



Impacts of neonicotinoid seed treatments on the wild bee community in agricultural field margins

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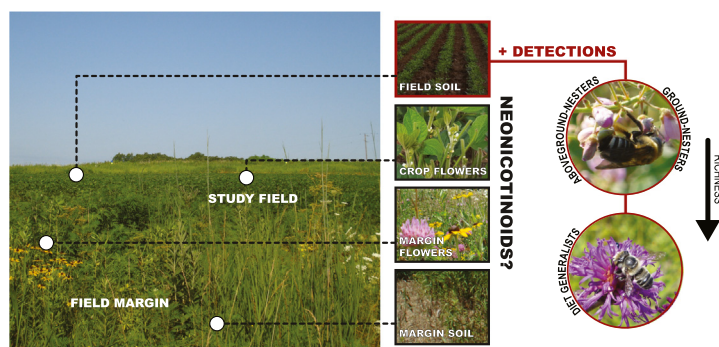
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HIGHLIGHTS

- Neonicotinoids were detected in 39% of treated and previously-treated fields.
- Field treatment had no significant effect on wild bee abundance or richness.
- Reduced species richness of wild bee guilds in fields with neonicotinoids present.
- Seed treatment insecticides may negatively impact bee community conservation.

GRAPHICAL ABSTRACT



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ABSTRACT

Wild bees support global agroecosystems via pollination of agricultural crops and maintaining diverse plant communities. However, with an increased reliance on pesticides to enhance crop production, wild bee communities may inadvertently be affected through exposure to chemical residues. Laboratory and semi-field studies have demonstrated lethal and sublethal effects of neonicotinoids on limited genera (e.g., *Apis*, *Bombus*, *Megachile*), yet full field studies evaluating impacts to wild bee communities remain limited. Here, we conducted a two-year field study to assess whether neonicotinoid seed treatment and presence in environmental media (e.g., soil, flowers) influenced bee nest and diet guild abundance and richness. In 2017 and 2018, we planted 23 Missouri agricultural fields to soybeans (*Glycine max*) using one of three seed treatments: untreated (no insecticide), treated (imidacloprid), or previously-treated (untreated, but neonicotinoid use prior to 2017). During both years, wild bees were collected in study field margins monthly (May to September) in tandem with soil and flowers from fields and field margins that were analyzed for neonicotinoid residues. Insecticide presence in soils and flowers varied over the study with neonicotinoids infrequently detected in both years within margin flowers (0%), soybean flowers (<1%), margin soils (<8%), and field soils (~39%). Wild bee abundance and species richness were not significantly different among field treatments. In contrast, neonicotinoid presence in field soils was associated with significantly lower richness (ground- and aboveground-nesting, diet generalists) of wild bee guilds. Our findings support that soil remains an underexplored route of exposure and long-term persistence of neonicotinoids in field soils may lead to reduced diversity in regional bee communities. Future reduction or elimination of neonicotinoid seed treatment use on areas managed for wildlife may facilitate conservation goals to sustain viable, diverse wild bee populations.

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1. Introduction

Agricultural fields surrounded by diverse floral resources provide food and nesting habitat that attract a broad spectrum of wild bees, which not only benefit crop yields, but further lead to maintenance and reproduction of wild plant communities (Blaauw and Isaacs, 2014; Kremen et al., 2002; Potts et al., 2016). Maintaining floral rich native grass and shrubland habitat leads to increased bee diversity, which is important in providing consistent crop and plant pollination services, as bee communities fluctuate across time (Kremen et al., 2002). However, agricultural intensification (e.g., use of synthetic pesticides) can lead to losses of regional pollinators via chemical contamination of resources or degradation of habitat (Long and Krupke, 2016; Potts et al., 2016). Pesticides may result in reduced bee abundance or species richness across a range of scales based on timing of application, pesticide loads, or limited availability of buffer habitat (Brittain et al., 2010; Main et al., 2020; Park et al., 2015).

Over the last decade, the neonicotinoid class of insecticides have become increasingly scrutinized due to their association with declines in managed and wild bee populations (Woodcock et al., 2017, 2016). As systemic plant insecticides, neonicotinoids are widely-used in agricultural production for protection of crops (e.g., oilseed, grain, vegetables) against a range of pests. Because of their high solubility and long half-life in soil, neonicotinoids are often transported from application fields to non-target field margins, adjacent floral communities, and wildlife habitat during crop planting and precipitation events (Hladik et al., 2018). The associated chemical fate provides potential for wild bee populations to interact with neonicotinoids via soil, plants (e.g., leaves, flowers), and pollen. Numerous studies have identified a range of neonicotinoid active ingredients in crops and non-target margin plant material (Goulson et al., 2015; Long and Krupke, 2016; Stewart et al., 2014). However, neonicotinoids in soils may persist for years (Hladik et al., 2018) and are often detected at levels ten times greater than concentrations found in pollen (Willis Chan et al., 2019).

The bulk of neonicotinoid pollinator literature has focused on neonicotinoid impacts to honeybees (Lundin et al., 2015). Compared to the highly managed, social honeybee, most wild bees are solitary; therefore, pesticide routes of exposure are more likely to differ among wild bee species. Differences between honeybees and native bees are based on life history traits (e.g., nest and diet preference), body size, timing of emergence relative to pesticide application, and functional guilds (Brittain and Potts, 2011; Williams et al., 2010). Ground-nesting bees may ingest contaminated soil particles by moving soil with their mandibles; whereas, cavity-nesting bees such as *Megachile* could ingest pesticides via chewing leaf material or floral petals during nest initiation (Kopit and Pitts-Singer, 2018). Adult bees may also consume neonicotinoids when foraging for pollen or nectar and provisioning their nests. If neonicotinoids more easily partition into some plants and not others, this could increase the risk of exposure to diet specialists that limit their foraging activity to a single floral genus. Despite the majority of bee species nesting in the ground, field-scale assessments of neonicotinoid seed treatment impacts on pollinators have overlooked the influence of soil contamination on wild bee communities (Anderson and Harmon-Threatt, 2019; Pisa et al., 2015; Willis Chan et al., 2019; Willis Chan and Raine, 2021).

Most studies evaluating neonicotinoids and wild bees have focused on social species (e.g., bumblebees) or a limited number of solitary bee genera [e.g., *Osmia* and *Megachile* (Blacquière et al., 2012; Rundlöf et al., 2015; Woodcock et al., 2017)]. Numerous laboratory studies evaluating wild bees have demonstrated negative impacts on bumblebees including decreased reproduction, reduced foraging ability, and diminished survival (see review by Pisa et al., 2015). However, the effects of neonicotinoid use on the vast majority of solitary, wild bee species remain unknown. Presently, few studies have evaluated neonicotinoid impacts on solitary species under field conditions (Pisa et al., 2015)

and there is limited research on impacts to wild bees at the landscape or population level (Rundlöf et al., 2015; Woodcock et al., 2017).

In the midwestern United States, corn (i.e., maize) and soybeans are widely cultivated using seed treated with a neonicotinoid active ingredient such as clothianidin, imidacloprid, or thiamethoxam (Douglas and Tooker, 2015). Although cropping is a common management practice on public lands, it is unclear whether years of consecutive seed treatment use impacts wild bees nesting near cultivated fields or if bees benefit by growers shifting from pesticide-treated to untreated crops. In a previous pilot-study evaluating a range of row-cropped fields, we found that overall richness of wild bees in field margins was negatively associated with neonicotinoid concentration in margin soil (Main et al., 2020). Here, we evaluated the effects of planting imidacloprid-treated or insecticide-free soybean seed on the abundance and richness of wild bee nesting and diet guilds found in agricultural field margins. To identify potential routes of exposure via environmental media, we quantified neonicotinoid concentrations in soils and flowers of agricultural fields and their surrounding margins. We hypothesized that (1) wild bee guild abundance and richness would be greatest in field margins surrounding soybean crops that had never been treated with a neonicotinoid (i.e. untreated fields) and (2) only ground-nesting bee abundance or richness would be reduced in fields with measurable neonicotinoid concentrations in soil.

2. Materials and methods

2.1. Study area and experimental treatments

In 2017 and 2018, we conducted a field experiment evaluating the impacts of multi-year planting of neonicotinoid seed treatments on abundance and richness of wild bee nesting and diet guilds at 23 agricultural fields. All agricultural fields were located on Missouri public lands [i.e., Conservation Areas (CA)] managed for wildlife. Site selection was limited to CA that were situated in two Major Land Resource Areas found in northern Missouri: the Central Claypan and the Iowa and Missouri Heavy Till Plain. Details of study CA and Missouri climate conditions are described in Main et al. (2019). At each CA, between three and six agricultural fields (field area range: 0.14–5.67 ha; mean: 1.86 ha; Table 1) were evaluated as part of the present study. Study fields were situated in a surrounding landscape matrix of woodland, grassland, and cropland that varied among CA. Although we had attempted to isolate study fields from one another by a minimum distance of 1 km, the availability of untreated fields on each CA became a limiting factor. Instead, each study field presented herein was a minimum distance of 500 m from any other experimental treatment. Solitary bees reportedly have a small foraging range (<600 m; Gathmann and Tschamtker, 2002) with many species foraging less than 300 m from their nest (Zurbuchen et al., 2010). However, we acknowledge that not all species are likely to be restricted by flight distance as larger genera such as *Bombus*, *Svastra*, or *Xylocopa* may have been able to move freely between treatment fields.

All study fields were surrounded by an existing margin that varied in area (field margin range: 0.13–2.8 ha; mean: 0.68 ha), but typically contained a mix of grasses and wildflowers. In both years, study fields were sown to soybeans [*Glycine max* (L.) Merr.] in early May, with a target planting rate of ~445,000 seeds per ha and field rows spaced ~46 cm apart. Fields were planted using either conventional or no-till agricultural practices. Each field was planted to one of three treatments: untreated ($n = 7$), previously-treated ($n = 8$), or neonicotinoid-treated ($n = 8$). Untreated fields had no prior neonicotinoid use, previously-treated fields had last used neonicotinoids in 2016, and treated fields had been continuously cropped using neonicotinoid seeds for >5 years. All treated study fields were sown using imidacloprid seed treatments at a rate of 0.10 kg AI/ha (Gaucho® Bayer Crop Science). At the time of study initiation, imidacloprid was used in almost half of all soybean seed treatments offered for sale across the state of Missouri. Since wild bees have diverse nesting preferences (e.g., soil, pith, wood) and may

Table 1

Description of Conservation Area study fields, surrounding land use, and pesticide treatment from 2017 to 2018. Treatments were as follows: untreated (U), previously-treated (PT), and treated (T).

Conservation Area	Field ID	Area (ha)		Treatment year		Crop 2016
		Field	Margin	2017–2018	2016	
Atlanta <i>n</i> = 4	AT UT1	2.00	0.43	U	U	Old field
	AT UT2	0.14	0.73	U	U	Grassland
	AT T2	1.78	0.32	T	T	Soybeans
	AT T3	0.73	0.36	PT	T	Soybeans
Fountain Grove <i>n</i> = 5	FG UT1	2.43	0.57	U	U	Grassland
	FG T1	1.01	0.21	T	T	Corn
	FG T2	2.59	0.90	T	T	Corn
	FG T3	3.24	1.14	PT	T	Corn
Indian Hills <i>n</i> = 3	FG T4	2.23	0.22	PT	T	Corn
	IH UT1	5.67	0.50	U	U	Hayfield
	IH T1	1.17	2.80	PT	T	Corn
	IH T3	2.06	1.02	T	T	Corn
Thomas Hill <i>n</i> = 5	THR UT1	0.93	0.23	U	U	Radishes
	THR T1	0.89	0.13	T	T	Milo
	THR T2	1.05	1.13	T	T	Corn
	THR T3	1.56	0.32	PT	T	Milo
Whetstone Creek <i>n</i> = 6	THR T4	1.61	0.94	PT	T	Corn
	WC UT1	1.62	1.08	U	U	Grassland
	WC UT2	1.21	0.23	U	U	Old field
	WC T1	1.62	1.42	PT	T	Corn
	WC T2	2.02	0.39	T	T	Corn
	WC T3	1.21	0.34	T	T	Corn
	WC T4	4.00	0.23	PT	T	Corn

interact with pesticides above and belowground (Kopit and Pitts-Singer, 2018), we collected soil and flowers from study fields and adjacent margins to quantify pesticide concentrations in relevant environmental media.

2.2. Soil and vegetation sampling

Each year (2017, 2018), we collected soil from the 23 planted study fields (total *n* = 115) and the surrounding field margins (total *n* = 92). Soil sampling occurred monthly during five discrete time periods throughout the agricultural growing season: pre-seeding (May; *n* = 46), post-seeding (June; *n* = 46), mid-growing (July; *n* = 46), soybean-flowering (August; *n* = 23), and pre-harvest (September; *n* = 46). Due to budget constraints and limited detections, margin soil samples collected in August were not analyzed. Field soil was haphazardly collected at the midway point (~23 cm) between soybean rows from a depth of 0 to 10 cm at ~15 locations throughout a study field (Main et al., 2020). Similarly, margin soils were collected at ~15 locations in a zigzag pattern at a minimum distance of 2 m from the field edge (Botías et al., 2015). All subsamples collected within a field or margin were composited (~1.5 kg) in a clean polyethylene bag before being stored in coolers with ice packs for transport to the laboratory. Individual study field and margin soil samples were thoroughly mixed in the laboratory, transferred to 50 mL Falcon centrifuge tubes (Fisher Scientific™), and frozen at −20 °C until chemical analysis.

At each field margin, one observer recorded vegetation characteristics using a 50 cm × 50 cm quadrat at six randomized locations along ~200 m transect within the margin. Vegetation characteristics were recorded during every site visit from May to September in 2017 and 2018. Measured variables included plant cover (%), vegetation height (cm), and floral richness. To determine pesticide concentrations in margin wildflowers (total *n* = 115) and soybean crops (August only; *n* = 23), we harvested flower heads each year during the same five time periods described above. Floral species varied between study field margins, but often included representatives from the families Asteraceae (e.g., genera: *Cirsium*, *Helianthus*, *Leucanthemum*, *Vernonia*, *Solidago*), Fabaceae (e.g., *Chamaecrista*, *Trifolium*), Lamiaceae (e.g., *Mentha*, *Morinda*), and Plantaginaceae (e.g., *Penstemon*). One observer would

randomly walk the margin haphazardly clipping flower heads from all plants that were in bloom, regardless of species. Although plant species may vary in their uptake of neonicotinoids (Botías et al., 2015), collected flower heads were composited to represent an overall sample per study field margin. Flowers were placed in polyethylene bags and stored in coolers with ice packs for transport to the laboratory. All margin flower samples were processed in the laboratory by finely cutting floral material into small pieces and were similarly stored as described above.

2.3. Wild bee collection and identification

Wild bees were collected monthly from study field margins during May through September in 2017 and 2018. Following a modified protocol described by Main et al. (2020), we monitored bee communities using SpringStar™ blue vane traps (BVT; SpringStar Inc., Woodinville, WA). BVT are an effective method for monitoring a wide variety of bees as some larger species may avoid other methods such as pan traps (Geroff et al., 2014). The traps contained no euthanizing agent and were placed at vegetation height every 30 m along an ~200 m field margin transect. Traps were deployed for 24 h beginning at 0700 h CST. It should be noted that bee communities respond differently to trap color, placement, and sampling method (e.g., pan trap, sweep-net, BVT; Joshi et al., 2015) and our chosen sampling method may have over- or underestimated the bee community reported here. Therefore, our bee data should be interpreted in light of the trap color, placement, and sampling effort selected.

After collection, individual traps were processed by placing bees in 50 mL Falcon centrifuge tubes where they were frozen at −20 °C until identification in the laboratory. Mounted specimens were placed under a microscope where technicians with taxonomic experience identified bees using a key of tallgrass prairie bees (Arduser, 2016). Individual specimens were further cross-validated using the online ID guide, Discover Life (Ascher and Pickering, 2016; last accessed December 12, 2019). More challenging species were evaluated by multiple technicians or via consult with a Midwest tallgrass prairie bee expert (M. Arduser, Missouri Department of Conservation — retired). All bees were identified to species or genera. An informal study collection is housed at the University of Missouri.

2.4. Pesticide analyses

Soil and flower samples collected from study fields and their surrounding margins were analyzed by the accredited USDA AMS National Science Laboratory in Gastonia, NC, USA. Soil and flower samples were extracted for analysis of agricultural pesticides using a refined methodology for the determination of neonicotinoid insecticides using an approach of the official pesticide extraction method (AOAC OMA 2007.01) also known as the QuEChERS method (Lehotay et al., 2005). Approximately 35 g of soil and 13 g of flowers were analyzed per individual sample. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method uses an acetonitrile and water solution to reduce the sample suppression or enhancement effects that matrices may create during chromatographic analysis. After a “clean up” to remove matrix components and filtration to remove particulates, separate aliquots of extract were analyzed for pesticide residues via liquid chromatography coupled with tandem-mass spectrometry (LC/MS/MS; Kamel, 2010; Stewart et al., 2014). Quantification was performed using external calibration standards prepared from certified standard reference material. Samples were analyzed for five neonicotinoid active ingredients including acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam. Despite being used in all planted seed treatments, we were unable to test for the fungicides penflufen, oxathiapiprolin, or prothioconazole due to poor recoveries or lack of reference materials. The limit of detection (LOD) ranged from 1 to 6 µg/kg (ppb) for the neonicotinoid active ingredients.

2.5. Statistical models

To analyze our data, we characterized bee functional guilds based on a simplified nesting preference [e.g., ground, aboveground (cavity, pith, wood, surface)] and diet specialization (e.g., diet generalist, specialist). Functional guilds were determined by consulting the existing literature (e.g., McCravy et al., 2019; Stephenson et al., 2018). Diet specialization was determined by either a bee species' known relationship to a specific host plant (e.g., *Melissodes denticulatus*) or an affinity for foraging on several plants from the same genus (e.g., *Peponapis pruinosa*). Bee abundance and total species richness (i.e., cumulative number of species collected per study field) were calculated for each functional guild. Due to our relatively small sample size of 23 fields, we estimated bee abundance per field by averaging captured bees among all traps within each field margin. We calculated the cumulative species richness per field for each sampling period using package 'vegan' in R 4.0.2 (R Core Team, 2020).

We fit a series of generalized linear mixed models in R (package 'glmmTMB'; Brooks et al., 2017) to evaluate the effect of treatment on the response variables of wild bee functional guild abundance and richness. Each model included a total of four explanatory variables: field treatment, neonicotinoid presence in soil, study year, and mean floral richness. Similarly, each model contained two random effects including *FieldID* nested in Conservation Area to account for spatial autocorrelation and *sampling period* to account for the repeated sampling of the same fields over time. A mixed effect model was constructed for the response variable of abundance and richness for each of four bee guilds (e.g., ground-nesting abundance, diet generalist richness). As our bee count data were zero-inflated, we selected a negative binomial distribution to reduce overdispersion in our models. All models were further screened for collinearity between predictor variables by evaluating the variance inflation factor (VIF), but all model VIF scores were <2 indicating low collinearity. Model fit was assessed by testing for outliers and through visualization of model residuals using the R package 'DHARMA' (Hartig, 2020). In glmmTMB, the individual significance of model terms is based on a Wald test that can test multiple parameters simultaneously and the model output produces an associated z score and P value for each variable. Model R code and data are provided in supplemental information.

Treatment was a categorical variable that identified all study fields as either treated, previously-treated, or untreated. As we were primarily interested in whether specific guild abundance and richness differed among treatments, we compared the aforementioned response variables in treated and previously-treated fields with untreated fields as a reference condition. We further accounted for the influence of pesticides by including a variable that represented the presence (i.e., detect/non-detect) of neonicotinoids in field soils. Neonicotinoid detections were infrequent (0–39%) in any environmental media (field or margin soil, margin flowers); therefore, due to the high level of censored data, only a categorical variable was included in our models. Neonicotinoid presence was based on each sampling period with any field >LOD listed as *detect* and any field <LOD (including trace detections) listed as *non-detect*. Based on bee ecological and life-history traits (Williams et al., 2010), offspring of ground-nesting bees may be most negatively impacted by pesticide presence as they spend the majority of the year belowground (Antoine and Forrest, 2020). In contrast, species that were categorized as aboveground nesters would be less likely to interact directly with contaminated soil as they nest in cavities, pith, wood, or grass. We further included mean floral richness measured monthly in field margins as a covariate since bees benefit from diverse floral habitat (Kremen et al., 2002), which may in turn be more important in structuring bee communities than pesticide-related variables. Finally, we controlled for study year (i.e., year) in our models. However, we did not interact year with any variables as there were substantial differences between years based on seasonal rainfall that likely affected floral resources and bee capture. Average monthly rainfall during May to September of 2017 was 130.2

mm; whereas, 2018 was considered a drought year with an average monthly precipitation of 4.94 mm.

3. Results

3.1. Pesticide concentrations in soils and flowers

Neonicotinoid concentrations in soils differed between years, and concentrations were greater in the second year of the study (2018). In 2017, imidacloprid and clothianidin were both detected in soils collected from previously-treated and treated fields (Table 2). The detection frequency and median concentrations varied with imidacloprid ranging from 0 to 75% detections per month (median: 3 µg/kg) in treated field soils and 13–50% (median: 3 µg/kg) in previously-treated field soils. Clothianidin was less frequently detected (0–13%) each month in both previously-treated and treated fields, with the greatest median concentration measured in a previously-treated field in September 2017 (27 µg/kg). By comparison, in 2018, imidacloprid and clothianidin were detected monthly in up to 100% and 50% of field soils, respectively (Table 2). Despite not being applied as a seed treatment in 2017 or 2018, the greatest median concentration of clothianidin was 20 µg/kg and 27.5 µg/kg in previously-treated and treated fields, respectively. Neither imidacloprid nor clothianidin were detected in soils of untreated fields in either study year. Imidacloprid and clothianidin were infrequently detected (overall: <8%) in field margin soils. All other neonicotinoids (acetamiprid, thiamethoxam) were detected in <3% of all soils collected from fields or margins in all years. No neonicotinoids were detected in margin flowers collected in 2017 or 2018. Similarly, neonicotinoids were detected in <4% of soybean flowers collected from study fields (both years), with no detections in 2017, but soybean flowers of one treated field contained imidacloprid in 2018 (168 µg/kg).

3.2. Wild bee communities on Conservation Areas

Over the entire study, a total of 8045 bees were collected from agricultural field margins, including 3549 bees collected in 2017 and 4496

Table 2

Frequency of detection (%) and median concentration (µg/kg) of clothianidin and imidacloprid in soils collected from study fields during May to September of 2017 and 2018. "–" indicates no median value due to neonicotinoids not being detected during that sampling period (month).

	Soil conc. (µg/kg)	Untreated (n = 7)		Prev. Treated (n = 8)		Treated (n = 8)	
		% detects	Median	% detects	Median	% detects	Median
2017	Clothianidin	% detects	Median	% detects	Median	% detects	Median
	May	0	–	13	6	0	–
	June	0	–	13	7	13	15
	July	0	–	13	10	13	12
	August	0	–	13	10	13	11
	September	0	–	13	27	13	8
	Imidacloprid	% detects	Median	% detects	Median	% detects	Median
	May	0	–	25	3	0	–
	June	0	–	13	4	50	8
	July	0	–	13	3	38	5
2018	Clothianidin	% detects	Median	% detects	Median	% detects	Median
	May	0	–	38	16	13	19
	June	0	–	0	–	0	–
	July	0	–	38	19	50	18
	August	0	–	38	20	50	27.5
	September	0	–	50	11	50	22.5
	Imidacloprid	% detects	Median	% detects	Median	% detects	Median
	May	0	–	13	8	25	6.5
	June	0	–	13	6	75	15
	July	0	–	0	–	100	10
	August	0	–	0	–	75	16
	September	0	–	38	4	100	12

collected in 2018. From May to September, a total of 27 genera and 31 genera were collected in 2017 and 2018, respectively (Table S1). *Melissodes*, *Bombus*, *Ceratina*, *Eucera*, and *Halictus* were the most abundant genera in both years (Fig. 1). The most frequently collected species or subgenus were as follows: (2017) *Melissodes bimaculatus*, *Lasioglossum (Dialictus) spp.*, *Ceratina calcarata*, *Melissodes desponsus*, and *Bombus pennsylvanicus*; (2018) *Melissodes bimaculatus*, *Eucera hamata*, *Ceratina mikmaqi*, *Halictus ligatus*, and *Ceratina calcarata*. Across both years, collected bees represented the two nest guilds as follows: ground-nesters (both years: 70%) and aboveground-nesting bees (both years: 29%). Cleptoparasitic species accounted for <1% of all bees captured each year. We further identified 81% and 84% of bees as diet generalists and 19% and 16% as diet specialists in 2017 and 2018, respectively. There were several infrequently caught species in both years as 12% (2017) and 22% (2018) of trapped bees were singletons.

3.3. Influence of abiotic and biotic factors on bee guild abundance

3.3.1. Nest guilds

Mean bee abundance by nesting guild was best explained by mean floral richness in field margins and sampling year. Nesting guilds were more abundant in margins dominated by fewer floral species with ground-nesting ($\beta = -0.09 \pm 0.04$, $z = -2.44$, $P = 0.01$) and aboveground-nesting ($\beta = -0.21 \pm 0.06$, $z = -3.76$, $P < 0.001$) bees responding positively to reduced floral richness. Similarly, mean bee abundance was greater for both nesting guilds in 2018 compared to the first year of the study in 2017 (Table 3). There was no significant effect of field treatment (Fig. 2) or presence of neonicotinoids in field soil on wild bee abundance for either nest guild.

3.3.2. Diet guilds

Similar to nest guild abundance, there were no significant differences between treatments for diet generalists or diet specialists (Fig. 2). Diet generalists were also more abundant in field margins containing fewer floral species ($\beta = -0.14 \pm 0.04$, $z = -3.97$, $P < 0.001$). In 2018, there were significantly greater numbers of wild bees in field

margins with more floral specialists ($\beta = 0.31 \pm 0.12$, $z = 2.65$, $P < 0.001$) and floral generalists ($\beta = 0.20 \pm 0.06$, $z = 3.30$, $P < 0.001$) compared to the previous year (2017).

3.4. Influence of abiotic and biotic factors on bee guild richness

3.4.1. Nest guilds

The presence of neonicotinoids in field soils was associated with fewer bee species in field margins. Bee species richness was significantly reduced for both ground-nesting ($\beta = -0.11 \pm 0.04$, $z = -2.61$, $P = 0.008$) and aboveground-nesting bees ($\beta = -0.37 \pm 0.06$, $z = -5.79$, $P < 0.001$) collected near fields with detectable neonicotinoid residues in soils. This amounted to ~11% fewer ground-nesting and ~31% fewer aboveground-nesting bees compared to field margins surrounding study fields with no detectable neonicotinoids (Fig. 3). There were also significantly more bee species belonging to both nesting guilds collected from field margins in 2018 (Table 3). Aboveground-nesting bees also benefited from diverse floral species in field margins with a greater number of bee species found in margins as floral richness increased ($\beta = 0.09 \pm 0.02$, $z = 3.61$, $P < 0.001$).

3.4.2. Diet guilds

The diversity of diet generalists was significantly reduced in field margins adjacent to study fields containing neonicotinoid residues (Table 3; Fig. 3). These field margins contained ~22% fewer species characterized as diet generalists compared to margins near uncontaminated field soil. However, field margins that had diverse floral communities also contained a greater number of diet generalist species ($\beta = 0.05 \pm 0.01$, $z = 3.04$, $P = 0.002$). The number of species considered diet generalists was also considerably greater in the second year of the study (2018; Table 3).

4. Discussion

Wild bees are critical components of global agroecosystems and maintaining diverse bee habitat is important for conserving pollinator

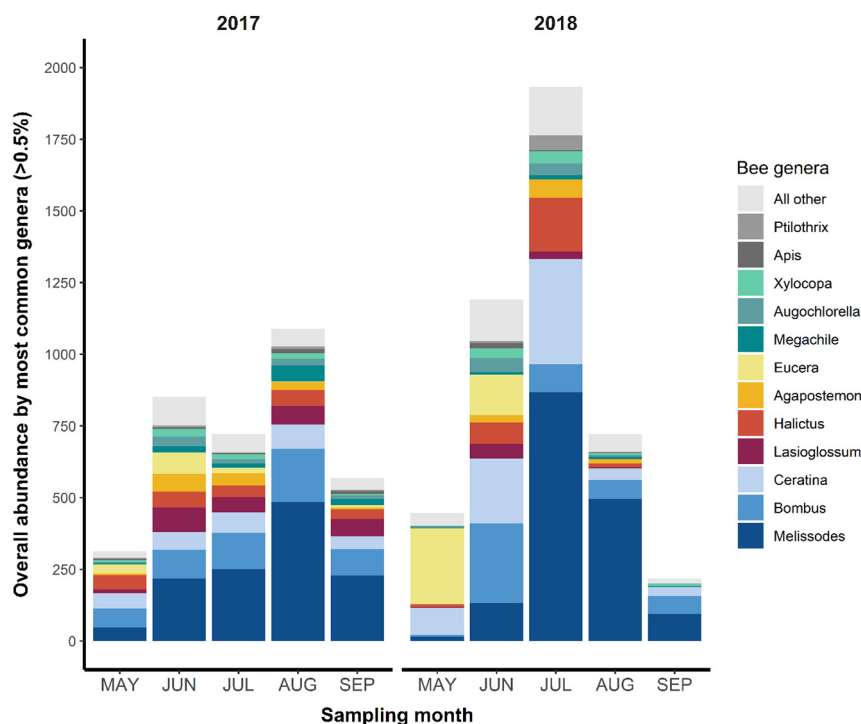


Fig. 1. Stacked bar chart of overall bee abundance by genera collected from study field margins in Conservation Areas in Missouri year 2017 and 2018. Only genera that equate to >0.5% of the total abundance per sampling period are presented. The category of *All other* represents all bee genera combined that are individually <0.5% of the total abundance. Sampling months are May, June (JUN), July (JUL), August (AUG), and September (SEP).

Table 3

Results of mixed-effects models analyzing wild bee abundance and richness by nest (ground, aboveground) and diet (generalist, specialist) guilds. Model fixed effects included field treatment [untreated, treated, previously-treated (PrevTreated)], presence of neonicotinoids in field soil (detect, non-detect), mean floral richness, and study year (2017, 2018). Data in **bold** indicate a significant explanatory variable. Neonics = neonicotinoids, (D) = detect.

Variables	Nest: Ground (abundance)				Variables	Diet: Generalist (abundance)			
	Coef.	±SE	z	P		Coef.	±SE	z	P
Treated ¹	−0.01	0.09	−0.11	0.91	Treated ¹	−0.01	0.13	−0.10	0.92
PrevTreated ¹	−0.03	0.08	−0.38	0.71	PrevTreated ¹	0.01	0.12	0.07	0.94
Soil neonics (D)	−0.07	0.08	−0.85	0.39	Soil neonics (D)	0.04	0.08	0.55	0.58
Floral richness	−0.09	0.04	−2.44	0.01	Floral richness	−0.14	0.04	−3.97	<0.001
Year (2018)	0.19	0.06	3.22	0.001	Year (2018)	0.20	0.06	3.30	<0.001
Variables	Nest: Aboveground (abundance)				Variables	Diet: Specialist (abundance)			
	Coef.	±SE	z	P		Coef.	±SE	z	P
Treated ¹	0.04	0.17	0.25	0.8	Treated ¹	−0.12	0.20	−0.59	0.56
PrevTreated ¹	0.14	0.16	0.92	0.36	PrevTreated ¹	−0.20	0.18	−1.13	0.26
Soil neonics (D)	0.01	0.12	0.07	0.94	Soil neonics (D)	−0.05	0.15	−0.33	0.74
Floral richness	−0.21	0.06	−3.76	<0.001	Floral richness	−0.10	0.07	−1.37	0.17
Year (2018)	0.27	0.09	2.96	0.003	Year (2018)	0.31	0.12	2.65	0.01
Variables	Nest: Ground (richness)				Variables	Diet: Generalist (richness)			
	Coef.	±SE	z	P		Coef.	±SE	z	P
Treated ¹	0.13	0.19	0.66	0.51	Treated ¹	0.23	0.18	1.25	0.21
PrevTreated ¹	−0.13	0.19	−0.66	0.50	PrevTreated ¹	−0.10	0.18	−0.58	0.56
Soil neonics (D)	−0.11	0.04	−2.61	0.008	Soil neonics (D)	−0.24	0.04	−6.03	<0.001
Floral richness	0.001	0.02	0.10	0.92	Floral richness	0.05	0.01	3.04	0.002
Year (2018)	0.09	0.03	2.82	0.005	Year (2018)	0.17	0.03	5.88	<0.001
Variables	Nest: Aboveground (richness)				Variables	Diet: Specialist (richness)			
	Coef.	±SE	z	P		Coef.	±SE	z	P
Treated ¹	0.32	0.19	1.69	0.09	Treated ¹	0.16	0.26	0.63	0.53
PrevTreated ¹	−0.04	0.19	−0.20	0.84	PrevTreated ¹	−0.16	0.26	−0.60	0.55
Soil neonics (D)	−0.37	0.06	−5.79	<0.001	Soil neonics (D)	−0.01	0.08	−0.15	0.88
Floral richness	0.09	0.02	3.61	<0.001	Floral richness	−0.02	0.03	−0.96	0.34
Year (2018)	0.20	0.05	4.23	<0.001	Year (2018)	0.08	0.06	1.30	0.20

¹ Reference condition = Untreated.

communities and pollination services (Deguines et al., 2014). Our results indicate that neonicotinoid presence in field soils is detrimental to maintaining species-rich bee communities in Midwestern agroecosystems. Although treatment had no significant effect on wild bee guilds, presence of neonicotinoids in study field soils resulted in significantly lower species richness for several bee guilds including ground-nesters, aboveground-nesters, and diet generalists. Our study indicates that field-measured declines in bee communities over two years may be associated with annual application or persistence of neonicotinoids in agricultural fields and specifically exposure of wild bees to pesticides in soil.

Soil remains an overlooked route of pesticide exposure for wild bees despite a number of studies demonstrating that neonicotinoids reduce abundance of ground-dwelling beneficial insects (see review by Pisa et al., 2015). Globally, the majority of solitary bee populations nest in the ground (Antoine and Forrest, 2020); therefore, numerous species may be chronically exposed to neonicotinoids where major row crop rotations (e.g., corn, soybeans) include annual use of seed treatments. Over both years of our project, 39% of soils collected from treated and previously-treated soybean fields contained neonicotinoids with concentrations up to 163 µg/kg (ppb) in 2018. In a recent laboratory study approximating chronic exposure to ground-nesting bees, soil imidacloprid concentrations <15 ppb were associated with reduced bee longevity, development speed, and mass (Anderson and Harmon-Threatt, 2019). Ground nesting bee exposure to neonicotinoids in soil could have major implications for maintaining viable wild bee populations in agricultural areas. Our models indicated that ground-nesting bee species richness declined in field margins where adjacent study fields contained neonicotinoids in soils. As developing offspring of solitary bees may remain in the ground for the majority of their life cycle

(Antoine and Forrest, 2020), our results suggest the potential for chronic exposure.

The significant declines of bee species richness in margins bordering fields containing neonicotinoid residues suggests that many bees in our study may have been exposed to insecticides through nesting in agricultural fields or contact with contaminated soils. Several genera of the

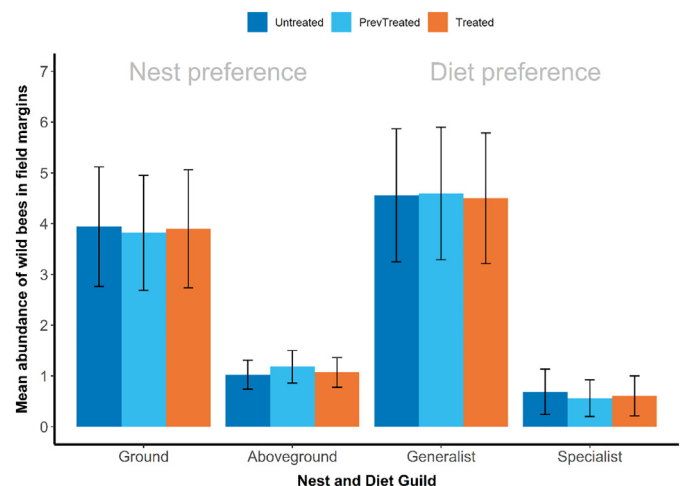


Fig. 2. Bar chart of mean bee abundance by field treatment (untreated, previously-treated [prev-treated], and treated). Bee abundance is categorized by nest (ground, aboveground) and diet (generalist, specialist) guilds. All bees were collected from Conservation Area study field margins in Missouri over both years (combined). Error bars represent the standard error of the mean. There were no significant differences between treatments.

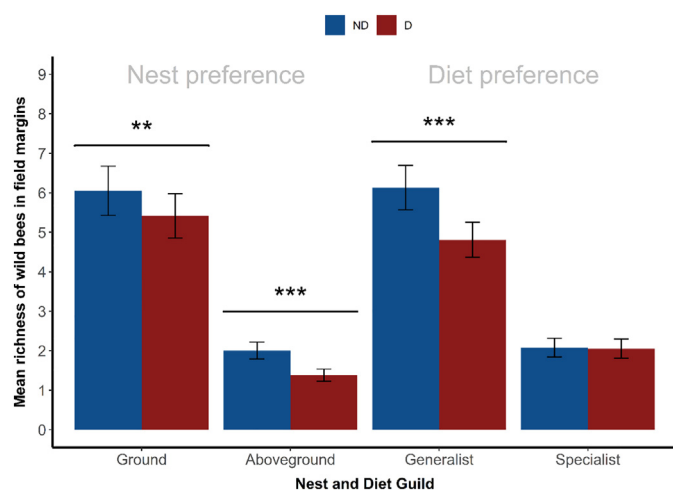


Fig. 3. Mean bee richness by nest (ground, aboveground) and diet (generalist, specialist) guild collected from margins adjacent to study fields in Conservation Areas in Missouri with non-detectable (ND) or detectable (D) levels of neonicotinoids in field soils. The presented data are for both years combined. Error bars denote the standard error of the mean. Results are based on outcomes of individual mixed-effects models. Significance is denoted as follows: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

tribe Eucerini (e.g., long-horned bees: *Eucera*, *Melissodes*, *Svastra*) were well-represented in our study and some long-horned species (e.g., *Peponapis pruinosa*, *Melissodes agilis*) are shown to preferentially nest in cultivated fields rather than less-disturbed floral margins (Julier and Roulston, 2009; Sardiñas et al., 2016). Similarly, other ground-nesting and diet generalist species we collected (e.g., *Halictus* spp., *Lasioglossum* (*Dialictus*) spp.) may be less selective and can populate heavily disturbed sites or preferentially nest in areas where vegetation is sparse and soil conditions are favorable (Antoine and Forrest, 2020; Kim et al., 2006; Sardiñas et al., 2016). It is not uncommon for wild bee communities collected near soybean fields to be comprised of a majority of mostly wild, solitary small and medium bee species (genera: *Halictus*, *Lasioglossum*, *Melissodes*; Gill and O'Neal, 2015). Solitary bees have a small foraging range (<600 m; Gathmann and Tscharrntke, 2002; Zurbuchen et al., 2010) and in mass-flowering crops, the majority of foraging movement can be concentrated in <1% of a bee's range (Sardiñas et al., 2016). In the present study, soybean fields were relatively small (~2 ha) and frequently measured <450 m across from one field edge to the other, increasing the potential of bees nesting in closer proximity to contaminated resources. To initiate nests, female ground-nesting bees excavate soils through use of their mandibles and forelegs (Antoine and Forrest, 2020) which may lead to direct exposure to pesticides via contact or ingestion (Kopit and Pitts-Singer, 2018). For example, exposure of the ground-nesting bee, *Eucera pruinosa* to soil-applied imidacloprid is shown to reduce nest initiation and offspring production by 85% and 89%, respectively (Willis Chan and Raine, 2021). As the solitary female is the sole reproductive entity of her nest, loss of potential offspring or an early death during nest initiation could lead to local population declines (Kopit and Pitts-Singer, 2018).

Soil concentrations fluctuated during the overall growing season, but due to infrequent detections, we were unable to specifically test the impact of soil concentration on wild bee guilds. To our knowledge, in the midwestern United States, neonicotinoids are mainly applied via seed coatings with limited use of foliar sprays or soil shanks (i.e., soil injections; Douglas and Tooker, 2015). The consistent detections of clothianidin – which was not applied during our study – may further validate the high potential for long-term persistence of neonicotinoids in cultivated soils (de Perre et al., 2015; Hladik et al., 2018). Several of our study fields were rotationally planted to thiamethoxam-treated corn before our experiment began. As clothianidin is a major metabolite of thiamethoxam, the presence of

clothianidin in field soils may be indicative of this previous thiamethoxam use. In contrast, with the vast difference in seasonal rainfall between study years, it is possible that surface and shallow sub-surface water transport may have moved neonicotinoids from nearby agricultural fields not associated with this experiment, thereby leading to contamination (Hladik et al., 2018). Therefore, regional bee communities are likely to be exposed to these insecticides regardless of whether treated seeds are directly planted in a field.

Richness of aboveground-nesting bees and diet generalist bees were also lower in study field margins where neonicotinoids were present in adjacent field soils. Aboveground-nesting species can interact with contaminated soil, water, and dust through direct contact or indirectly to construct their nests (e.g., mason bees: *Osmia* spp.; Kopit and Pitts-Singer, 2018). We did not control for wind deposition of field soil or planter dust during the course of our study and it is possible that these exposure routes may have contributed to our results. Neonicotinoids have been uniformly measured in planter dust at concentrations >1.4 $\mu\text{g}/\text{m}^2$ up to 100 m from the edge of corn fields in Indiana with higher deposition downwind of the treated field (Krupke et al., 2017). Erosion of surface soil by wind has also been measured in fields with a history of seed treatment use (Limay-Rios et al., 2016) and this particulate may land on bee habitat or foraging plants.

Several studies have measured neonicotinoid concentrations in field margin and crop flowers (Botías et al., 2015; Stewart et al., 2014) that may contribute to pesticide exposure. In our study, we found no detection of neonicotinoids in margin plants over both years; however, we cannot eliminate bee interactions with plant components containing neonicotinoids as a potential route of exposure that may further explain the reduced species richness of several guilds. It is unlikely that neonicotinoids did not partition into the floral media of Conservation Areas. Rather, the decision to aggregate margin floral tissues may have diluted our ability to detect neonicotinoids that were likely present in nectar, pollen, or leaves of plants. We acknowledge that our analytical limits of detection (1 to 6 ppb) likely further limited us from identifying the contribution of neonicotinoid exposure via floral resources, especially at low concentrations. Recent studies have demonstrated that even within a five-year moratorium on neonicotinoid use, insecticide residues were detected in canola nectar (Wintermantel et al., 2020) and clothianidin residues in leaf tissues and nectar may be dependent on the plant species and seasonality (Mogren and Lundgren, 2016).

Despite our initial hypothesis, we found no effect of treatment for all nesting and diet guilds measured in this study; specifically, the abundance and richness of wild bee guilds were not significantly greater in untreated fields. This was in opposition to other neonicotinoid field studies that identified “treatment” as a significant determinant of reductions in wild bee density, solitary bee nesting, and reproduction (Rundlöf et al., 2015; Woodcock et al., 2017). We propose two explanations for this finding. First, in order to evaluate fields with no history of neonicotinoid application, most of our untreated study fields were created from hayfields, old fields, and grassland sites in 2016. Creating experimental fields may have provided an initial disturbance that inadvertently reduced or altered bee communities. Habitat degradation or habitat loss through intensive tillage and mowing of floral and nesting resources can lead to lower pollinator density and diversity (Potts et al., 2016; Williams et al., 2010). Wild bee communities exhibit a range of nesting requirements including availability of cover, proximity to floral resources (including weeds), soil hardness, and soil moisture (Antoine and Forrest, 2020; Julier and Roulston, 2009; Main et al., 2019; Potts et al., 2016; Williams et al., 2010). Secondly, despite never being treated with a neonicotinoid insecticide, several of these same fields were found to contain neonicotinoid residues in collected field and margin soils during a pilot study conducted in 2016 (Main et al., 2020). It is unclear whether our introduced disturbance or previous exposure to neonicotinoids used in surrounding agricultural operations may have precluded our ability to identify an effect of treatment in our current study.

4.1. Implications for wild bee conservation

A criticism of field-based studies that measure neonicotinoid residues is that they often occur over a limited temporal scale or it is unclear to what extent bees will be exposed to neonicotinoids measured in the environment (Lundin et al., 2015). Our study results show over two seasons that presence of neonicotinoids in field soils reduced richness of wild bee nest and diet guilds living in or near agricultural fields on public lands, but annual treatment did not. Despite our findings, our results may indicate a “best case scenario” as, compared to fields in our study, production agriculture fields on private lands are likely to be significantly larger with limited margin habitat availability. Further study is necessary to determine if neonicotinoid seed treatment use on private lands have an even greater impact on wild bee communities. Indeed, small and isolated patches of pollinator habitat or floral resources provide wild bees with inadequate relief from neonicotinoid exposure in agricultural regions (Mogren and Lundgren, 2016). On public lands, agricultural crops can provide valuable food and habitat resources for a range of wildlife species but use of neonicotinoid seed treatments may inadvertently diminish habitat resources for wild bees and other beneficial insects. Therefore, to reduce impacts of neonicotinoid seed treatments to wild bee communities, land managers may consider focusing on eliminating the use of pesticides on areas managed for conservation. As wild bees contribute essential ecosystem services in agroecosystems, they should be considered sentinel species requiring conservation.

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CRediT authorship contribution statement

Anson R. Main: Conceptualization, Investigation, Formal analysis, Writing – original draft, Visualization. **Elisabeth B. Webb:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Keith W. Goynes:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Robert Abney:** Investigation, Writing – review & editing. **Doreen Mengel:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anderson, N.L., Harmon-Threatt, A.N., 2019. Chronic contact with realistic soil concentrations of imidacloprid affects the mass, immature development speed, and adult longevity of solitary bees. *Sci. Rep.* 9, 1–9. <https://doi.org/10.1038/s41598-019-40031-9>.

- Antoine, C.M., Forrest, J.R.K., 2020. Nesting habitat of ground-nesting bees: a review. *Ecol. Entomol.*, ean.12986 <https://doi.org/10.1111/een.12986>.
- Arduser, M.S., 2016. Identification and Ecology of Tallgrass Prairie Bees.
- Ascher, J.S., Pickering, J., 2016. Discover life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). [WWW Document]. URL: <http://www.discoverlife.org/mp/20q?search=Apoidea>. (Accessed 12 December 2019).
- Blaauw, B.R., Isaacs, R., 2014. Flower plantings increase wild bee abundance and the pollination services provided to a pollination-dependent crop. *J. Appl. Ecol.* 51, 890–898. <https://doi.org/10.1111/1365-2664.12257>.
- Blacquière, T., Smagghe, G., van Gestel, C.A.M., Mommaerts, V., 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology* 21, 973–992. <https://doi.org/10.1007/s10646-012-0863-x>.
- Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., Goulson, D., 2015. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. *Environ. Sci. Technol.* 49, 12731–12740. <https://doi.org/10.1021/acs.est.5b03459>.
- Brittain, C., Potts, S.G., 2011. The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. *Basic Appl. Ecol.* 12, 321–331. <https://doi.org/10.1016/j.BAAE.2010.12.004>.
- Brittain, C.A., Vighi, M., Bommarco, R., Settele, J., Potts, S.G., 2010. Impacts of a pesticide on pollinator species richness at different spatial scales. *Basic Appl. Ecol.* 11, 106–115. <https://doi.org/10.1016/j.BAAE.2009.11.007>.
- Brooks, M., Kristensen, K., van Benthem, K., Magnusson, A., Berg, C., Nielsen, A., Skaug, H., Maechler, M., Bolker, B., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378–400.
- Deguines, N., Jono, C., Baude, M., Henry, M., Julliard, R., Fontaine, C., 2014. Large-scale trade-off between agricultural intensification and crop pollination services. *Front. Ecol. Environ.* 12, 212–217. <https://doi.org/10.1890/130054>.
- Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environ. Sci. Technol.* 49, 5088–5097.
- Gathmann, A., Tschamtké, T., 2002. Foraging ranges of solitary bees. *J. Anim. Ecol.* 71, 757–764. <https://doi.org/10.1046/j.1365-2656.2002.00641.x>.
- Geroff, R.K., Gibbs, J., McCravy, K.W., 2014. Assessing bee (Hymenoptera: Apoidea) diversity of an Illinois restored tallgrass prairie: methodology and conservation considerations. *J. Insect Conserv.* 18, 951–964. <https://doi.org/10.1007/s10841-014-9703-z>.
- Gill, K.A., O'Neal, M.E., 2015. Survey of soybean insect pollinators: community identification and sampling method analysis. *Environ. Entomol.* 44 (3), 488–498.
- Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* (80-.) 347.
- Hartig, F., 2020. DHARMa: Residual Diagnostics for Hierarchical (Multi-level/Mixed) Regression Models.
- Hladik, M.L., Main, A.R., Goulson, D., 2018. Environmental risks and challenges associated with neonicotinoid insecticides. *Environ. Sci. Technol.* 52, 3329–3335. <https://doi.org/10.1021/acs.est.7b06388>.
- Joshi, N.K., Leslie, T., Rajotte, E.G., Kammerer, M.A., Otieno, M., Biddinger, D.J., 2015. Comparative trapping efficiency to characterize bee abundance, diversity, and community composition in apple orchards. *Ann. Entomol. Soc. Am.* 108, 785–799.
- Julier, H.E., Roulston, T.H., 2009. Wild bee abundance and pollination service in cultivated pumpkins: farm management, nesting behavior and landscape effects. *J. Econ. Entomol.* 102, 563–573. <https://doi.org/10.1603/029.102.0214>.
- Kamel, A., 2010. Refined methodology for the determination of neonicotinoid pesticides and their metabolites in honey bees and bee products by liquid chromatography-tandem mass spectrometry (LC-MS/MS). *J. Agric. Food Chem.* 58, 5926–5931.
- Kim, J., Williams, N., Kremen, C., 2006. Effects of cultivation and proximity to natural habitat on ground-nesting native bees in California sunflower fields. *J. Kans.* 79, 309–320.
- Kopit, A.M., Pitts-Singer, T.L., 2018. Routes of pesticide exposure in solitary, cavity-nesting bees. *Environ. Entomol.* 47, 499–510. <https://doi.org/10.1093/ee/nvy034>.
- Kremen, C., Williams, N.M., Thorp, R.W., 2002. Crop pollination from native bees at risk from agricultural intensification. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16812–16816.
- Krupke, C.H., Holland, J.D., Long, E.Y., Eitzer, B.D., 2017. Planting of Neonicotinoid-treated Maize Poses Risks for Honey Bees and Other Non-target Organisms Over a Wide Area Without Consistent Crop Yield Benefit. <https://doi.org/10.1111/1365-2664.12924>.
- Lehotay, S.J., DeKok, A., Hiemstra, M., van Bodegraven, P., 2005. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection. *J. AOAC Int.* 88, 595–614.
- Limay-Rios, V., Forero, L.G., Xue, Y., Smith, J., Baute, T., Schaafsma, A., 2016. Neonicotinoid insecticide residues in soil dust and associated parent soil in fields with a history of seed treatment use on crops in southwestern Ontario. *Environ. Toxicol. Chem.* 35, 303–310. <https://doi.org/10.1002/etc.3257>.
- Long, E.Y., Krupke, C.H., 2016. Non-cultivated plants present a season-long route of pesticide exposure for honey bees. *Nat. Commun.* 7, 1–12. <https://doi.org/10.1038/ncomms11629>.
- Lundin, O., Rundlöf, M., Smith, H.G., Fries, I., Bommarco, R., 2015. Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. *PLoS One* 10, 1–20. <https://doi.org/10.1371/journal.pone.0136928>.
- Main, A.R., Webb, E.B., Goynes, K.W., Mengel, D., 2019. Field-level characteristics influence wild bee functional guilds on public lands managed for conservation. *Glob. Ecol. Conserv.* 17. <https://doi.org/10.1016/j.gecco.2019.e00598>.
- Main, A.R., Webb, E.B., Goynes, K.W., Mengel, D., 2020. Reduced species richness of native bees in field margins associated with neonicotinoid concentrations in non-target soils. *Agric. Ecosyst. Environ.* 287, 106693. <https://doi.org/10.1016/j.agee.2019.106693>.
- McCravy, K.W., Geroff, R.K., Gibbs, J., 2019. Bee (Hymenoptera: Apoidea: Anthophila) functional traits in relation to sampling methodology in a restored tallgrass prairie. *Florida Entomol.* 102, 134. <https://doi.org/10.1653/024.102.0122>.

- Mogren, C.L., Lundgren, J.G., 2016. Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status. *Sci. Rep.* 6, 1–10.
- Park, M.G., Blitzer, E.J., Gibbs, J., Losey, J.E., Danforth, B.N., 2015. Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proc. R. Soc. B Biol. Sci.* 282. <https://doi.org/10.1098/rspb.2015.0299>.
- de Perre, C., Murphy, T.M., Lydy, M.J., 2015. Fate and effects of clothianidin in fields using conservation practices. *Environ. Toxicol. Chem.* 34, 258–265. <https://doi.org/10.1002/etc.2800>.
- Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M., Morrissey, C.A., Noome, D.A., Settele, J., Simon-Delso, N., Stark, J.D., der Sluijs, J.P., Van Dyck, H., Wiemers, M., 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ. Sci. Pollut. Res.* 22, 68–102. <https://doi.org/10.1007/s11356-014-3471-x>.
- Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J., Vanbergen, A.J., 2016. Safeguarding pollinators and their values to human well-being. *Nature* 540, 220–229. <https://doi.org/10.1038/nature20588>.
- R Core Team, 2020. *R: A Language and Environment for Statistical Computing*.
- Rundlöf, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J., Smith, H.G., 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* 521, 77.
- Sardiñas, H.S., Tom, K., Ponisio, L.C., Rominger, A., Kremen, C., 2016. Sunflower (*Helianthus annuus*) pollination in California's Central Valley is limited by native bee nest site location. *Ecol. Appl.* 26, 438–447. <https://doi.org/10.1890/15-0033>.
- Stephenson, P.L., Griswold, T.L., Arduser, M.S., Dowling, A.P.G., Kremen, D.G., 2018. Checklist of bees (Hymenoptera: Apoidea) from managed emergent wetlands in the lower Mississippi Alluvial Valley of Arkansas. *Biodivers. Data J.*, e24071 <https://doi.org/10.3897/BDJ.6.e24071>.
- Stewart, S.D., Lorenz, G.M., Catchot, A.L., Gore, J., Cook, D., Skinner, J., Mueller, T.C., Johnson, D.R., Zawislak, J., Barber, J., 2014. Potential exposure of pollinators to neonicotinoid insecticides from the use of insecticide seed treatments in the mid-southern United States. *Environ. Sci. Technol.* 48, 9762–9769. <https://doi.org/10.1021/es501657w>.
- Williams, N.M., Crone, E.E., Roulston, T.H., Mincley, R.L., Packer, L., Potts, S.G., 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. *Biol. Conserv.* 143, 2280–2291. <https://doi.org/10.1016/j.biocon.2010.03.024>.
- Willis Chan, D.S., Raine, N.E., 2021. Population decline in a ground-nesting solitary squash bee (*Eucera pruinosa*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*). *Sci. Rep.* 11, 1–11.
- Willis Chan, D.S., Prosser, R.S., Rodríguez-Gil, J.L., Raine, N.E., 2019. Assessment of risk to hoary squash bees (*Peponapis pruinosa*) and other ground-nesting bees from systemic insecticides in agricultural soil. *Sci. Rep.* 9, 1–13. <https://doi.org/10.1038/s41598-019-47805-1>.
- Wintermantel, D., Odoux, J.F., Decourtye, A., Henry, M., Allier, F., Bretagnolle, V., 2020. Neonicotinoid-induced mortality risk for bees foraging on oilseed rape nectar persists despite EU moratorium. *Sci. Total Environ.* 704, 135400.
- Woodcock, B.A., Isaac, N.J.B., Bullock, J.M., Roy, D.B., Garthwaite, D.G., Crowe, A., Pywell, R.F., 2016. Impacts of neonicotinoid use on long-term population changes in wild bees in England. *Nat. Commun.* 7, 12459.
- Woodcock, B.A., Bullock, J.M., Shore, R.F., Heard, M.S., Pereira, M.G., Redhead, J., Ridding, L., Dean, H., Sleep, D., Henrys, P., Peyton, J., Hulmes, S., Hulmes, L., Sárospataki, M., Saure, C., Edwards, M., Genersch, E., Knäbe, S., Pywell, R.F., 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. *Science* (80-.) 356, 1393–1395.
- Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., Dorn, S., 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biol. Conserv.* 143, 669–676. <https://doi.org/10.1016/j.biocon.2009.12.003>.