($F_{3,77}=1.56$, $P=0.206$), and 2016 double cropped soybean ($F_{3,77}=0.78$, $P=0.507$). In 2017,
286 taxa evenness was reduced by imidacloprid in pre-planting plots ($F_{3,15}=2.53$, $P=0.096$) and by all
287 pesticide seed treatments in post-planting plots ($F_{3,77}=3.76$, $P=0.014$) (Table 1).

288 *Sticky cards*: Arthropod activity density ~8cm above the ground was measured during three one-
289 week intervals using sticky cards. Data from the second sampling date at Queenstown for
290 double-cropped soybean could not be included because the cards were misplaced before
291 processing. After averaging subsamples, 34,413 individuals were identified from across crops
292 and sites (Table S10). Shannon diversity indices were not impacted by the pesticide treatments
293 for most crops: 2015-2016 wheat ($F_{3,77}=1.11$, $P=0.351$), 2016 double cropped soybean
294 ($F_{3,61}=1.59$, $P=0.201$), 2017 maize (post-planting) ($F_{3,77}=1.96$, $P=0.127$). However, in 2015 full-
295 season soybean ($F_{3,77}=2.36$, $P=0.078$), sticky card captures were less diverse in imidacloprid
296 plots than control plots. Treatments also did not impact taxa evenness for most crops: 2015
297 soybean ($F_{3,77}=0.73$, $P=0.537$), 2016 double cropped soybean ($F_{3,77}=1.64$, $P=0.189$), 2017 maize
298 (post-planting) ($F_{3,77}=0.27$, $P=0.847$); except in wheat ($F_{3,77}=2.28$, $P=0.082$), where the
299 thiamethoxam treatment exhibited higher taxa evenness (Table 1).

300 *Sweep net*: Sweep nets were used to collect canopy-dwelling taxa in full-season and double-
301 cropped soybean, with 3,892 individuals identified (Table S11). In full-season soybean, diversity
302 was not impacted by the pesticide treatments ($F_{3,14}=1.78$, $P=0.197$), while in double-cropped
303 soybean ($F_{3,15}=3.6$, $P=0.039$), the thiamethoxam treatment exhibited lower arthropod diversity

304 than the control (Table 1). Taxa evenness was lower than the control in all three treatments in
 305 2015 ($F_{3,14}=3.95$, $P=0.031$), but not 2016 ($F_{3,15}=3.18$, $P=0.055$, no contrast differences).

306 Table 1. Treatment means for Shannon Index and evenness values for litter extraction (LE), sticky card (SC) and
 307 sweep net (SN) arthropod communities. Analysis of variance was used with date and treatment as fixed effects and
 308 location, row (location) and column (location) as random effects. Contrasts were used to compare the fungicide
 309 (FUN), imidacloprid (IMI) and thiamethoxam (THI) treatments to the control (CON), * indicates $P<0.05$, **
 310 indicates $P<0.01$, *** indicates $P<0.001$ and N.S. indicates no significance. FS = full season and DC = double
 311 cropped soybean.

Metric	Crop	Sample Type	Treatment Mean \pm S.E.			
			CON	FUN	IMI	THI
Shannon Index	FS Soybean (2015)	SC	2.02 \pm 0.06	1.97 \pm 0.07 ^{N.S.}	1.94 \pm 0.07*	2.04 \pm 0.07 ^{N.S.}
	Winter Wheat (2015-2016)	LE	1.27 \pm 0.05	1.36 \pm 0.05 ^{N.S.}	1.17 \pm 0.07 ^{N.S.}	1.28 \pm 0.05 ^{N.S.}
	DS Soybean (2016)	SN	2.24 \pm 0.11	2.13 \pm 0.05 ^{N.S.}	2.09 \pm 0.07 ^{N.S.}	1.90 \pm 0.08**
	Maize Pre-Plant (2017)	LE	1.71 \pm 0.10	1.63 \pm 0.07 ^{N.S.}	1.3 \pm 0.14*	1.45 \pm 0.06*
	Maize Post-Plant (2017)	LE	1.47 \pm 0.09	1.42 \pm 0.11 ^{N.S.}	1.22 \pm 0.07**	1.36 \pm 0.10 ^{N.S.}
Evenness	FS Soybean (2015)	SN	0.67 \pm 0.06	0.77 \pm 0.04*	0.74 \pm 0.05*	0.77 \pm 0.05**
	Winter Wheat (2015-2016)	SC	0.72 \pm 0.03	0.72 \pm 0.02 ^{N.S.}	0.74 \pm 0.02 ^{N.S.}	0.75 \pm 0.03*
	DC Soybean (2016)	SN	0.83 \pm 0.04	0.84 \pm 0.02 ^{N.S.}	0.83 \pm 0.03 ^{N.S.}	0.77 \pm 0.04 ^{N.S.}
	Maize Pre-Plant (2017)	LE	0.74 \pm 0.04	0.71 \pm 0.03 ^{N.S.}	0.61 \pm 0.07*	0.65 \pm 0.05 ^{N.S.}
	Maize Post-Plant (2017)	LE	0.73 \pm 0.03	0.61 \pm 0.04*	0.58 \pm 0.03**	0.61 \pm 0.04*

312

313 3.2.1.2 Community disturbances

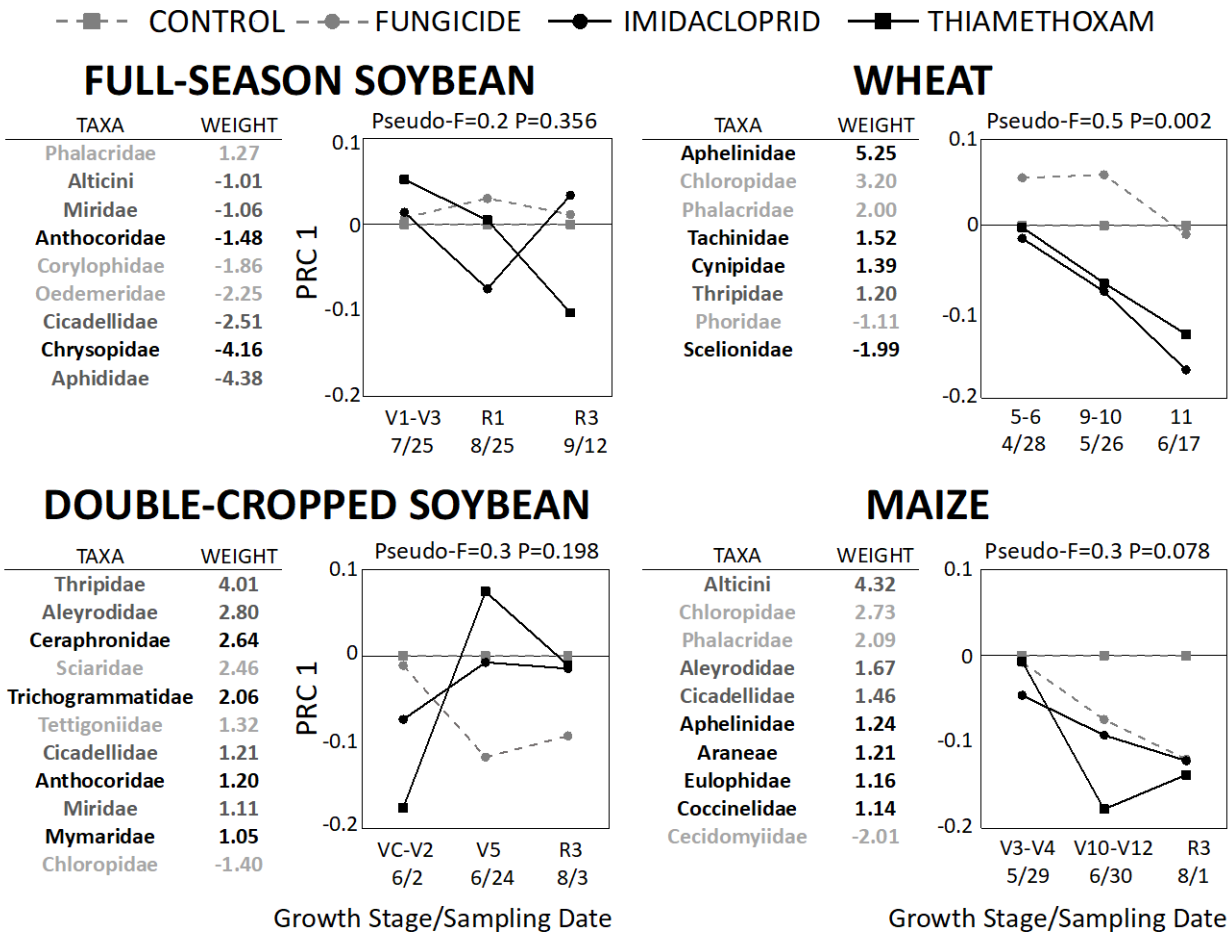
314 *Pitfall traps*: Pitfall trapped communities exhibited no disturbance by the pesticide treatments
 315 over time for any crop: 2015 full-season soybean (Pseudo-F=0.1, $P=0.924$), 2015-2016 winter
 316 wheat (Pseudo F=0.2, $P=0.712$), 2016 double-cropped soybean (Pseudo-F=0.2, $P=0.814$), 2017
 317 maize (Pseudo F=0.2, $P=0.278$) (Fig. S2).

318 *Litter extraction:* Litter communities were similarly not disturbed across sampling dates by the
319 pesticide treatments: 2015 full-season soybean (Pseudo-F=0.2, P=0.946), 2015-2016 winter
320 wheat (Pseudo-F=0.2, P=0.976), 2016 double-cropped soybean (Pseudo-F=0.3, P=0.064), 2017
321 maize (Pseudo-F=0.5, P=0.198) (Fig. S3). Though the insecticide treatment canonical
322 coefficients were often below the control on the first sampling date, community responses
323 varied.

324 *Sticky cards:* Although sticky card communities were not impacted by pesticide treatments in
325 2015 full-season (Pseudo-F=0.2, P=0.356) and 2016 double-cropped (Pseudo-F=0.3, P=0.198)
326 soybean, increasing insecticide treatment community declines were observed over the sampling
327 dates in wheat (Pseudo-F=0.5, P=0.002), with all pesticides causing increasing declines over
328 time in maize (Pseudo-F=0.3, P=0.078) (Fig. 1).

329

330



331

332 Fig. 1. Principal Response Curve analysis of sticky card data for all crops. Date*treatment served as the explanatory
 333 variable, with date and site*replicate used as covariates. Subsamples were averaged by taxa for each replicate, and
 334 only taxa with overall means greater than one were included. Ants (Formicidae) were also excluded due to their
 335 highly clumped distribution. A Monte-Carlo permutation procedure with N=499 was used to calculate the Pseudo-F
 336 statistic. Taxon weights indicate which groups most contributed to the observed community response. Higher
 337 positive weights indicate that taxon abundances in the treated plots followed the trend depicted by the response
 338 curve, whereas higher negative values indicate the opposite. Taxon weights between -1 and 1 were excluded due to
 339 weak response or lack of correlation with the trends shown. Beneficial groups are shown in black, economic pests in
 340 dark gray, and other groups in light gray.

341 *Sweep net*: Sweep net collected arthropods were not impacted by pesticide treatments in full-
342 season soybean (First axis Pseudo-F=0.4, P=0.412); however, multiple beneficial taxa (e.g.,
343 Coccinellidae, Anthocoridae, Araneae) were reduced by the insecticide treatments in double-
344 cropped soybean (First axis Pseudo-F=0.9 P=0.004) (Fig 2).

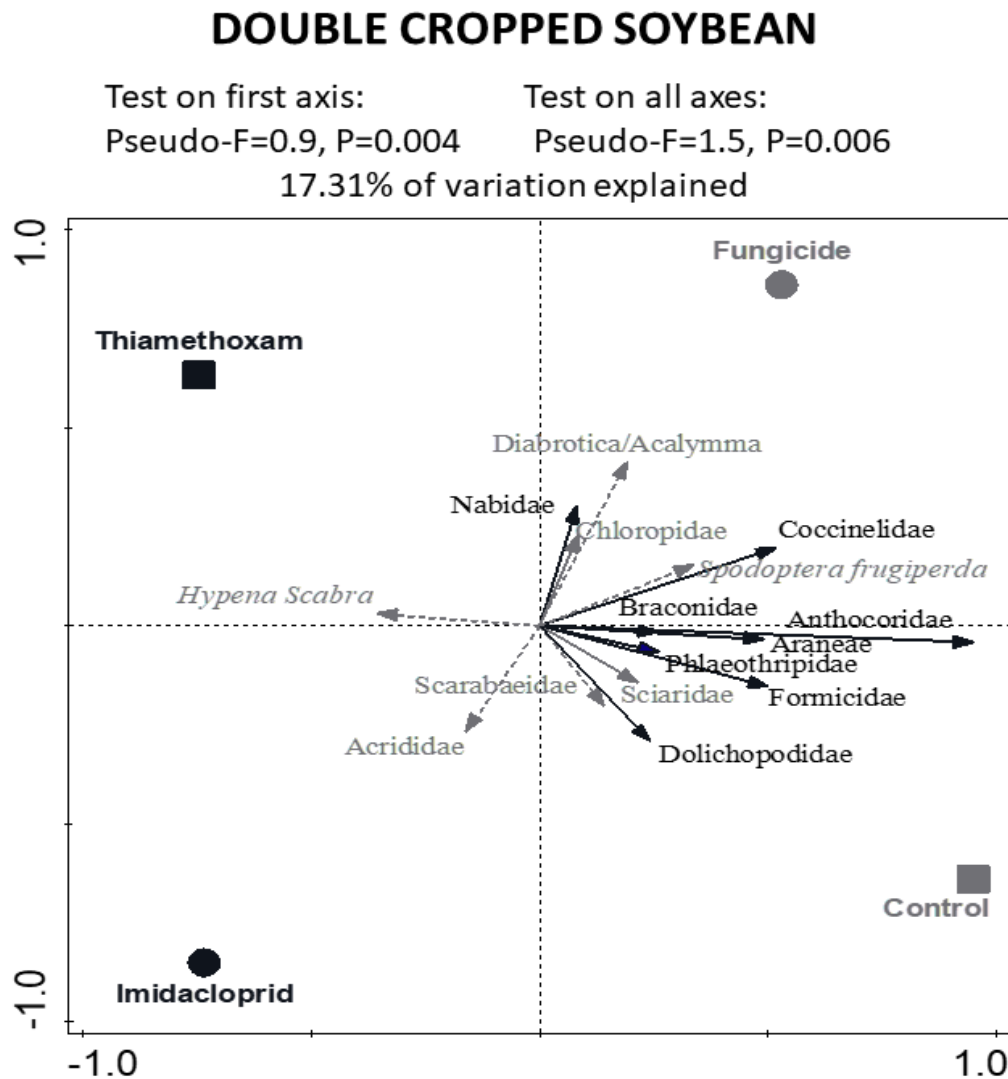


Fig. 2. Redundancy Analysis of sweep net data from 2016 double cropped soybean. Treatment served as the explanatory variable and the site*replicate interaction was used as a covariate. The horizontal axis is the first axis. Only the 15 taxa that most contributed are shown. A Monte-Carlo permutation procedure with N=499 was used to calculate a Pseudo-F statistic. Beneficial groups are shown in black, economic pests in gray with dotted lines, and other groups in gray with solid lines.

345

346 3.2.2 *Effects of seed treatments on individual taxa within crops*

347 2015 Full-season soybean: Visual scouting revealed fewer predatory thrips (Phlaeothripidae,
348 $F_{3,15}=4.46$, $P=0.020$) within both insecticide treated plots; however, only thiamethoxam reduced
349 leafhoppers (Cicadellidae, $F_{3,15}=8.77$, $P=0.001$) and pestiferous thrips (Thripidae, $F_{3,15}=8.21$,
350 $P=0.002$) (Table 2).

351 2015-2016 Winter wheat: Imidacloprid treated plots exhibited higher pitfall trap abundances of
352 free-living predatory mites (Mesostigmata, $F_{3,77}=2.4$, $P=0.074$) and saprovorous mites (Acari -
353 Orbatida +Tarsonemidae, $F_{3,77}=3.23$, $P=0.027$) (Table 2). However, rove beetles (Staphylinidae,
354 $F_{3,77}=3.03$, $P=0.035$) were less abundant in the imidacloprid treatment litter extractions than the
355 control, and sticky cards in imidacloprid treated plots captured fewer aphelinid ($F_{3,77}=4.27$,
356 $P=0.008$) and braconid wasps ($F_{3,77}=2.98$, $P=0.037$). Fewer phytophagous thrips (Thripidae,
357 $F_{3,77}=3.18$, $P=0.029$) were captured on sticky cards in both insecticide treatments, and visual
358 scouting indicated that aphids (Aphididae, $F_{3,46}=16.45$, $P<0.001$) were controlled by both
359 treatments in the fall/winter. However, cereal leaf beetle (*Oulema melanopus*, $F_{3,77}=5.47$,
360 $P=0.002$) abundances were higher in imidacloprid treated plots compared to the control in the
361 spring.

362

363

364

365

366

367

368

369 Table 2. Treatment means of individual taxa from pitfall traps (PT), litter extraction (LE), sticky cards (SC), sweep
 370 nets (SN) and visual counts (VC). Data was averaged across treatment dates for sticky card, pitfall trap, litter and
 371 soybean and wheat visual count samples; in maize visual counts, each date was analyzed separately due to
 372 differences in sampling methodology. Analysis of variance was used with date and treatment as fixed effects and
 373 location, row (location) and column (location) as random effects. Contrasts were used to compare the fungicide
 374 (FUN), imidacloprid (IMI) and thiamethoxam (THI) treatments to the control (CON), * indicates P<0.05, **
 375 indicates P<0.01, *** indicates P<0.001 and N.S. indicates not significant.

Crop	Guild	Sample Type	Taxon	Treatment Mean ± S.E.			
				CON	FUN	IMI	THI
2015 Full Season Soybean	Predator	VC	Phlaeothripidae	5.5±1.3	5.3±1.4 ^{N.S.}	2.9±1.1*	2.3±0.9*
	Pest	VC	Cicadellidae	3.8±1.5	4.3±0.8 ^{N.S.}	2.6±0.9 ^{N.S.}	0.5±0.3*
			Thripidae	13.0±4.0	13.4±2.7 ^{N.S.}	7.5±1.8 ^{N.S.}	4.3±0.8*
2015-2016 Winter Wheat	Predator	PT	Mesostigmata	5.1±1.3	5.0±1.2 ^{N.S.}	8.0±1.8*	5.9±1.6 ^{N.S.}
		LE	Staphylinidae	2.3±0.5	2.4±0.6 ^{N.S.}	1.0±0.2*	1.3±0.2 ^{N.S.}
	Parasitoid	SC	Aphelinidae	6.1±1.8	5.7±1.3 ^{N.S.}	2.3±0.6*	2.7±0.6 ^{N.S.}
			Braconidae	2.3±0.4	1.9±0.4 ^{N.S.}	1.4±0.3**	2.0±0.3 ^{N.S.}
	Pest	SC	Thripidae	20.8±6.4	17.0±5.3 ^{N.S.}	14.1±4.5**	18.3±8.5*
		VC Fall	Aphididae	5.9±1.2	8.9±2.7	1.0±0.5***	0.8±0.3***
		VC Spring	<i>Oulema melanopus</i>	1.8±0.5	2.4±0.6 ^{N.S.}	3.2±0.7**	2.4±0.9 ^{N.S.}
Other	PT	Acari (Oribatida + Tarsonemidae)	2.8±0.7	4.1±0.8 ^{N.S.}	7.0±1.8**	4.3±0.9 ^{N.S.}	
2016 Double Cropped Soybean	Predator	LE	Staphylinidae	0.9±0.2	1.7±0.3**	1.2±0.3 ^{N.S.}	0.9±0.2 ^{N.S.}
		SN	Araneae	5.5±1.4	3.5±0.4 ^{N.S.}	2.8±0.9*	2.4±0.5**
			Anthocoridae	4.4±1.1	3.9±1.1 ^{N.S.}	1.1±0.4*	0.8±0.4**
			Coccinellidae	1.4±0.7	1.4±0.5 ^{N.S.}	0.1±0.1*	0.4±0.3*
	VC	Phlaeothripidae	7.1±1.5	3.9±0.5 ^{N.S.}	5.0±0.9 ^{N.S.}	3.9±1.6**	
	Parasitoid	PT	Scelionidae	3.5±0.5	3.0±0.5 ^{N.S.}	2.1±0.4*	3.0±0.5 ^{N.S.}
		SN	Braconidae	1.8±0.5	0.9±0.5 ^{N.S.}	0.6±0.5**	1.5±0.7 ^{N.S.}
	Pest	SC	Thripidae	14.6±3.0	9.7±2.2*	14.1±3.4 ^{N.S.}	11.9±3.8 ^{N.S.}
	Other	LE	Collembola	53.3±12.0	78.8±15.3 ^{N.S.}	87.6±17.0*	75.2±13.3 ^{N.S.}
SC		Chloropidae	58.3±10.5	40.1±7.7*	39.0±7.4**	52.2±12.4 ^{N.S.}	
2017 Maize	Predator	PT	Araneae	7.0±1.8	4.4±1.0 ^{N.S.}	3.1±0.5**	3.0±0.4**
		VC July	Araneae	8.3±1.6	11.9±2.2 ^{N.S.}	13.8±1.6**	14.5±1.9***
			Chrysopidae	11.0±1.6	9.0±1.5**	8.6±2.2 ^{N.S.}	10.9±2.5 ^{N.S.}
	Pest	SC	Alticini	2.5±0.7	1.4±0.5*	0.9±0.3**	0.6±0.1**
	Other	PT	Acari	7.8±2.1	13.9±6.1 ^{N.S.}	16.0±4.9**	11.6±4.6 ^{N.S.}
SC		Phalacridae	1.9±0.4	1.6±0.3 ^{N.S.}	1.2±0.3*	1.2±0.3*	

376

377

378 *2016 Double-cropped soybean*: In litter extractions, rove beetles (Staphylinidae, $F_{3,77}=3.29$,
379 $P=0.025$) were more abundant in the fungicide treatment compared to the control, and more
380 collembola ($F_{3,77}=2.29$, $P=0.085$) were extracted from the litter in the imidacloprid treatment
381 compared to the control (Table 2). Fewer scelionid ($F_{3,77}=2.33$ $P=0.081$) parasitoid wasps were
382 pitfall trapped and fewer braconid wasps ($F_{3,15}=4.08$, $P=0.026$) were captured in sweep net
383 samples within imidacloprid treated plots. Visual scouting detected fewer predatory thrips
384 (Phlaeothripidae, $F_{3,46}=3.04$, $P=0.038$) within thiamethoxam treated plots. Spiders (Araneae,
385 $F_{3,15}=4.09$, $P=0.026$), minute pirate bugs (Anthocoridae, $F_{3,77}=5.58$, $P=0.009$), and lady beetles
386 (Coccinellidae, $F_{3,15}=4.24$, $P=0.023$) were suppressed in sweep net samples from both insecticide
387 treatments. Phytophagous thrips (Thripidae, $F_{3,77}=3.36$, $P=0.024$) sticky card captures were
388 reduced in the fungicide treatment. Dark-winged fungus gnat (Sciaridae, $F_{3,61}=2.54$, $P=0.064$
389 sticky card captures were lower in the imidacloprid treatment, with fewer grass flies
390 (Chloropidae, $F_{3,61}=3.54$, $P=0.020$) in the fungicide and imidacloprid treatments.

391 *2017 Maize*: Similar to winter wheat, more saproverous mites (Acari - Oribatida + Tarsonemidae,
392 $F_{3,77}=2.94$, $P=0.038$) were captured in imidacloprid treated pitfall traps (Table 2). Higher
393 abundances of spiders (Araneae, $F_{3,15}=16.79$, $P<0.001$) were detected with visual sampling in
394 both insecticide treated plots, in contrast to the fewer spiders ($F_{3,77}=3.55$, $P=0.018$) that were
395 captured in pitfall traps. Green lacewings (Chrysopidae, $F_{3,15}=3.69$, $P=0.036$) were less abundant
396 in fungicide visual samples. All pesticide treatments captured fewer flea beetles (Chrysomelidae
397 - Alticini, $F_{3,77}=4.64$, $P=0.005$) on sticky cards than the control, and fewer shining flower beetles
398 (Phalacridae, $F_{3,77}=2.84$, $P=0.040$) were collected in both insecticide treatments.

399

400

401 *3.3 Crop sampling*

402 To evaluate treatment impacts on plant growth rates and health, plant height, stand count and
403 yield were measured in all the crops, with NDVI and the number of tillers also measured in
404 wheat (Table 3). Stand count was improved in imidacloprid treated plots compared to the control
405 in full-season soybean ($F_{3,15}=15.11$, $P<0.001$) and in both insecticide treatments in maize
406 ($F_{3,15}=6.65$, $P=0.005$). The plant height was also greater in all three pesticide treatments
407 compared to the control in maize ($F_{3,15}=4.18$, $P=0.011$). Plant height, NDVI and tiller counts in
408 wheat were not impacted by the treatments, but stand count was lower in the thiamethoxam
409 treatment than the control ($F_{3,46}=2.32$, $P=0.087$). In wheat, the fungicide and imidacloprid
410 treatments increased yield in comparison to the control ($F_{3,15}=5.16$, $P=0.012$), but yield benefits
411 were not observed in any other crop.

412

413

414

415

416

417

418

419 Table 3. The effect of seed treatments on plant health parameters and yield for each crop. Analysis of variance was
 420 used with treatment as a fixed effect and location, row (location) and column (location) as random effects. † and ^
 421 represent cases with two and four sampling dates, respectively. In these cases, treatment and date were used as fixed
 422 effects. For effect differences of $P < 0.10$, contrasts were used to compare the fungicide (FUN), imidacloprid (IMI)
 423 and thiamethoxam (THI) treatments to the control (CON). * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates
 424 $P < 0.001$ and N.S. indicates not significant. Results where contrasts were performed are bolded.

425

Metric	Treatment Mean \pm S.E.				D.F	Treatment F-value, P-value
	CON	FUN	IMI	THI		
<i>2015 Full-season Soybean</i>						
Stand Count (plants 2m ⁻¹)	15.5\pm1.4	16.8\pm1.4 N.S.	20.2\pm1.6 ***	15.3\pm1.6 N.S.	3,15	15.11, <0.001
Plant Height (cm)	26.4 \pm 1.0	26.7 \pm 0.9	27.8 \pm 1.2	27.6 \pm 0.8	3,15	0.78, 0.524
Yield (kg ha ⁻¹)	3901 \pm 212	4113 \pm 198	4129 \pm 180	3974 \pm 210	3,15	0.63, 0.609
<i>2015 – 2016 Winter Wheat</i>						
Stand Count (plants m ⁻¹) †	42.0\pm2.6	40.0\pm3.4 N.S.	42.0\pm2.5 N.S.	38.3\pm2.5 *	3,46	2.32, 0.087
Plant Height (cm)	13.5 \pm 0.4	13.0 \pm 0.2	13.5 \pm 0.3	13.1 \pm 0.3	3,15	1.42, 0.276
No. of Tillers (tillers 0.9m ⁻¹) †	137 \pm 4	147 \pm 8	132 \pm 6	134 \pm 7	3,46	1.33, 0.275
NDVI ^	0.40 \pm 0.01	0.40 \pm 0.01	0.40 \pm 0.01	0.40 \pm 0.01	3,108	0.06, 0.981
Yield (kg ha ⁻¹)	2845\pm277	3373\pm201 *	3584\pm213 **	3383\pm389 N.S.	3,15	5.16, 0.012
<i>2016 Double-cropped Soybean</i>						
Stand Count (plants 2m ⁻¹) †	16.6 \pm 0.7	17.9 \pm 0.7	16.7 \pm 0.7	18.0 \pm 0.6	3,46	2.01, 0.126
Plant Height (cm)	52.0 \pm 3.0	56.4 \pm 1.6	55.4 \pm 3.0	54.6 \pm 3.6	3,15	1.22, 0.336
Yield (kg ha ⁻¹)	3068 \pm 184	3165 \pm 179	3203 \pm 169	3148 \pm 178	3,15	0.31, 0.821
<i>2017 Maize</i>						
Stand Count (plants 2m ⁻¹)	11.6\pm0.5	11.8\pm0.4 N.S.	12.2\pm0.4 **	12.0\pm0.5 **	3,15	6.65, 0.005
Plant Height (cm) †	29.4\pm4.0	31.2\pm4.0 *	31.9\pm4.2 **	31.5 \pm 4.2 *	3,46	4.18, 0.011
Yield (kg ha ⁻¹)	8850 \pm 485	9595 \pm 204	9583 \pm 711	9502 \pm 520	3,15	0.58, 0.640

426

427 4. Discussion

428 We conducted a three-year field study evaluating pesticide seed treatment impacts in a full-
 429 season soybean, winter wheat, double-cropped soybean and maize rotation. Our specific goals
 430 were to quantify neonicotinoid residues in the soil and in winter annual flowers, which underlies
 431 the magnitude of non-target impacts on the arthropod community. We also quantified benefits to
 432 plant growth and yield to determine whether treatments were economically justified. Trace
 433 amounts of insecticide were present in one winter annual species in one site year, which did not
 434 correspond with our treatments. Low levels of insecticide residues were present in the soil, with
 435 the highest levels observed in the final year, suggesting some accumulation. Pesticide seed
 436 treatments variably impacted the arthropod community throughout the study. Shannon diversity

437 indices, taxa evenness, and PRC analyses demonstrated occasional community disturbances of a
438 relatively small magnitude, and pesticide seed treatments also impacted individual taxa.
439 However, there was little consistency between crops and sampling methods. Overall, the
440 imidacloprid treatment had stronger impacts than the thiamethoxam treatment, and the fungicide
441 only treatment also occasionally impacted arthropod communities. Pest pressure was very low
442 throughout the study, and while the treatments occasionally improved early season plant growth,
443 we only observed yield benefits in winter wheat, which we attributed to the fungicide component
444 since it occurred across pesticide treatments.

445 *4.1 Environmental persistence and routes of exposure to neonicotinoid residues*

446 *4.1.1 Persistence in plants*

447 Neonicotinoid insecticides are systemic (Nauen et al., 2008), with active ingredient taken up by
448 crop plants and distributed throughout their tissues (Alford & Krupke, 2017; Myers & Hill,
449 2014). In maize and soybean, neonicotinoid seed treatment active ingredients only remain active
450 in plant tissue for three to four weeks post planting (Alford & Krupke, 2017; Myers & Hill,
451 2014). In fall planted winter wheat, the exact activity period is unknown; Zhang et al. (2016)
452 found trace amounts of imidacloprid and clothianidin in seed treated winter wheat up to 200 days
453 after planting and observed successful control of cereal aphids throughout the growing period.
454 The presence of insecticide in plant tissue over a longer period could be a source of exposure for
455 non-target beneficials arthropods such as lady beetles and minute pirate bugs that supplement
456 their diet with plant material, or parasitoids that rely on nectar as a food source (Gontijo et al.,
457 2015; Moscardini et al., 2014; Moser & Obrycki, 2009). Neonicotinoids could remain active for
458 much longer in winter wheat than in maize and soybean, because of low temperatures and plant
459 dormancy during the winter and early spring.

460 Neonicotinoid residues can also be taken up from the soil by non-target plants, such as
461 wildflowers and inter-seeded cover crops (Botías et al., 2015; Bredeson & Lundgren, 2019;
462 Krupke, Hunt, Eitzer, Andino, & Given, 2012; Pecenka & Lundgren, 2015); these are important
463 resources for pollinators, and could be another source of neonicotinoid exposure (Bretagnolle &
464 Gaba, 2015; Mandelik, Winfree, Neeson, & Kremen, 2016). Since these non-target plants were
465 sampled during peak planting and crop production seasons, aerial deposition cannot be separated
466 from uptake. To mitigate this issue, we sampled in late winter. Trace levels of imidacloprid were
467 present in *S. media* flower samples at Beltsville in 2017. Neonicotinoid levels were below the
468 detection threshold for our analysis (5 ppb) and did not correspond with our treatments. Previous
469 studies quantifying residues within non-target plants often detected levels of less than 5 ppb
470 (Bredeson & Lundgren, 2019; Pecenka & Lundgren, 2015); therefore, despite low soil residues,
471 winter annual flowers may uptake small amounts of active ingredient.

472 *4.1.2 Persistence in soil*

473 In soil, the half-life of neonicotinoids can vary greatly, ranging from 28-1250 days for
474 imidacloprid, and 7-353 days for thiamethoxam (Goulson, 2013), with temperature, sunlight, and
475 soil texture, organic matter and moisture content impacting persistence (Bonmatin et al., 2015).
476 Persistence in soil also varies by the amount of active ingredient used, which can differ greatly
477 between crops due to different treatment and seeding rates. We did not detect high levels of
478 neonicotinoid residues in the soil, but the highest levels of both insecticides were observed after
479 2017 maize planting, suggesting the possibility of some accumulation across crops, as
480 hypothesized. This was further supported by higher imidacloprid levels in imidacloprid treated
481 plots than surrounding plots prior to 2017 maize planting at Beltsville. Overall, imidacloprid was
482 detected more often than thiamethoxam, with some detected before the start of the study at

483 Beltsville, even though imidacloprid was not used in that field the previous year. This difference
484 in soil persistence is likely due to imidacloprid's longer half-life. High moisture content,
485 temperature and sunlight are all positively correlated with neonicotinoid breakdown, and
486 thiamethoxam and imidacloprid also have high leaching potential (Bonmatin et al., 2015). Given
487 the high summer temperatures and precipitation in Maryland, the low levels of neonicotinoid
488 residues in our plots could be caused by rapid microbial and photolytic breakdown of residues, or
489 by leaching and run-off.

490 *4.2 Non-target impacts of NSTs on arthropods*

491 *4.2.1 Impacts on communities over time*

492 The arthropod community was impacted variably in different crops and sampling types. Pitfall
493 trap data did not show community disturbance through PRC analysis, diversity, or evenness
494 metrics. Litter communities exhibited lower diversity in both insecticide treatments and lower
495 evenness in the imidacloprid treatment before planting maize in 2017, as well as lower diversity
496 in the imidacloprid treatment and lower evenness in all three pesticide treatments after planting
497 maize. Our hypothesis that the soil community would experience the strongest impacts was not
498 supported, as we generally observed a greater impact on foliar taxa. However, the results for the
499 litter community provide some support for the hypothesis that the soil community would
500 experience greater disturbance later in the study due to accumulation of active ingredients in the
501 soil and litter. Peck (2009) used pitfall traps and soil core extractions to measure the impact of
502 repeated imidacloprid applications in turf, and found that only taxa extracted from soil cores
503 were impacted, with reduced abundance of collembola, true bugs, ground beetles and rove
504 beetles. Conversely, Disque et al. (2018) observed deviation from the control in PRC analysis of
505 pitfall data but not litter data from clothianidin treated maize. While litter or soil core extractions

506 are absolute samples of the community at a given time and place, pitfall traps measure activity
507 density; therefore, these sampling methods may vary in their effectiveness in capturing treatment
508 impacts.

509 Sticky card, sweep net, and visual sample data exhibited pesticide treatment impacts on
510 the arthropod community. The PRC analysis for sticky cards showed increasing disturbance over
511 time in both winter wheat and maize, with no recovery over the sampling period. Given the short
512 period of neonicotinoid activity in crop plants, we expected sticky card and foliar communities to
513 recover, as observed by Disque et al. (2018) in maize; our results suggest that NSTs may have
514 longer term effects on sticky card arthropod communities than previously observed. Redundancy
515 analysis of sweep net data demonstrated community disturbances in 2016 double-cropped
516 soybean but not 2015 full-season soybean. In 2016, diversity was lower in the thiamethoxam
517 treatment than the control. Taxon diversity and community evenness are both correlated with
518 lowered pest pressure (Lundgren & Fausti, 2015); by reducing diversity and evenness, NSTs
519 could interfere with natural pest control. We did not observe increases in pest abundances that
520 would suggest a breakdown of natural pest control, except for Cereal leaf beetle *Oulema*
521 *melanopus* in imidacloprid treated wheat, which did not correspond with reductions in diversity
522 and evenness. Instead, treatments may have impacted control of Cereal leaf beetle by natural
523 enemies. For example, neonicotinoids could reduce the presence of alternate prey for natural
524 enemies, preventing their population from building up sufficiently to control Cereal leaf beetle
525 (Yoo & O'Neil, 2009), or exposure to neonicotinoids could reduce the ability of parasitoid wasps
526 to parasitize hosts (Rogers & Potter, 2003).

527

528

529 *4.2.2. Impacts on individual taxa within a crop*

530 Through the different crops and sampling methods, we observed impacts on taxa that fill various
531 ecological roles in the agricultural arthropod community. The soil community was dominated by
532 mites and collembola, which demonstrated numerically higher abundances in the pesticide
533 treatments throughout the study. Previous studies have found that neonicotinoids can have both
534 positive and negative impacts on collembola (Peck, 2009; Zaller et al., 2016) and can stimulate
535 fecundity in mites (Pisa et al., 2015). Disque et al. (2018) also observed increased collembola
536 and mite activity density in a similar study conducted with clothianidin treated maize, which
537 could be attributed to a trophic cascade caused by natural enemy suppression, or changes in the
538 fungal community caused by the fungicide components. Both soil mites and collembola play a
539 key role in breaking down soil organic matter (Crossley, Mueller, & Perdue, 1992), and by
540 altering the abundance or activity of these organisms, NSTs could further disturb
541 agroecosystems.

542 In pitfall and litter samples, we observed suppression of two prominent groups of
543 generalist predators in insecticide treated crops, rove beetles and spiders. Spider abundance was
544 also reduced in both insecticide treatments in double-cropped soybean sweep net samples. The
545 low toxicity of neonicotinoids towards arachnids (Douglas & Tooker, 2016) suggests that
546 changes in spider density were driven by prey scarcity or other community interactions. In
547 contrast, spider abundance was higher in the maize visual samples from the insecticide treated
548 plots. These contradictory results could be due to variation in behavioral responses to the active
549 ingredients; Easton and Goulson (2013) found that spiders were attracted to low doses of
550 imidacloprid, but were repelled by a high dose. In sweep net and visual samples, we observed
551 reduced abundance or activity density of various predators that have previously been shown to be

552 impacted by NSTs, such as minute pirate bugs (Anthocoridae), lady beetles (Coccinellidae), and
553 predatory thrips (Phlaeothripidae) (Albajes, López, & Pons, 2003; Amjad, Azam, Sarwar, Malik,
554 & Sattar, 2018; Disque et al., 2018; Gontijo et al., 2015; Seagraves & Lundgren, 2012; Zhang et
555 al., 2016). The imidacloprid treatment also suppressed aphelinid and braconid wasps captured on
556 sticky cards in winter wheat, and braconid wasps in double cropped soybean sweep net samples.
557 Parasitoids are especially important for controlling cereal aphids in wheat (Schmidt et al., 2003),
558 therefore suppression of aphid natural enemies could have economic implications.

559 *4.2.3 Potential for sublethal impacts*

560 While we only measured abundance or activity density, neonicotinoids can negatively impact
561 behavior, condition, reproductive success and survival of non-target arthropods (Main, Webb,
562 Goyne, & Mengel, 2018). Examples of sublethal impacts on predatory taxa include reduced
563 survival, longevity and oviposition in lady beetles (Papachristos & Milonas, 2008), delayed
564 development and reduced fecundity and survival in minute pirate bugs (Gontijo et al., 2015), and
565 paralysis, impaired walking, and increased grooming in ground beetles (Kunkel, Held, & Potter,
566 2001). In parasitoids, exposure to sublethal doses of neonicotinoids can decrease longevity,
567 disrupt courtship behavior, impair mate and host-finding ability, as well as reduce parasitism
568 rates and the ratio of female offspring (Frewin, Schaafsma, & Hallett, 2014; Moscardini et al.,
569 2014; Stapel, Cortesero, & Lewis, 2000; Tappert, Pokorny, Hofferberth, & Ruther, 2017). Such
570 sublethal effects may have contributed to the patterns observed in our study. The increasing
571 disruption of the community over time that occurred in wheat and maize sticky cards could be
572 due to the pesticides reducing reproductive success, which would emerge over multiple
573 generations.

574

575 4.2.3 Differences between pesticides

576 In addition to impacts due to both insecticide treatments, there were several instances where only
577 the imidacloprid treatment exhibited impacts. For example, thiamethoxam did not affect
578 parasitoids, while imidacloprid reduced braconid, aphelinid and scelionid wasps. Burgess and
579 King (2015) previously found that the LC₅₀ of imidacloprid was 4.5 times lower than that of
580 thiamethoxam for a parasitoid of houseflies. Imidacloprid has a longer half-life and is an older
581 chemical than thiamethoxam (Bonmatin et al., 2015; Simon-Delso et al., 2015), which could
582 explain its greater non-target impacts.

583 We included a fungicide only treatment in order to isolate effects of seed applied
584 fungicides, and the results supported our hypothesis that these fungicides would impact the
585 arthropod community. For example, in double-cropped soybean, the fungicide only treatment
586 solely reduced phytophagous thrips abundance on sticky cards and increased abundance of rove
587 beetles in litter samples. Additionally, the fungicide only treatment exhibited similar impacts to
588 one or both insecticide + fungicide treatments in certain cases, such as similar deviations from
589 the control in several PRC analyses. To the best of our knowledge, few studies have evaluated
590 the persistence of seed applied fungicides in agroecosystems, or their impact on the arthropod
591 community, even though they can be moderately toxic to arthropods (MDA, 2012). Both
592 fungicide and insecticide seed treatments decreased earthworm surface activity and increased
593 collembola surface activity in wheat (Van Hoesel et al., 2017; Zaller et al., 2016). Given that the
594 fungicide treatments consist of several active ingredients, those ingredients could interact
595 synergistically with each other or with the insecticides to impact the arthropod community. The
596 effects of fungicides on arthropod health have been investigated in pollinators; clothianidin can
597 synergistically interact with the fungicide propiconazole leading to higher mortality in multiple

598 bee species (Sgolastra et al., 2017). In addition, fungicides could alter arthropod abundance by
599 interfering with entomopathogenic fungi, thereby altering disease pressure (Lagnaoui &
600 Radcliffe, 2009). In our study, the soil community was dominated by fungivore taxa (mites and
601 collembola). Therefore, fungicides could also affect arthropods through changes in fungal
602 diversity and abundance, impacting resources available for fungivores. Regardless of the
603 mechanism, our results clearly demonstrate that seed applied fungicides can disrupt arthropod
604 communities in agroecosystems.

605 *4.3 Economic impacts*

606 Although NSTs suppressed pests throughout the study, namely thrips (Thripidae) and
607 leafhoppers (Cicadellidae) in soybean, aphids (Aphididae) in early season wheat, and flea beetles
608 (Chrysomelidae – Alticini) in maize, these pests were not present at economically damaging
609 levels. As we predicted, the insecticides did not improve yield through pest suppression. The
610 insecticide treatments improved early season stand density and plant height, but these benefits
611 did not translate to yield increases. In wheat, the fungicide and imidacloprid treatments
612 significantly increased yield, which was also numerically higher in the thiamethoxam treatment.
613 Given that yield improvements occurred across the pesticide treatments, it was likely a result of
614 the fungicide products and could be achieved without insecticides. Our results are consistent with
615 several previous findings that NSTs may not provide economic benefits in the absence of early
616 season pest pressure (Cox et al., 2007; Myers & Hill, 2014; Wilde et al., 2007). This suggests
617 that the use of NSTs in Maryland grain production may not be warranted outside of specific
618 instances of high pest pressure.

619

620 **5. Conclusions and Future Directions**

621 We found that NSTs can disturb arthropod communities in Maryland grain systems, despite low
622 levels of neonicotinoid residues in the agroecosystem, and the communities occasionally were
623 unable to recover by the end of the sampling period. While we observed treatment impacts on
624 diversity metrics as well as individual taxa abundances, results were inconsistent between crops
625 and sampling methods, making it difficult to elucidate the underlying mechanisms. The
626 persistence of neonicotinoids in the environment can vary greatly, and the effects on the
627 community in our study suggest that areas with greater neonicotinoid persistence and
628 accumulation could experience much stronger disturbances. This study is among the first to
629 document disruption of arthropod communities by seed applied fungicides that are used with
630 NSTs, which have received little attention with regards to their impacts on arthropods. Given
631 their inclusion in all commercial neonicotinoid seed treatments, there is an urgent need to further
632 understand their role. Without a corresponding increase in pest pressure (Douglas & Tooker,
633 2015), NST treated maize and soybean acreage has increased, with many of these acres not
634 previously treated with insecticides. Between 2011 and 2014, the overall quantity of
635 neonicotinoids applied to maize also doubled, indicating an increase in the rate of products used
636 (Tooker, Douglas, & Krupke, 2017). Despite minimal or no benefits in many cases, NST use has
637 continued to grow. Unfortunately, there is little availability of maize without NSTs in the US,
638 leaving farmers with limited choices (Alford & Krupke, 2017). Given the levels of NST
639 contamination in the environment and the impacts on non-target arthropod communities, tactics
640 must be developed to minimize overuse.

641

642

643 **6. Author Contributions**

644 A.D., G.D., and K.H. developed and carried out the study, conducted data analysis and wrote the
645 manuscript. M.L. participated in data collection and manuscript preparation.

646 **7. Acknowledgements**

647 This project was supported by the Hatch/Multistate funds [project no. MD-ENTM-
648 8887/project accession no. 1009567 and project no. MD-ENTO-9589/project accession no.
649 1012455] from the USDA National Institute of Food and Agriculture, by USDA NIFA award
650 number 2015-38640- 23777 through the North East SARE program under sub-award number
651 GNE16- 11B-29994, and by the Maryland Grain Producers Utilization Board and the Maryland
652 Soybean Board.

653 Outreach efforts and publication costs associated with this project were supported by the Crop
654 Protection and Pest Management Program [grant no. 2017-70006-27171/project accession no.
655 1013913] from the USDA National Institute of Food and Agriculture. Opinions, findings,
656 conclusions, or recommendations expressed in this publication are those of the author(s) and do
657 not necessarily reflect the view of these organizations.

658 We would like to thank Kevin Conover, John Draper, and the staff at the Central Maryland and
659 Wye Research and Education Centers for planting and maintaining our study plots, and Terry
660 Patton, Matt Dimmock, Robert Starkenburg and the other members of the Hamby and Dively
661 Labs who contributed to this project. We would also like to thank Stephanie Yarwood, Daniel
662 Gruner, and Robert Kratochvil for their constructive reviews.

663

664 8. Data Accessibility

665 Data will be made available through the Dryad Digital Repository.

666 9. References

- 667 Albajes, R., López, C., & Pons, X. (2003). Predatory fauna in cornfields and response to
668 imidacloprid seed treatment. *Journal of Economic Entomology*, 96(6), 1805–1813.
- 669 Alford, A., & Krupke, C. H. (2017). Translocation of the neonicotinoid seed treatment
670 clothianidin in maize. *PLoS ONE*, 12(3), 1–19. doi: 10.1371/journal.pone.0173836
- 671 Amjad, A., Azam, I., Sarwar, M. K., Malik, M. F., & Sattar, A. (2018). A review of imidacloprid
672 toxicity in coccinellids. *Arthropods*, 7(1), 1–10.
- 673 Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreuzweiser, D. P., Krupke, C., ...
674 Tapparo, A. (2015). Environmental fate and exposure; neonicotinoids and fipronil.
675 *Environmental Science and Pollution Research*, 22(1), 35–67.
- 676 Botias, C., David, A., Hill, E., & Goulson, D. (2016). Contamination of wild plants near
677 neonicotinoid seed-treated crops, and implications for non-target invertebrates. *Science*
678 *of the Total Environment*, 566–567, 269–278.
- 679 Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., & Goulson, D. (2015).
680 Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees.
681 *Environmental Science and Technology*, 49(21), 12731–12740.
- 682 Bredeson, M. M., & Lundgren, J. G. (2019). Neonicotinoid insecticidal seed-treatment on corn
683 contaminates interseeded cover crops intended as habitat for beneficial insects.
684 *Ecotoxicology*, 28(2), 222–228.
- 685 Bretagnolle, V., & Gaba, S. (2015). Weeds for bees? A review. *Agronomy for Sustainable*
686 *Development*, 35(3), 891–909.
- 687 Burgess, E. R., & King, B. H. (2015). Compatibility of the parasitoid wasp *Spalangia endius*
688 (Hymenoptera: Pteromalidae) and insecticides against *Musca domestica* (Diptera:
689 Muscidae) as evaluated by a new index. *Journal of Economic Entomology*, 108(3), 986–
690 992.
- 691 Cox, W. J., Cherney, J. H., & Shields, E. (2007). Clothianidin seed treatments inconsistently
692 affect corn forage yield when following soybean. *Agronomy Journal*, 99(2), 543–548.
- 693 Crossley, D. A., Mueller, B. R., & Perdue, J. C. (1992). Biodiversity of microarthropods in
694 agricultural soils: relations to processes. *Agriculture, Ecosystems and Environment*, 40,
695 37–46.

- 696 Disque, H. H., Hamby, K. A., Dubey, A., Taylor, C., & Dively, G. P. (2018). Effects of
697 clothianidin-treated seed on the arthropod community in a mid-Atlantic no-till corn
698 agroecosystem. *Pest Management Science*, 75, 969–978
- 699 Douglas, M. R., Rohr, J. R., & Tooker, J. F. (2015). Neonicotinoid insecticide travels through a
700 soil food chain, disrupting biological control of non-target pests and decreasing soya bean
701 yield. *Journal of Applied Ecology*, 52(1), 250–260.
- 702 Douglas, M. R., & Tooker, J. F. (2015). Large-scale deployment of seed treatments has driven
703 rapid increase in use of neonicotinoid insecticides and preemptive pest management in
704 U.S. field crops. *Environmental Science and Technology*, 49(8), 5088–5097.
- 705 Douglas, M. R., & Tooker, J. F. (2016). Meta-analysis reveals that seed-applied neonicotinoids
706 and pyrethroids have similar negative effects on abundance of arthropod natural enemies.
707 *PeerJ*, 4, e2776.
- 708 Easton, A. H., & Goulson, D. (2013). The Neonicotinoid insecticide imidacloprid repels
709 pollinating flies and beetles at field-realistic concentrations. *PLoS ONE*, 8(1), 8–11.
- 710 Erdle, K., Mistele, B., & Schmidhalter, U. (2011). Comparison of active and passive spectral
711 sensors in discriminating biomass parameters and nitrogen status in wheat cultivars. *Field
712 Crops Research*, 124(1), 74–84.
- 713 Frewin, A. J., Schaafsma, A. W., & Hallett, R. H. (2014). Susceptibility of *Aphelinus certus*
714 (Hymenoptera: Aphelinidae) to neonicotinoid seed treatments used for soybean pest
715 management. *Journal of Economic Entomology*, 107(4), 1450–1457.
- 716 Godfray, H. C. J., Blacquière, T., Field, L. M., Hails, R. S., Petrokofsky, G., Potts, S. G., ...
717 McLean, A. R. (2014). A restatement of the natural science evidence base concerning
718 neonicotinoid insecticides and insect pollinators. *Proceedings of the Royal Society B:
719 Biological Sciences*, 281(1786), 20140558.
- 720 Gontijo, P. C., Moscardini, V. F., Michaud, J., & Carvalho, G. A. (2015). Non-target effects of
721 two sunflower seed treatments on *Orius insidiosus* (Hemiptera: Anthocoridae). *Pest
722 Management Science*, 71(4), 515–522.
- 723 Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides.
724 *Journal of Applied Ecology*, 50(4), 977–987.
- 725 Hallmann, C. A., Foppen, R. P. B., Turnhout, C. A. M. Van, Kroon, H. De, Jongejans, E., van
726 Turnhout, C. A. M., ... Jongejans, E. (2014). Declines in insectivorous birds are
727 associated with high neonicotinoid concentrations. *Nature*, 511(7509), 341–343.
- 728 Khani, A., Ahmadi, F., & Ghadamyari, M. (2012). Side effects of imidacloprid and abamectin on
729 the mealybug destroyer *Cryptolaemus montrouzieri*. *Trakia Journal of Sciences*, 10(3),
730 30–35.
- 731 Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G., & Given, K. (2012). Multiple routes of
732 pesticide exposure for honey bees living near agricultural fields. *PLoS ONE*, 7(1).

- 733 Kunkel, B. A., Held, D. W., & Potter, D. A. (2001). Lethal and sublethal effects of bendiocarb,
734 halofenozide, and imidacloprid on *Harpalus pennsylvanicus* (Coleoptera: Carabidae)
735 following different modes of exposure in turfgrass. *Journal of Economic Entomology*,
736 94(1), 60–67.
- 737 Lagnaoui, A., & Radcliffe, E. B. (2009). Potato fungicides interfere with entomopathogenic
738 fungi impacting population dynamics of green peach aphid. *American Journal of Potato*
739 *Research*, 75(1), 19–25.
- 740 Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a
741 rainforest food web. *Proceedings of the National Academy of Sciences*, 115(44),
742 E10397–E10406.
- 743 Lundgren, J. G., & Fausti, S. W. (2015). Trading biodiversity for pest problems. *Scientific*
744 *Advancements*, 1, e1500558.
- 745 Main, A. R., Webb, E. B., Goyne, K. W., & Mengel, D. (2018). Neonicotinoid insecticides
746 negatively affect performance measures of non-target terrestrial arthropods: a meta-
747 analysis. *Ecological Applications*, 28(5), 1232–1244.
- 748 Mandelik, Y., Winfree, R., Neeson, T., & Kremen, C. (2016). Complementary habitat use by
749 wild bees in agro-natural landscapes. 22(5), 1535–1546.
- 750 Minnesota Department of Agriculture (2012). Sedaxane. Retrieved from New Active Ingredient
751 Review website: [https://www.mda.state.mn.us/sites/default/files/inline-files/nair-](https://www.mda.state.mn.us/sites/default/files/inline-files/nair-sedaxane.pdf)
752 [sedaxane.pdf](https://www.mda.state.mn.us/sites/default/files/inline-files/nair-sedaxane.pdf)
- 753 Morrissey, C. A., Mineau, P., Devries, J. H., Sanchez-Bayo, F., Liess, M., Cavallaro, M. C., &
754 Liber, K. (2015). Neonicotinoid contamination of global surface waters and associated
755 risk to aquatic invertebrates: A review. *Environment International*, 74, 291–303.
- 756 Moscardini, V. F., Gontijo, P. C., Michaud, J. P., & Carvalho, G. A. (2014). Sublethal effects of
757 chlorantraniliprole and thiamethoxam seed treatments when *Lysiphlebus testaceipes* feed
758 on sunflower extrafloral nectar. *BioControl*, 59(5), 503–511.
- 759 Moser, S. E., & Obrycki, J. J. (2009). Non-target effects of neonicotinoid seed treatments;
760 mortality of coccinellid larvae related to zoophytophagy. *Biological Control*, 51(3), 487–
761 492.
- 762 Mullin, C. A., Saunders, M. C., Leslie, T. W., Biddinger, D. J., & Fleischer, S. J. (2005). Toxic
763 and behavioral effects to Carabidae of seed treatments used on Cry3Bb1- and Cry1Ab/c-
764 protected corn. *Environmental Entomology*, 34(6), 1626–1636.
- 765 Myers, C., & Hill, E. (2014). Benefits of neonicotinoid seed treatments to soybean production.
766 Environmental Protection Agency. Website:
767 [https://www.epa.gov/sites/production/files/2014-](https://www.epa.gov/sites/production/files/2014-10/documents/benefits_of_neonicotinoid_seed_treatments_to_soybean_production_2.pdf)
768 [10/documents/benefits_of_neonicotinoid_seed_treatments_to_soybean_production_2.pdf](https://www.epa.gov/sites/production/files/2014-10/documents/benefits_of_neonicotinoid_seed_treatments_to_soybean_production_2.pdf)

- 769 Nauen, R., Jeschke, P., & Copping, L. (2008). In Focus: Neonicotinoid insecticides. Pest
770 Management Science, 64(11), 1081.
- 771 Papachristos, D. P., & Milonas, P. G. (2008). Adverse effects of soil applied insecticides on the
772 predatory coccinellid *Hippodamia undecimnotata* (Coleoptera: Coccinellidae). Biological
773 Control, 47(1), 77–81.
- 774 Pecenka, J. R., & Lundgren, J. G. (2015). Non-target effects of clothianidin on monarch
775 butterflies. Science of Nature, 102(3–4).
- 776 Peck, D. C. (2009). Long-term effects of imidacloprid on the abundance of surface- and soil-
777 active nontarget fauna in turf. Agricultural and Forest Entomology, 11(4), 405–419.
- 778 Pisa, L. W., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Downs, C. A., Goulson, D.,
779 ... Wiemers, M. (2015). Effects of neonicotinoids and fipronil on non-target
780 invertebrates. Environmental Science and Pollution Research International, 22(1), 68–
781 102.
- 782 Rogers, M. E., & Potter, D. A. (2003). Effects of spring imidacloprid application for white grub
783 control on parasitism of Japanese beetle (Coleoptera: Scarabaeidae) by *Tiphia vernalis*
784 (Hymenoptera: Tiphidae). Journal of Economic Entomology, 96, 1412–1419.
- 785 Schmidt, M. H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M., & Tschardt, T. (2003).
786 Relative importance of predators and parasitoids for cereal aphid control. Proceedings of
787 the Royal Society B: Biological Sciences, 270(1527), 1905–1909.
- 788 Seagraves, M. P., & Lundgren, J. G. (2012). Effects of neonicotinoid seed treatments on soybean
789 aphid and its natural enemies. Journal of Pest Science, 85(1), 125–132.
- 790 Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M. T., Tosi, S., Bogo, G., ... Bosch, J. (2017).
791 Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-
792 inhibiting fungicide in three bee species. Pest Management Science, 73(6), 1236–1243.
- 793 Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs,
794 C., ... Wiemers, M. (2015). Systemic insecticides (Neonicotinoids and fipronil): Trends,
795 uses, mode of action and metabolites. Environmental Science and Pollution Research,
796 22(1), 5–34.
- 797 Stapel, J. O., Cortesero, A. M., & Lewis, W. J. (2000). Disruptive sublethal effects of
798 insecticides on biological control: Altered foraging ability and life span of a parasitoid
799 after feeding on extrafloral nectar of cotton treated with systemic insecticides. Biological
800 Control, 17(3), 243–249.
- 801 Stewart, S. D., Lorenz, G. M., Catchot, A. L., Gore, J., Cook, D., Skinner, J., ... Barber, J.
802 (2014). Potential exposure of pollinators to neonicotinoid insecticides from the use of
803 insecticide seed treatments in the mid-southern United States. Environmental Science and
804 Technology, 48(16), 9762–9769.

- 805 Sur, R., & Stork, A. (2003). Uptake, translocation and metabolism of imidacloprid in plants.
806 *Bulletin of Insectology*, 56(1), 35–40.
- 807 Tappert, L., Pokorny, T., Hofferberth, J., & Ruther, J. (2017). Sublethal doses of imidacloprid
808 disrupt sexual communication and host finding in a parasitoid wasp. *Scientific Reports*, 7,
809 42756.
- 810 Tooker, J. F., Douglas, M. R., & Krupke, C. H. (2017). Neonicotinoid seed treatments:
811 Limitations and compatibility with Integrated Pest Management. *Agricultural and*
812 *Environmental Letters*, 2, 170026.
- 813 Van den Brink, P. J., & Ter Braak J. F., C. (1999). Principal Response Curves: Analysis of time-
814 dependent multivariate responses of biological community to stress. *Environmental*
815 *Toxicology and Chemistry*, 18(2), 138–148.
- 816 Van Hoesel, W., Tiefenbacher, A., König, N., Dorn, V. M., Hagenguth, J. F., Prah, U., ... Zaller,
817 J. G. (2017). Single and combined effects of pesticide seed dressings and herbicides on
818 earthworms, soil microorganisms, and litter decomposition. *Frontiers in Plant Science*, 8,
819 215.
- 820 Wilde, G., Roozeboom, K., Ahmad, A., Claassen, M., Gordon, B., Heer, W., ... Witt, M. (2007).
821 Seed treatment effects on early-season pests of corn and on corn growth and yield in the
822 absence of insect pests. *Journal of Agricultural and Urban Entomology*, 24(4), 177–193.
- 823 Yoo, H. J. S., & O'Neil, R. J. (2009). Temporal relationship between the generalist predator,
824 *Orius insidiosus*, and its two major prey in soybean. *Biological Control*, 48, 168–180.
- 825 Zaller, J. G., König, N., Tiefenbacher, A., Muraoka, Y., Querner, P., Ratzenböck, A., ... Koller,
826 R. (2016). Pesticide seed dressings can affect the activity of various soil organisms and
827 reduce decomposition of plant material. *BMC Ecology*, 16(1), 37.
- 828 Zhang, P., Zhang, X., Zhao, Y., Wei, Y., Mu, W., & Liu, F. (2016). Effects of imidacloprid and
829 clothianidin seed treatments on wheat aphids and their natural enemies on winter wheat.
830 *Pest Management Science*, 72(6), 1141–1149.