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# **RESEARCH ARTICLE**



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# Ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation

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# Abstract

- 1. While many studies have investigated non-target impacts of neonicotinoid seed treatments (NSTs), they usually take place within a single crop and focus on specific pest or beneficial arthropod taxa.
- 2. We compared the impacts of three seed treatments to an untreated control: imidacloprid + fungicide products, thiamethoxam + fungicide products and fungicide products alone in a 3-year crop rotation of full-season soybean, winter wheat, doublecropped soybean and maize. Specifically, we quantified neonicotinoid residues in the soil and in weedy winter annual flower buds and examined treatment impacts on soil and foliar arthropod communities as well as on plant growth and yield.
- 3. Unquantifiably low amounts of insecticide were found in winter annual flowers of one species in one site year, which did not correspond with our treatments. Although low levels of insecticide residues were present in the soil, residues were not persistent. Residues were highest in the final year of the study, suggesting some accumulation.
- 4. We observed variable impacts of NSTs on the arthropod community; principle response curve and redundancy analyses exhibited occasional treatment effects, with treatments impacting the abundance of various taxa, including predators and parasitoids. Overall, foliar taxa were more impacted than soil taxa, and the fungicides occasionally affected communities and individual taxa.
- 5. Pest pressure was low throughout the study, and although pest numbers were reduced by the insecticides, corresponding increases in yield were not observed.
- 6. Synthesis and applications. Pesticide seed treatments can impact arthropod taxa, including important natural enemies even when environmental persistence and active ingredient concentrations are low. The foliar community in winter wheat showed that in some cases, these impacts can last for several months after planting. Given the low pest pressure and lack of yield improvement in full-season soybean, double-cropped soybean, winter wheat and maize, we did not observe benefits that could justify the risks associated with neonicotinoid seed treatment (NST) use. Our results suggest that NSTs are not warranted in Maryland grain production, outside of specific instances of high pest pressure.

### KEYWORDS

fungicide seed treatment, *Glycine max* L. Merr., imidacloprid, neonicotinoid seed treatment, thiamethoxam, *Triticum* spp., *Zea mays* L.

## 1 | INTRODUCTION

Declines in arthropod biomass have been documented at multiple locations and are likely linked to habitat loss, climate change and agrochemical pollutants (Hallmann et al., 2014; Lister & Garcia, 2018). Since their introduction in the 1990s, neonicotinoid insecticides have become the most heavily used insecticide class worldwide, due to their low vertebrate toxicity, systemic nature and versatility of application methods (Nauen, Jeschke, & Copping, 2008). Neonicotinoid seed treatments (NSTs) are especially popular; by 2011, NSTs were used in 79%-100% of maize Zea mays L. and 34%-44% of soybean Glycine max L. Merr. planted in the USA (Douglas & Tooker, 2015). When neonicotinoids are applied as NSTs, less than 20% of the active ingredients are taken up by the plant (Alford & Krupke, 2017; Sur & Stork, 2003), instead largely remaining in the soil, where their environmental fate is not fully understood. The half-lives of neonicotinoids in soil vary considerably and they may persist and accumulate for multiple years post planting (Bonmatin et al., 2015). Due to their water solubility, neonicotinoids can also leach into groundwater and run off into waterbodies; neonicotinoid residues are frequently detected at levels above ecological thresholds in waterbodies that are adjacent to or receive runoff from crop lands (Morrissey et al., 2015). In addition, neonicotinoids may contaminate non-crop plants. Several studies have found neonicotinoid residues in plants growing near treated fields, but it is difficult to determine whether the active ingredients were taken up from the soil or deposited aerially (Basley & Goulson, 2018; Botías et al., 2015; Pecenka & Lundgren, 2015; Stewart et al., 2014). Due to the widespread use, environmental persistence, and mobility of the active ingredients from NSTs they are common pesticide pollutants.

NSTs pollution can negatively impact many non-target organisms. Although NSTs require relatively low active ingredient concentrations and can reduce non-target exposure due to pesticide drift, they have similar impacts on non-target arthropod abundance as soil and foliar pyrethroid applications (Douglas & Tooker, 2016). Beneficial natural enemies may be exposed to NST active ingredients indirectly by consuming herbivores or directly, either through physical contact or by feeding on plant material or nectar (Gontijo, Moscardini, Michaud, & Carvalho, 2015; Khani, Ahmadi, & Ghadamyari, 2012; Moscardini, Gontijo, Michaud, & Carvalho, 2014; Moser & Obrycki, 2009; Papachristos & Milonas, 2008; Seagraves & Lundgren, 2012). For example, the presence of neonicotinoids in the soil can suppress predatory ground beetles (Coleoptera: Carabidae) through direct contact with active ingredients (Pisa et al., 2015; Simon-Delso et al., 2015), or by ingestion of contaminated prey (Douglas, Rohr, & Tooker, 2015). Work characterizing the impact of neonicotinoids typically focuses on specific pest or beneficial taxa; however, the interconnected arthropod community should also be evaluated as a whole. Increased taxon diversity and evenness is associated with reduced pest pressure (Lundgren & Fausti, 2015); therefore, community-level impacts of NSTs could disrupt natural pest control. In maize, clothianidin-treated seed altered the overall arthropod community after planting, with several beneficial predators decreasing in abundance (Disque, Hamby, Dubey, Taylor, & Dively, 2018). Neonicotinoids can also negatively impact pollinators, which exhibit acute toxicity at high doses as well as sublethal impacts such as impaired memory, impaired foraging ability and increased parasite loads (Decourtye et al., 2004; Godfray et al., 2014; Henry et al., 2012; Pettis, Vanengelsdorp, Johnson, & Dively, 2012; Rundlöf et al., 2015; Vidau et al., 2011; Whitehorn, O'Connor, Wackers, & Goulson, 2012). Because pollinators often rely on noncrop floral resources, uptake by non-crop plants may be an important route of exposure (Basley & Goulson, 2018; Botias, David, Hill, & Goulson, 2016; Dively & Kamel, 2012). Given the risks associated with NST pollution, consideration must be given to their use in multiple crops, their potential long-term environmental persistence and their effects on arthropod communities when evaluating non-target impacts.

In addition to the many risks associated with NSTs, they often provide limited benefits. Active ingredients from NSTs generally remain bioactive in plant tissue for 3–4 weeks post planting, so they only provide protection against early season soil and seedling pests (Alford & Krupke, 2017; Myers & Hill, 2014). Additionally, many of the pests targeted by NSTs are sporadic pests that rarely cause economic losses (Papiernik, Sappington, Luttrell, Hesler, & Allen, 2018). NSTs are frequently used prophylactically and growers may not recoup the cost of treatment unless significant early season pest pressure occurs (Cox, Cherney, & Shields, 2007; Myers & Hill, 2014; Wilde et al., 2007). The economic benefits of NSTs vary greatly based on region and cropping system and must be evaluated on a case by case basis (Papiernik et al., 2018).

In this study, we evaluated the impacts of repeated use of two popular NSTs [Gaucho 600 (imidacloprid), and Cruiser 5FS (thiamethoxam)] during a 3-year grain crop rotation common to the mid-Atlantic United States: full-season soybean, winter wheat, double-cropped soybean and maize. Given that NSTs are most commonly used in maize but are less widely used in soybean and wheat, this represents a worst-case scenario where NSTs are used repeatedly in all three crops. Because commercial NSTs always include fungicides in addition to insecticides, we included a fungicide-only treatment as well as an untreated control in order to isolate the impacts of the fungicides from those of the insecticides. To the best of our knowledge, this is among the first studies to quantify the impacts of seed applied fungicides on the arthropod community. The location and concentration of pesticide active ingredients drive non-target effects; therefore, we quantified the persistence of neonicotinoids in the soil and determined whether weedy winter annual flowers uptake residues. We hypothesized that higher levels of neonicotinoid residues would be present in the soil later in the study due to accumulation from multiple crops. Our second objective was to evaluate the impacts of pesticide seed treatments on the overall arthropod community and on individual arthropod taxa. We anticipated the strongest impacts on the soil community, given the potential soil persistence of active ingredients and the short activity period in plant tissue. We expected community disturbance early on with recovery during each cropping cycle as observed previously in maize (Disgue et al., 2018), but hypothesized that disturbance in the soil community would increase over the course of the study due to potential cumulative impacts of repeated NST use. We also hypothesized that the fungicide-only treatment could also impact the arthropod community, due to direct toxicity of seed-applied fungicides towards arthropods (Minnesota Department of Agriculture, 2012) or indirect alteration of crop fungal communities. Our final objective was to measure the economic value of the treatments in terms of plant growth metrics and yield to determine whether the environmental risks of NSTs are justified by economic benefits in mid-Atlantic grain production. We did not expect the insecticide treatment to significantly improve yield because Maryland tends to have low pressure from pests targeted by NSTs; however; neonicotinoids may stimulate plant growth in the absence of pest pressure (Jeschke, Nauen, Schindler, & Elbert, 2010), which could improve growth parameters and yield.

# 2 | MATERIALS AND METHODS

The study was conducted at the Wye Research and Education Center in Queenstown, MD, USA (38°54'02.80"N, 76°08'22.06"W) and the Central Maryland Research and Education Center in Beltsville, MD, USA (39°01'08.11"N, 76°49'25.10"W) and compared treatments over a 3-year rotation of four crops at each site. The four treatments were untreated seeds (control), fungicide products alone (varied by crop; Syngenta), fungicide products + imidacloprid insecticide (Gaucho 600; Bayer Crop Science) and fungicide products + thiamethoxam insecticide (Cruiser<sup>®</sup> 5FS; Syngenta). Full-season soybean was planted in spring 2015, winter wheat in autumn 2015, double-cropped soybean in summer 2016 and maize in spring 2017. At each site, four replicate plots of each treatment measuring 9.1 m × 15.2 m were arranged in a Latin square (Figure S1). The plot rows were separated by rows of untreated grain that provided space for the planter to turn. Plot columns were separated by 0.91 m bare strips to delimit plots and facilitate sampling. To determine cumulative effects of repeated treatments, each treatment replicate was planted in the same location for each crop in the rotation. Standard no-till agronomic practices

for the region were followed throughout, except cover crops were not planted during the study to promote the growth of winter annual plants within the plots. No foliar fungicides or insecticides were applied, with the exception of wheat, where the fungicide Caramba (metconazole; BASF Agricultural) was applied twice during the flowering stage at the Queenstown site to control Fusarium head blight. Weeds were controlled through pre-plant and early season herbicide applications of products including Authority First DF (sulfentrazone, cloransulam-methyl; FMC Corporation), GlyStar Plus (glyphosate; Albaugh, Inc.) and Makaze (glyphosate; Loveland Products). The field at Beltsville was previously planted with untreated soybean and at Queenstown with neonicotinoid seed-treated maize. The seeding rate, variety and active ingredient rate for each treatment and crop are listed in Tables S1 and S2. Due to differences in seeding and application rates, the amount of active ingredient per acre varied slightly between soybean and maize, with wheat concentrations almost double that of the other crops.

# 2.1 | Residue analysis

In spring 2016 and 2017, we collected flower buds from winter annual plants growing within the experimental plots for neonicotinoid residue analysis. Winter annual species were chosen based on abundance and attractiveness to pollinators. In 2016, common henbit *Lamium amplexicaule* L. was collected at Beltsville and common chickweed *Stellaria media* L. Vill. at Queenstown. In 2017, we collected common chickweed at Queenstown and both species at Beltsville. Soil was collected for residue analysis before and shortly after soybean and maize were planted in 2015 and 2017, and in March 2016, while wheat was dormant (see Table 1 for sampling dates). Further details about material collection are included in Section 1.1 of Appendix S1.

Residue samples (3 g per sample for flowers, ~100 g per sample for soil) were sent to the USDA National Science Laboratory (Gastonia, NC, USA) for analysis, where they were tested for imidacloprid, thiamethoxam and clothianidin, another popular neonicotinoid that is also a breakdown product of thiamethoxam (Simon-Delso et al., 2015). Briefly, neonicotinoid residues were extracted with a refined official pesticide extraction method [AOAC OMA 2007.0, the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe)], using an acetonitrile and water solution. Extraction was followed by enhance matrix reduction (EMR) clean-up and analysis using certified standard reference materials and liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS) utilizing the precursor and product ions of analytes of interest. The USDA National Science Laboratory reported detection levels were 1 ppb for imidacloprid, 1 ppb for thiamethoxam and 1 ppb for clothianidin in flowers in 2016, and 10 ppb for imidacloprid, 5 ppb for thiamethoxam and 30 ppb for clothianidin in flowers in 2017. In soil, the USDA National Science Laboratory detection level was 5 ppb for imidacloprid, 10 ppb for thiamethoxam and 15 ppb for clothianidin.

**TABLE 1** Neonicotinoid residues insoil samples collected in 2015, 2016 and2017. The detection level was 5 ppb forimidacloprid, 10 ppb for thiamethoxamand 15 ppb for clothianidin. Nd, notdetected. Trace indicates that theinsecticide was present but at levels belowthe quantification threshold. Pre-plantingdata from Queenstown are not includedfor 2015 soybean or 2017 maize as noinsecticides were detected. For 2015 and2017, the two values indicate data fromthe two pooled replicate samples, while in2016, all the replicates were pooled into asingle sample

		Insecticide residue (ppb)						
Site	Treatment	Imidacloprid	Thiamethoxam	Clothianidin				
Full-season soybean: Pre-plant-12/5/2015 and 21/5/2015								
Beltsville	Control	8, trace	nd, nd	trace, nd				
	Fungicide	6,7	nd, nd	nd, nd				
	Imidacloprid	trace, trace	nd, nd	nd, nd				
	Thiamethoxam	7, 6	nd, nd	trace, nd				
Full-season soybean: Post-plant-3/6/2015 and 12/6/2015								
Beltsville	Control	10, trace	nd, nd	trace, nd				
	Fungicide	trace, 8	nd, nd	nd, nd				
	Imidacloprid	8, trace	nd, trace	nd, nd				
	Thiamethoxam	trace, 8	nd, trace	trace, nd				
Queenstown	Control	nd, nd	nd, nd	nd, nd				
	Fungicide	nd, nd	nd, nd	nd, nd				
	Imidacloprid	trace, nd	nd, nd	nd, nd				
	Thiamethoxam	nd, nd	16, nd	nd, nd				
Winter wheat: Dormancy—2/3/2016 and 7/3/2016								
Beltsville	Control	trace	nd	nd				
	Fungicide	trace	nd	nd				
	Imidacloprid	7	nd	nd				
	Thiamethoxam	trace	nd	nd				
Queenstown	Control	nd	nd	nd				
	Fungicide	nd	nd	nd				
	Imidacloprid	trace	nd	nd				
	Thiamethoxam	nd	nd	nd				
Maize: Pre-plant-10/4/2017 and 12/4/2017								
Beltsville	Control	7, nd	nd, nd	nd nd				
	Fungicide	nd, nd	nd, nd	nd, nd				
	Imidacloprid	8,9	nd, nd	nd, nd				
	Thiamethoxam	trace, nd	nd, nd	nd, nd				
Maize: Post-plant-30/5/2017 and 31/5/2017								
Beltsville	Control	7, nd	nd, nd	nd, nd				
	Fungicide	trace, nd	nd, nd	nd, nd				
	Imidacloprid	11, 35	nd, nd	nd, nd				
	Thiamethoxam	12, trace	17, nd	23, nd				
Queenstown	Control	nd, nd	nd, nd	nd, nd				
	Fungicide	nd, nd	nd, nd	nd, nd				
	Imidacloprid	14, 26	nd, nd	nd, nd				
	Thiamethoxam	nd, nd	15, 16	nd, nd				

# 2.2 | Arthropod sampling

Throughout the study the epigeal and soil invertebrate community was measured using pitfall traps (3 subsamples per plot) and surface litter extractions (4 subsamples pooled into two Berlese funnel extractions per plot). Samples were collected three times during each growing season. A small number of pitfall traps were lost due to animal activity in the field. However, we successfully collected at least one subsample per plot in each case. Activity density of aerial and foliar arthropods close to the ground was measured through sticky cards (3 subsamples per plot). In soybean, arthropod abundance in the plant canopy was measured by sweep netting, where 15 sweeps were taken in a straight line through the centre of each plot once per season. Samples from one 2015 sweep net imidacloprid replicate at Beltsville and one 2016 sticky card double-cropped soybean sampling date at Queenstown were misplaced prior to processing. We also conducted visual inspections of plants to quantify pest pressure and beneficial arthropods in all crops. Data from subsamples within replicates were averaged for analysis for all sample types. The sampling timeline can be found in Tables S3–S6 and further details can be found in Section 1.2 of Appendix S1.

# 2.3 | Crop sampling

We measured the impact of NSTs on plant growth by recording stand density and plant height in all crops. In wheat, we also counted the number of tillers and measured the normalized difference vegetative index (NDVI), which can be used to indirectly measure crop biomass (Erdle, Mistele, & Schmidhalter, 2011). These metrics were included to test manufacturer claims that neonicotinoids can increase plant health and growth even in the absence of insect pests, and determine whether NSTs could be beneficial for Maryland farmers regardless of pest pressure (Jeschke et al., 2010). We also measured yield at the time of harvest. Details for each crop are included in Section 1.3 of Appendix S1.

### 2.4 | Statistical analysis

## 2.4.1 | Arthropod data analysis

For arthropod sampling, taxa were identified to family in most cases, and adults and immatures were combined for all taxa. Insects from the following orders that could not be identified to family were excluded from all analyses: Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera. Ants (Hymenoptera: Formicidae) were excluded from principal response curve (PRC) and individual taxon analyses for sticky cards, pitfall traps and litter due to their highly clumped distribution in the soil, which makes it difficult to correlate their abundance in the soil to treatment effects. However, they were included in the redundancy analysis (RDA) for sweep net sampling which captures activity on the plant.

To characterize the impact of treatment over time, arthropod community composition was analysed in CANOCO 5 (Microcomputer Power) using PRC analysis for pitfall traps, litter extraction and sticky card data for each crop, similar to previous studies (Disque et al., 2018). Briefly, PRC multivariate analysis is based on RDA (Van den Brink & Ter Braak, 1999), with adjustments for the change in community response over time. In our study, total abundances for each taxon were averaged over subsamples within a replicate plot for each site prior to analysis. Taxa where the sum of individuals across sampling dates and sites for a crop was less than one were excluded from the PRC. For each crop and sample type, the date × treatment interaction term was used as an explanatory variable, and date and the site × column interaction were used as covariates to restrict data shuffling due to known spatial variability across columns. Canonical coefficients were generated for each date and plotted over time to evaluate the community response to the treatments relative to the untreated control; the control is plotted along the horizontal axis (representing time), and the magnitude (represented by canonical coefficients plotted on the vertical axis) and shape of curves represent the deviation of treatments from the control. The analysis also generates taxon-specific weights for the individual taxa that exhibit the strongest effects; taxa with high positive weights are more likely to follow the pattern depicted in the PRC, while taxa with high negative weights exhibit an opposite response. A Monte Carlo permutation procedure with N = 499 was used to test the null hypothesis that the canonical coefficients of the treatment response equalled zero for all sampling times, and to calculate a Pseudo-F statistic, as performed in previous studies (Disgue et al., 2018). Due to the sticky cards from the first sampling date at Queenstown being misplaced, only data from Beltsville were included for the first date for double-cropped soybean sticky card PRC. Because sweep net samples were conducted on a single date, captures were analysed using RDA (Van den Brink & Ter Braak, 1999).

The PRC and RDA analyses were followed by analysis of variance of key arthropod taxa (JMP Pro 13.2.1; SAS Institute Inc.) within crops and sample types, which were selected if they met all the following criteria: taxon weight >1 or <-1 in the PRC for at least one crop; total abundance ≥10 individuals across all treatments and sampling dates for that crop and sample type; mean abundance >1 individual per treatment for at least one treatment within that crop and sample type. For each crop and sample type, treatment, site and column (nested within site) were included as fixed effects due to known spatial variability between columns. For pitfall trap, litter extraction and sticky card data collected on multiple dates, mean abundances for each replicate plot from all three sampling dates were summed across dates for analysis. For visual counts, data from multiple sampling dates were summed for double-cropped soybean and wheat; however, in wheat, data from the two winter sampling dates and the three spring/summer sampling dates were summed separately. Visual counts were only conducted once in full-season soybean, while in maize, each date was analysed separately due to variation in sampling methods. Sticky card data from the first sampling date for double-cropped soybean were excluded as samples from one site were misplaced before identification. The assumption of normality was tested using a Shapiro-Wilk test, and data were transformed as necessary. The assumption of homoscedasticity was tested using Levene's test and weighted least squares methods (Weighting factor: [residual variance]<sup>-1</sup> of the fixed effect that most deviated from homoscedasticity) were used when needed. To evaluate effect size, fungicide, imidacloprid and thiamethoxam treatments were compared to the control through a Hedge's g effect test using the cohen.d function (EFFSIZE package; Torchiano, 2019) in the R statistical program (Version 3.5.1; R Core Team, 2018). For pitfall trap, litter, sticky card and sweep net samples, if the ANOVA for a taxon was

significant for any crop, effect sizes were calculated for all crops where that taxon was present, to allow for comparison between crops. This was not done for visual count data as sampling methods and collected taxa were not comparable across crops. Effect sizes were also calculated for collembola and soil mites in pitfall traps and litter data regardless of significance level, as they comprised up to 80% of the total soil arthropod abundance. When reporting data from ANOVAs, data are reported for the treatment effect unless the overall model was not significant, in which case model statistics are reported.

### 2.4.2 | Crop data analysis

Plant height, stand count and yield data were analysed with analysis of variance, using the model and methods described in the previous section for arthropod taxa. For NDVI and tiller counts, date and date × treatment were also included as fixed effects, as data were collected on multiple sampling dates. The date × treatment was dropped from the model when not significant. When p < .05 for the treatment effect, the fungicide, imidacloprid and thiamethoxam treatments were compared to the control through contrasts.

### 3 | RESULTS

### 3.1 | Residue analysis

### 3.1.1 | Winter annual flowers

The USDA National Science Laboratory reported detection level was 1 ppb for imidacloprid, 1 ppb for thiamethoxam and 1 ppb for clothianidin in flowers in 2016. In 2016, neonicotinoid residues were not found in any samples. In 2017, the reported detection level was 10 ppb for imidacloprid, 5 ppb for thiamethoxam and 30 ppb for clothianidin in flowers. In 2017, unquantifiably low amounts (<10 ppb) of imidacloprid were found in five of the chickweed samples from Beltsville, specifically two control samples and one from each of the other treatments. Detections did not exhibit a spatial relationship with the treatments.

# 3.1.2 | Soil

In soil, the reported detection level was 5 ppb for imidacloprid, 10 ppb for thiamethoxam and 15 ppb for clothianidin. Before planting in 2015, low levels (≤10 ppb) of imidacloprid were present in several replicates at Beltsville (Table 1). Similar levels of imidacloprid were detected after treated soybean was planted, and unquantifiably low amounts of thiamethoxam and clothianidin were found in one thiamethoxam- and one imidacloprid-treated replicate. At Queenstown, no residues were detected prior to planting, and after planting only one thiamethoxam replicate and one imidacloprid replicate contained residues. In 2016,

during wheat dormancy, unquantifiably low levels of imidacloprid were found in all plots at Beltsville, with higher amounts (7 ppb) detected in the imidacloprid-treated plots. In contrast, at Queenstown, unquantifiably low amounts of imidacloprid were detected only in the imidacloprid-treated plots. Before maize was planted in 2017, low levels of imidacloprid were present in both imidacloprid sample replicates, and one control and thiamethoxam sample replicate at Beltsville. At Queenstown, no residues were detected prior to maize planting. After maize was planted, imidacloprid was detected across multiple treatments at Beltsville, and in the imidacloprid-treated plots at Queenstown, with higher levels (≥10 ppb) present in the imidacloprid-treated plots at both sites. Thiamethoxam was detected in both thiamethoxam replicates (15–16 ppb) at Queenstown, and thiamethoxam (17 ppb) and clothianidin (23 ppb) were found in one thiamethoxam sample replicate from Beltsville.

### 3.2 | Arthropod sampling

### 3.2.1 | Community impacts

### 2015 Full-season soybean

In total, we analysed 9,750 individuals from pitfall traps, 22,112 from litter extraction, 13,979 from sticky cards and 2,320 from sweep nets (Tables S7–S10). Arthropod communities did not respond to the pesticide treatments in pitfall trap (Pseudo-F = 0.1, p = .924; Figure S2), litter (Pseudo-F = 0.2, p = .946; Figure S3) or sticky card (Pseudo-F = 0.2, p = .356) PRC analyses (Figure 1). Similarly, no treatment impacts on the arthropod community (First axis Pseudo-F = 0.4, p = .412) occurred in RDA analysis for sweep net data (Figure S4).

### 2016 Double-cropped soybean

We analysed 24,760 individuals from pitfall traps, 23,135 from litter, 9,790 from sticky cards (excluding the first date at Queenstown, where the samples were misplaced) and 1,549 from sweep nets (Tables S7–S10). Pesticide treatments did not impact arthropod communities over the season for pitfall trap (Pseudo-F = 0.2, p = .814; Figure S2) or sticky card (Pseudo-F = 0.4p = .198; Figure 1) PRC analyses. Litter data (Pseudo-F = 0.3, p = .064) revealed impacts during the early season for all three treatments, with an increase in the abundance of collembola and predatory mites (Mesostigmata; Figure S3). The insecticide treatments altered the arthropod community, reducing abundances of several taxa (First axis Psuedo-F = 0.9 p = .004) in RDA analysis of sweep net data (Figure S4).

### 2015-2016 Winter wheat

We analysed a total of 9,438 individuals from pitfall traps, 18,529 from litter extraction and 5,273 from sticky cards (Tables S7–S9). PRC analysis revealed no community responses to the pesticide treatments in pitfall trap (Pseudo F = 0.2, p = .712; Figure S2) or litter communities (Pseudo-F = 0.2, p = .976; Figure S3). However, the



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

sticky card community increasingly declined in response to insecticide treatments over the sampling dates (Pseudo-F = 0.5, p = .002; Figure 1).

### 2017 Maize

In total, we analysed 9,448 individuals from pitfall traps, 5,536 from litter extraction, and 5,238 from sticky cards (Tables S7–S9). Pesticide treatments did not impact arthropod communities over time in pitfall trap (Pseudo F = 0.2, p = .278; Figure S2) or litter extraction (Pseudo-F = 0.5, p = .198; Figure S3) PRC analyses. All pesticide treatments caused increasing declines over time for sticky card taxa (Pseudo-F = 0.3, p = .016; Figure 1).

# 3.2.2 | Effects of seed treatments on individual taxa within crops

#### 2015 Full-season soybean

Soil taxa—None of the measured taxa from pitfall traps (PT) or litter (LE) were significantly impacted by the treatments (Mesostigmata LE model  $F_{10,21} = 1.63$ , p = .167; Mesostigmata PT  $F_{3,21} = 0.39$ , p = .760; Staphylinidae LE  $F_{3,21} = 0.54$ , p = .662; Acari LE model  $F_{10,21} = 1.68$ , p = .152; Acari PT  $F_{3,21} = 1.34$ , p = .288; Collembola LE model  $F_{10,21} = 1.00$ , p = .473; Collembola PT model  $F_{10,21} = 1.82$ , p = .119; Figure S5).

Foliar taxa—The abundance of predatory thrips was reduced in both insecticide treatments compared to the control (Phlaeothripidae VC  $F_{3,21} = 15.16$ , p < .001; Figure 2). Planthoppers were suppressed by the thiamethoxam treatment (Cicadellidae VC  $F_{3,21} = 6.79$ , p = .002) while plant thrips were suppressed by both insecticide treatments (Thripidae VC  $F_{3,21} = 5.41$ , p = .006). Lady beetles (Coccinellidae SN model  $F_{10,20} = 0.59$ , p = .804), Aphelinidae (SC  $F_{3,21} = 0.75$ , p = .533), Chloropidae (SC model  $F_{10,21} = 0.47$ , p = .890), Phalacridae (SC model  $F_{10,21} = 0.31$ , p = .969) and Sciaridae (SC model  $F_{10,21} = 0.67$ , p = .738) were not impacted.

### 2016 Double-cropped soybean

Soil taxa—Pesticide treatments did not impact any pitfall trap or litter taxa in double-cropped soybean (Mesostigmata LE model  $F_{10,21} = 1.87$ , p = .108; Mesostigmata PT  $F_{3,21} = 0.69$ , p = .567; Staphylinidae LE  $F_{3,21} = 2.78$ , p = .066; Acari LE  $F_{3,21} = 0.51$ , p = .677; Acari PT model  $F_{10,21} = 1.99$ , p = .088; Collembola LE  $F_{3,21} = 2.83$ , p = .063; Collembola PT model  $F_{10,21} = 1.47$ , p = .219; Figure S5).

Foliar taxa—Lady beetles (Coccinellidae SN  $F_{3,21}$  = 8.67, p < .001) and predatory thrips (Phlaeothripidae VC  $F_{3,21}$  = 9.66, p < .001) were reduced in all three pesticide treatments. Plant thrips (Thripidae VC  $F_{3,21}$  = 11.54, p < .001) were suppressed in the thiamethoxam treatment but increased in the imidacloprid treatment, while dark winged fungus gnats were reduced somewhat in the fungicide and imidacloprid treatments (Sciaridae SC



**FIGURE 2** Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control through analysis of variance followed by Hedge's g effect test ( $\pm$ 95% confidence intervals) for sweep net (SN), sticky card (SC), and visual count (VC) taxa in full-season (FS) and double-cropped (DC) soybean. The values in parentheses indicate mean taxon abundance  $\pm$  standard error for the control. \**p* < .05, \*\**p* < .01, \*\*\**p* < .001 for the ANOVA treatment effect. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively

 $F_{3,21}$  = 3.70, p = .028; Figure 2). Sticky card-collected Aphelinidae (SC model  $F_{10,21}$  = 1.15, p = .372), Aleyrodidae (model  $F_{10,21}$  = 0.99, p = .479) and Chloropidae (model  $F_{10,21}$  = 0.87, p = .573) were not impacted.

### 2015-2016 Winter wheat

Soil taxa—The abundance of rove beetles from litter extraction was strongly reduced in both insecticide treatments (Staphylinidae LE  $F_{3,21} = 6.36$ , p = .003; Figure 3). No other taxa were impacted (Mesostigmata LE  $F_{3,21} = 1.00$ , p = .413; Mesostigmata PT model  $F_{10,21} = 2.16$ , p = .066; Acari LE model  $F_{10,21} = 0.77$ , p = .658; Acari PT model  $F_{10,21} = 1.81$ , p = .120; Collembola LE model  $F_{10,21} = 1.56$ , p = .186; Collembola PT  $F_{3,21} = 0.38$ , p = .771; Figure 3).

Foliar taxa—Sticky card-collected Aphelinid wasps ( $F_{3,21} = 18.54$ , p < .001) were strongly suppressed in both insecticide treatments (Figure 4). In winter, visually counted aphids (Aphididae) were strongly suppressed in both insecticide treatments ( $F_{3,21} = 7.93$ , p = .001), while in spring, they were suppressed in the imidacloprid treatment, but increased in the fungicide-only treatment ( $F_{3,21} = 4.55$ , p = .013). Sticky card-collected grass flies (Chloropidae) increased in the fungicide-only treatment ( $F_{3,21} = 6.41$ , p = .003), while shining flower beetles (Phalacridae) increased in the fungicide-only treatment and were reduced in the thiamethoxam treatment ( $F_{3,21} = 8.59$ , p = .001). Aleyrodidae (model  $F_{10,21} = 1.04$ , p = .446) and Sciaridae ( $F_{3,21} = 1.50$ , p = .208) collected using sticky cards were not impacted.

#### 2017 Maize

Soil taxa—Pesticide treatments did not impact any pitfall trap or litter taxa in maize (Mesostigmata LE model  $F_{10,21} = 1.04$ , p = .444; Mesostigmata PT  $F_{10,21} = 1.16$ , p = .347; Staphylinidae LE model  $F_{10,21} = 0.56$ , p = .824; Acari LE model  $F_{10,21} = 1.08$ , p = .420; Acari PT  $F_{3,21} = 0.30$ , p = .824; Collembola LE model  $F_{10,21} = 1.28$ , p = .304; Collembola PT Model  $F_{10,21} = 0.46$ , p = .711; Figure 3).

Foliar taxa—In July visual counts, spiders (Araneae) increased in abundance in all three pesticide treatments ( $F_{3,21} = 4.77$ , p = .011), while sticky card-collected whiteflies (Aleyrodidae) decreased in all three treatments ( $F_{3,21} = 3.73$ , p = .027; Figure 4). None of the other sticky card taxa were impacted (Aphelinidae model  $F_{10,21} = 1.32$ , p = .281; Aphididae  $F_{3,21} = 1.43$ , p = .262; Chloropidae  $F_{3,21} = 1.37$ , p = .278; Phalacridae  $F_{3,21} = 3.02$ , p = .053; Sciaridae  $F_{3,21} = 0.68$ , p = .576).

# 3.3 | Crop sampling

To evaluate treatment impacts on plant growth rates and health, plant height, stand count and yield were measured in all the crops (Table 2), with NDVI and the number of tillers also measured in wheat. Stand count was improved in imidacloprid-treated plots compared to the control in full-season soybean ( $F_{3,21} = 12.46$ , p < .001) and in both insecticide treatments in maize ( $F_{3,21} = 5.51$ , p = .006), but not in wheat ( $F_{3,21} = 0.39$ , p = .760) or double-cropped soybean ( $F_{3,21} = 1.21$ , p = .331). The plant height was



**FIGURE 3** Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control through analysis of variance followed by Hedge's g effect test ( $\pm$ 95% confidence intervals) for litter (LE) and pitfall trap (PT) taxa in winter wheat and maize. The values in parentheses indicate mean taxon abundance  $\pm$  standard error for the control. \**p* < .05, \*\**p* < .01, \*\*\**p* < .001 for the ANOVA treatment effect. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively. Acari refers specifically to the mite order Oribatida and the family Tarsonemidae



**FIGURE 4** Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control for foliar taxa. Data were analysed through analysis of variance followed by Hedge's g effect test (±95% confidence intervals) for sticky card (SC) and visual count (VC) taxa in winter wheat and maize. The values in parentheses indicate mean taxon abundance ± standard error for the control. \*p < .05, \*\*p < .01, \*\*\*p < .001 for the ANOVA for each taxon. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively

also greater in all three pesticide treatments compared to the control in maize ( $F_{3,21}$  = 9.04, p < .001), but not in full-season soybean (model  $F_{10,21}$  = 0.80, p = .628), double-cropped soybean

(model  $F_{10,21}$  = 1.31, p = .290) or wheat ( $F_{3,21}$  = 1.42, p = .265). NDVI ( $F_{3,114}$  = 0.06, p = .983) and tiller counts (model  $F_{11,52}$  = 1.24, p = .286) were not impacted by the treatments in wheat, and yield **TABLE 2** The effect of seed treatments on plant health parameters and yield for each crop. Analysis of variance was used with treatment, location and column (location) as fixed effects. For effect differences of p < .05, contrasts were used to compare the fungicide (FUN), imidacloprid (IMI) and thiamethoxam (THI) treatments to the control (CON). Results where contrasts were performed are bolded. NA indicates that the overall ANOVA was not significant

	Treatment, M ± SE				Treatment			
Metric	CON	FUN	IMI	тні	F-value, p-value			
Stand count (plants (2 m) <sup>-1</sup> in maize and soybean, plants m <sup>-1</sup> in wheat; $df = 3,21$ )								
Full-season Soybean	15.5 ± 1.4	$16.8 \pm 1.4^{N.S.}$	20.2 ± 1.6***	$15.3 \pm 1.6^{N.S.}$	12.46, <0.001			
Winter wheat	45.9 ± 2.6	45.6 ± 5.0	46.9 ± 2.8	$43.8 \pm 2.3$	0.39, 0.760			
Double-cropped soybean	17.8 ± 1.2	$18.2 \pm 1.0$	16.9 ± 0.7	$18.6 \pm 0.7$	1.21, 0.331			
Maize	11.6 ± 0.5	11.8 ± 0.4 <sup>N.S.</sup>	$12.2 \pm 0.4^{**}$	12.0 ± 0.5**	5.51, 0.006			
Plant height (cm; <i>df</i> = 3,21)								
Full-season soybean	26.4 ± 1.0	26.7 ± 0.9	27.8 ± 1.2	27.6 ± 0.8	NA			
Winter wheat	$13.5 \pm 0.4$	$13.0 \pm 0.2$	$13.5 \pm 0.3$	$13.1 \pm 0.3$	1.42, 0.265			
Double-cropped soybean	52.0 ± 3.0	56.4 ± 1.6	55.4 ± 3.0	54.6 ± 3.6	NA			
Maize	$14.4 \pm 0.5$	15.8 ± 0.6**	15.7 ± 0.6***	15.3 ± 0.7**	9.04, 0.001			
Yield (kg/ha; <i>df</i> = 3,21)								
Full-season soybean	2,973 ± 465	3,184 ± 517	3,159 ± 486	3,020 ± 461	0.400, 0.755			
Winter wheat	2,845 ± 277	3,373 ± 201	3,584 ± 213	3,383 ± 389	NA			
Double-cropped soybean	3,068 ± 184	3,165 ± 179	3,203 ± 169	3,148 ± 178	NA			
Maize	8,850 ± 485	9,595 ± 204	9,583 ± 711	9,502 ± 520	NA			

Abbreviation: N.S., not significant.

p < .05; p < .01; p < .01; p < .001.

benefits were not observed in full-season soybean ( $F_{3,21}$  = 0.400, p = .755), winter wheat (model  $F_{10,21}$  = 1.48, p = .215), doublecropped soybean (model  $F_{10,21}$  = 1.76, p = .132) or maize (model  $F_{10,21}$  = 1.40, p = .248).

# 4 | DISCUSSION

We conducted a 3-year field study evaluating pesticide seed treatment impacts in a full-season soybean, winter wheat, double-cropped soybean and maize rotation. Our specific goals were to quantify neonicotinoid residues in the soil and in winter annual flowers, which underlies the magnitude of non-target impacts on the arthropod community. In addition to characterizing non-target impacts, we also quantified benefits to plant growth and yield to determine whether treatments were economically justified. Unquantifiably low amounts of insecticide were present in one winter annual species in one site year, which did not correspond with our treatments. Low levels of insecticide residues were present in the soil, with the highest levels observed in the final year, suggesting some accumulation. Pesticide seed treatments variably impacted the arthropod community throughout the study. PRC and RDA analyses demonstrated occasional deviations from the control community of a relatively small magnitude, and pesticide seed treatments also impacted individual taxa. However, there was little consistency between crops and sampling methods. Overall, insecticide treatments had a stronger impact on foliar taxa than on soil taxa, and the fungicides also occasionally impacted arthropod communities. Pest pressure was very low throughout the study, and while the treatments occasionally improved early season plant growth, we did not observe yield differences in any crop.

# 4.1 | Environmental persistence and routes of exposure to neonicotinoid residues

## 4.1.1 | Uptake by plants

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

### 4.1.2 | Persistence in soil

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

High moisture content, temperature and sunlight are all positively correlated with neonicotinoid breakdown, and thiamethoxam and imidacloprid also have high leaching potential (Smalling, Hladik, Sanders, & Kuivila, 2018). Given the high summer temperatures and precipitation in Maryland, the low levels of neonicotinoid residues in our plots could be caused by rapid microbial and photolytic breakdown of residues, or by leaching and run-off. Soil testing prior to the start of the study indicated that our plots had low organic matter content, which is correlated with reduced sorption of neonicotinoids, another potential cause for low residue levels (Smalling et al., 2018). We found relatively low residue levels compared to some other studies (Bonmatin et al., 2015), and the levels we found were below the known acute toxicity thresholds for various terrestrial arthropods (Douglas & Tooker, 2016; Pisa et al., 2015). However, chronic exposure to neonicotinoid residues in the soil at levels similar to those that we detected, including levels below our quantification thresholds, can impact development and survival in solitary ground-nesting bees (Anderson & Harmon-Threatt, 2019), and can lead to bioaccumulation and DNA damage in earthworms (Chevillot et al., 2017). Therefore, even these low residue levels could lead to non-target impacts over time.

# 4.2 | Non-target impacts of pesticide seed treatments on arthropods

Our hypothesis that the soil community would experience the strongest impacts from pesticide seed treatments was not supported. We observed minimal impacts on soil community activity density as measured through pitfall traps and litter extraction; neither PRC nor individual taxon analyses exhibited responses to pesticides, except for a trend of increased mites and collembola in double-cropped soybean litter and increased mites in maize pitfall traps that was consistent across all pesticide treatments, and a large reduction of rove beetles in the insecticide-treated wheat. The lack of impact on soil taxa is consistent with the low levels of insecticide residues we found in the soil, which were generally below the threshold for acute toxicity towards arthropods (Douglas & Tooker, 2016; Pisa et al., 2015). However, as mentioned earlier, chronic exposure to the low levels of insecticides that we detected could sub-lethally impact soil-dwelling organisms over time. Due to a much higher seeding rate, NST effects on rove beetles in wheat may result from the higher rate of active ingredient which was almost double the amount applied in soybean or maize.

Other studies have described variable NSTs impacts on soil taxa in maize and soybeans (Atwood, Mortensen, Koide, & Smith, 2018; Disque et al., 2018). Clothianidin-treated maize reduced the activity density of scelionid wasps, ants, carabid beetles and staphylinid beetles early in the season with effects diminishing over the course of the season in PRC analysis of pitfall data (Disgue et al., 2018). In contrast, orbatid soil mites as well as isotomid and entomobryid collembola activity density increased relative to the control (Disgue et al., 2018). In maize and soybean rotations, responses of arthropod communities extracted from soil cores and litter bags varied between crops, years and functional guilds, with occasional positive responses to pesticide seed treatments (thiamethoxam and fungicide seed treatments) in detritivore and predator guilds, reduced predator richness and diversity in 1 year of the study, and no effect on herbivores in any year 2 weeks after planting (Atwood et al., 2018). Additionally, it has been suggested that individual studies of pesticide non-target impacts lack the power to detect effects due to relatively small sample size and high variability in arthropod community datasets (Douglas & Tooker, 2016). A meta-analysis across 20 studies revealed small negative effects [effect size  $d = -0.30 \pm 0.10$  (95%) confidence interval)] on natural enemy abundance associated with NSTs, with a trend towards soil taxa being more impacted than foliar taxa (Douglas & Tooker, 2016).

However, we detected stronger small, medium and large effect size positive and negative responses to pesticide seed treatments for foliar taxa as measured by sticky cards, sweep netting and visual samples. Redundancy analysis of sweep net data demonstrated NST impacts on arthropod abundances in 2016 double-cropped soybean but not 2015 full-season soybean. Community impacts were driven by reductions in predatory taxa such as lady beetles, minute pirate bugs and predatory thrips, indicating that the insecticide treatments had strong negative impacts on natural enemies. Given the short period of neonicotinoid activity in crop plants (3–4 weeks post planting in maize and soybean; Alford & Krupke, 2017; Myers & Hill, 2014), we expected foliar communities to recover rapidly, as observed by Disque et al. (2018) in maize. In contrast, PRC analysis for sticky cards showed increasing deviations from the control community over time in insecticide-treated winter wheat and for all pesticide treatments in maize, with no recovery over the sampling period. In maize, the group that contributed most to this deviation was flea beetles, suggesting that the community disturbance was driven by a reduction in pest abundance.

The results in wheat are more surprising, as wheat was sampled in April, May and June, 26-32 weeks post planting. The period of activity of NSTs in wheat is not as well defined as in maize and soybean; neonicotinoids could remain active for much longer in winter wheat, because of low temperatures and plant dormancy during winter and early spring. Unfortunately, we were unable to directly sample foliage for insecticide residues during our study. However, Zhang et al. (2016) found low levels (10-22 ppb) of imidacloprid and clothianidin in seed-treated winter wheat up to 28 weeks after planting and observed successful control of cereal aphids throughout the growing period. The presence of insecticide in plant tissue over a longer period could be a source of exposure for non-target beneficials such as lady beetles and minute pirate bugs that supplement their diet with plant material, or parasitoids that rely on nectar as a food source (Gontijo et al., 2015; Moscardini et al., 2014; Moser & Obrycki, 2009). In our study, the strongest drivers of the effects observed in the PRC analysis for wheat were aphelinid wasps, which were greatly reduced in both insecticide treatments. This family contains many important aphid parasitoids, which play a key role in controlling cereal aphids in wheat (Pike et al., 1997; Schmidt et al., 2003). Although the insecticide treatments reduced aphid abundance in winter, this strong effect was no longer apparent in spring, so prey scarcity does not explain impacts in spring. It is possible that during the later sampling dates, insecticide residues were too low to control aphids but high enough to impact their parasitoids.

In foliar sweep net and visual samples from soybeans, we also observed reduced abundance or activity density of lady beetles (Coccinellidae), which are known to be impacted by neonicotinoids (Amjad, Azam, Sarwar, Malik, & Sattar, 2018; Disque et al., 2018; Zhang et al., 2016), as well as predatory thrips; some of these impacts occurred across all three pesticide treatments and thus may have been driven by the fungicide treatments. In contrast, spider abundance was higher in the maize visual samples from the insecticide-treated plots, and to a lesser extent the fungicide-treated plots. Arachnids are less susceptible to neonicotinoids than insects (Douglas & Tooker, 2016), and Easton and Goulson (2013) found that spiders were attracted to low doses of imidacloprid, which could explain the increased abundance of spiders. Another possibility is that sublethal impacts of the pesticides on insects made insect prey easier to capture (Main, Webb, Goyne, & Mengel, 2018), thereby improving resource availability and increasing spider abundance.

Overall, we did not see any evidence of cumulative impacts over time in soil or foliar taxa. The taxa that were impacted varied from crop to crop, and no taxa were consistently impacted throughout the study. When possible, residue analysis of foliar tissue should be conducted to better understand the variation in pesticide seed treatment impacts between crops.

# 4.3 | Impacts of fungicides on arthropods

In order to isolate effects of fungicides from those of insecticides, we examined fungicide seed treatments alone, which also impacted the arthropod community. In double-cropped soybean litter samples and maize sticky card samples, the impact of the fungicide-only treatment on the community was similar to that of the imidacloprid treatment in PRC analyses. In addition, the fungicide treatment exhibited similar impacts as one or both insecticide treatments in double-cropped soybean individual taxa analyses, reducing abundance of predatory thrips, lady beetles and dark-winged fungus gnats as well as increasing the abundance of spiders in maize. In other studies, both fungicide and insecticide seed treatments decreased earthworm surface activity and increased collembola surface activity in wheat (Van Hoesel et al., 2017; Zaller et al., 2016). In our study, there were also cases where only the fungicide treatment impacted certain taxa, such as increased abundance of aphids in wheat in spring, along with increased activity density of grass flies and shining flower beetles in wheat individual taxa analyses.

To the best of our knowledge, few studies have evaluated the persistence of seed applied fungicides in agroecosystems, or their impact on the arthropod community, even though they can be moderately toxic to arthropods (Minnesota Department of Agriculture, 2012) and vary in their mobility as well as likelihood for leaching (Smalling et al., 2018). Given that the fungicide treatments consist of several active ingredients, those ingredients could interact synergistically with each other or with the insecticides to impact the arthropod community. The effects of fungicides on arthropod health have been investigated in pollinators; clothianidin can synergistically interact with the fungicide propiconazole increasing mortality in multiple bee species (Sgolastra et al., 2017). In addition, fungicides could alter arthropod abundance by interfering with entomopathogenic fungi, thereby altering disease pressure (Lagnaoui & Radcliffe, 2009). In our study, the soil community was dominated by fungivore taxa (mites and collembola). Therefore, fungicides could also affect arthropods through changes in fungal diversity and abundance, impacting resources available for fungivores. Regardless of the mechanism, our results clearly demonstrate that seed applied fungicides can disrupt arthropod communities in agroecosystems.

### 4.4 | Economic impacts

Throughout the study, we did not experience pressure from any of the foliar pests for which NSTs are labelled, as exhibited in our visual scouting data. This is typical for Maryland; although NSTs suppressed thrips (Thripidae) and leafhoppers (Cicadellidae) in soybean, and aphids (Aphididae) in early season wheat, these pests were not present at economically damaging levels. Indeed, many of the pests for which NSTs are labelled are considered sporadic pests that most growers do not typically scout for or actively manage; for some of these pests, effective alternative management strategies such as early planting and crop rotation exist (Hesler, Sappington, Luttrell, Allen, & Papiernik, 2018; Papiernik et al., 2018; Sappington, Hesler, Clint Allen, Luttrell, & Papiernik, 2018). However, soil pests such as wireworms (Elateridae) and white grubs (Scarabeidae), can require NST applications because they have multi-year life cycles and their damage cannot be mitigated with rescue treatments. In our case, scouting for grubs and wireworms before the start of the study in 2015 and shortly after planting maize in 2017 indicated very low soil pest pressure (<1 individual per plot). As we predicted, the insecticide seed treatments did not improve yield through pest suppression.

In some cases, NSTs improved early season stand density and plant height, supporting the claim that NSTs can stimulate growth and improve plant health even in the absence of pest pressure (Jeschke et al., 2010). All three pesticide seed treatments also increased plant height in maize. However, these early season agronomic benefits did not translate to yield increases. Our results are consistent with several previous findings that NSTs may not provide economic benefits in the absence of early season pest pressure (Cox et al., 2007; Mourtzinis et al., 2019; Myers & Hill, 2014; Wilde et al., 2007). This suggests that the use of NSTs in Maryland grain production may not be warranted outside of specific instances of high pest pressure.

# 5 | CONCLUSIONS

We found that NSTs can impact arthropod communities in Maryland grain systems, despite low levels of neonicotinoid residues in the agroecosystem. The communities occasionally were unable to recover by the end of the sampling period, which in wheat was 32 weeks after planting. We observed suppression of predators and parasitoids that play an important role in controlling insect pests, which could have harmful management consequences. Although the levels of insecticide residues found in the soil were low, chronic exposure to those levels of insecticides has the potential to negatively impact important organisms such as pollinators and earthworms. We also cannot discount the possibility of insecticide runoff into nearby waterways, where the toxicity towards aquatic arthropods can alter aquatic food webs and cause trophic cascades (Miles, Hua, Sepulveda, Krupke, & Hoverman, 2017; Morrissey et al., 2015; Yamamuro et al., 2019). Given the lack of economically damaging pests throughout our study, we did not observe any yield benefits that could justify the risks associated with NST use. Without a corresponding increase in pest pressure (Douglas & Tooker, 2015), NST-treated maize and soybean acreage has increased, and many of these acres were previously untreated with insecticides. The Acute Insecticide Toxicity Loading on US agricultural lands has increased 48- and 4-fold for oral and contact toxicity from 1992 and 2014, primarily due to the use of neonicotinoids in maize and soybean (DiBartolomeis, Kegley, Mineau, Radford, & Klein, 2019). Between 2011 and 2014, the overall quantity of neonicotinoids applied to maize also doubled, indicating an increase in the rate of products used (Tooker, Douglas, & Krupke, 2017). Despite minimal or no

benefits in many cases, NST use has continued to grow. Unfortunately, there is little availability of maize without NSTs in the US, leaving farmers with limited choices (Alford & Krupke, 2017). Given the levels of NST contamination in the environment and the impacts on non-target arthropod communities, tactics must be developed to minimize overuse.

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### AUTHORS' CONTRIBUTIONS

A.D., G.P.D. and K.A.H. developed and carried out the study, conducted data analysis and wrote the manuscript; M.T.L. participated in data collection and manuscript preparation. All authors have reviewed the final manuscript and given approval for publication.

### DATA AVAILABILITY STATEMENT

Data are available via the Dryad Digital Repository https://doi. org/10.5061/dryad.wm37pvmj7 (Dubey, Lewis, Dively, & Hamby, 2020).

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# SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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