



## Reduced species richness of native bees in field margins associated with neonicotinoid concentrations in non-target soils

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### ABSTRACT

Native bees are in decline as many species are sensitive to habitat loss, climate change, and non-target exposure to synthetic pesticides. Recent laboratory and semi-field assessments of pesticide impacts on bees have focused on neonicotinoid insecticides. However, field studies evaluating influences of neonicotinoid seed treatments on native bee communities of North America are absent from the literature. On four Conservation Areas of Missouri, we sampled row-cropped (treated,  $n = 15$ ) and reference (untreated,  $n = 9$ ) agricultural fields, and their surrounding field margins for neonicotinoids in soil and non-target vegetation (i.e., native wildflowers). Wildflowers were further collected and screened for the presence of fungicides. Concurrently, we sampled native bees over three discrete time points throughout the agricultural growing season to assess potential impacts of seed treatment use on local bee populations over time. Neonicotinoids were detected in 87% to 100% of treated field soils and 22% to 56% of reference field soils. In adjacent field margin soils, quantifiable concentrations were measured near treated (53% to 93% detection) and untreated fields (33% to 56% detection). Fungicides were detected in < 40% of wildflowers, whereas neonicotinoids were rarely detected in field margin vegetation (< 7%). Neonicotinoid concentrations in margin soils were negatively associated with native bee richness ( $\beta = -0.21$ ,  $P < 0.05$ ). Field margins with a combination of greater neonicotinoid concentrations in soil and fungicides in wildflowers also contained fewer wild bee species ( $\beta = -0.21$ ,  $P < 0.001$ ). By comparison, bee abundance was positively influenced by the number of wildflower species in bloom with no apparent impact of pesticides. Results of this study indicate that neonicotinoids in soil are a potential route of exposure for pollinator communities, specifically ground-nesting species. Importantly, native bee richness in non-target field margins may be negatively affected by the use of neonicotinoid seed treatments in agroecosystems.

### 1. Introduction

Globally, pollinator communities – both wild and domesticated – are in decline (Gill et al., 2016; Potts et al., 2010). Pollination is an invaluable ecosystem service that not only maintains diverse wild plant communities, but further increases agricultural productivity (Garibaldi et al., 2013). Approximately 80% of all wild plant species and 75% of all crop types used for human consumption rely, to some extent, on insect pollination (Klein et al., 2007). Numerous factors are likely contributing to pollinator declines, including land-use changes (e.g., habitat loss, fragmentation), invasive species (e.g., plants, pests), climate change, and environmental stressors (e.g., agrochemicals, land management decisions; Gill et al., 2016; Potts et al., 2016). Research

efforts have primarily focused on understanding honeybee declines compared to wild bee populations, due to widespread reliance and economic value of honeybee pollination services. Since the mid-2000s, many studies have concentrated on colony collapse disorder through evaluation of metrics such as bee physiology, pests, fungi, and pathogens (e.g., *Varroa* mites, *Nosema*; vanEngelsdorp et al., 2009), and pesticide levels, including fungicides and insecticides (e.g., neonicotinoids; Alaux et al., 2010; Potts et al., 2016). Wild insect pollinators, including native bees and butterflies, are critically important beneficial guilds that remain less well-understood (Potts et al., 2010); however, studies suggest that wild bees may enhance overall crop pollination (Greenleaf and Kremen, 2006). Indeed, wild insects may pollinate crops more effectively than honeybees with greater increases in yields

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(Garibaldi et al., 2013).

Agricultural intensification – including increased chemical inputs and cultivation of marginal land – may reduce native pollinator communities and their effectiveness to provide ecosystem services (Kremen et al., 2002; Potts et al., 2016). Surrounding landscape composition and function are important components of species-rich agroecosystems, with more organic systems supporting a greater diversity of native pollinators (Holzschuh et al., 2008; Kennedy et al., 2013; Weibull and Östman, 2003). The quality of habitat resources, habitat connectivity, and local management practices (e.g., maintenance of vegetation communities) are critical for overall health of pollinator populations (Kennedy et al., 2013; Main et al., 2019; Williams and Kremen, 2007). Although landscape heterogeneity is arguably important for pollinator success, the potential effects of pesticides on non-target pollinators from extensive use on agroecosystems is an issue of growing concern (Brittain et al., 2010; Gill et al., 2012).

Native bee and butterfly communities may be threatened by the growing popularity and rapid increase in neonicotinoid insecticide applications (e.g., seed treatments). Neonicotinoids are systemic insecticides which are translocated into plant tissues from soil and can negatively impact non-target insects, cover crops for beneficial insects, and margin plant communities (Botías et al., 2016; Bredeson and Lundgren, 2019; Rundlöf et al., 2015; Stewart et al., 2014). However, study results have reached mixed conclusions. Negative effects of neonicotinoid use on wild bees, including reduced bee density (Rundlöf et al., 2015), offspring production in solitary bees (*Osmia bicornis*; Sandrock et al., 2014) or queen production in bumblebees (*Bombus terrestris*; Whitehorn et al., 2012) have been reported. Other research has indicated neonicotinoids have no detrimental effects on reproduction or development of native bees (Peters et al., 2016; Sterk et al., 2016). Importantly, treated-seed may not only contain neonicotinoids, but also contain other active ingredients, additives, fungicides, adjuvants, and lubricants (Myers and Hill, 2014) which could lead to interactions between neonicotinoids and other chemical stressors. Field studies remain limited and most investigations have examined a small subset of species or have relied extensively on single-species toxicity testing (Pisa et al., 2015). Even less is understood about potential effects of neonicotinoid seed treatments on native pollinator abundance and species richness in agroecosystems.

In the Midwestern United States (US), neonicotinoids are extensively used in production of major row crops such as maize (i.e., corn) and soybeans. In 2011, of the US area planted to soybeans and maize, ~44% of soybean and 79–100% of maize crops were treated with a neonicotinoid insecticide (Douglas and Tooker, 2015). Although modern agricultural practices tend to maximize field area for planting, in some regions, uncultivated strips of land (i.e., field margins) containing wildflowers, grasses, and shrubs are maintained. Field margins provide habitat for birds, predatory insects, and pollinators (Kennedy et al., 2013). However, these margins may become contaminated by agrochemicals used on adjacent crops (Goulson, 2013). Neonicotinoids are highly water soluble (Bonmatin et al., 2015), may leach, and are readily transported during rainfall (Chrétien et al., 2017) and snowmelt events (Main et al., 2016). As neonicotinoids can persist in soil even years after application (de Perre et al., 2015; Jones et al., 2014), off-site movement could lead to potential neonicotinoid accumulation and persistence in margin soils (Jones et al., 2014). Transport of neonicotinoid-contaminated soil particles from fields to local margins may be furthered by wind erosion (Limay-Rios et al., 2016; Schaafsma et al., 2015). Far less is known about neonicotinoid behavior in agricultural field and field margin soils of major cropping regions such as the Midwestern US. Accumulation by non-target plants could affect native pollinators foraging in these areas as wild plant pollen sources were previously shown to contain neonicotinoid concentrations up to 86 µg/kg (Botías et al., 2015). As most native bees in the US are solitary, ground-nesting, and forage close to their nest, it is unclear how neonicotinoid use may affect native bee abundance and/or richness in

agroecosystem fields and field margins.

Across the Midwestern US, thousands of hectares (ha) of public land are annually cultivated for production of agricultural crops, food plots, and cover for wildlife (e.g., ~3176 ha, Nebraska Game and Parks; > 20,000 ha, Missouri Dept. of Conservation). Many of these conservation and/or wildlife areas have historically used neonicotinoid seed treatments on crops such as maize, soybean, and sunflowers. However, it is unclear as to what extent neonicotinoids occurred in Conservation Area soils, whether they would persist (if detected), or if Area managers would need to take mitigating actions regardless of seed treatment application. Here, we present the results of a baseline field study evaluating the potential impacts of neonicotinoid seed treatment use on wild bee communities found on public lands throughout north-central Missouri. We compared wild bee and pesticide concentration data collected from treated row crop fields and field margins against data collected from untreated, uncultivated reference fields and their surrounding margins. To assess the potential for neonicotinoid seed treatments to impact local native bee communities, we evaluated (1) potential routes of exposure in fields and margins based on neonicotinoid concentrations detected in soil and plants (all study fields), and (2) changes in pollinator abundance and richness over time in those same fields and margins. We accomplished this by tracking concentrations in soil and plants through discrete phases of the agricultural growing season (e.g., from pre-seeding to harvest), where we overlapped sampling dates with collection of local pollinators. Compared to margins of fields sown with neonicotinoid treated seeds, we hypothesized that neonicotinoid concentrations would be lower in margins surrounding untreated reference fields. However, we anticipated that we would observe a negative association between field margin concentrations and bee abundance/richness, regardless of cultivation type or where concentrations were observed. We used baseline data collected during this study to inform a future experimental evaluation of neonicotinoid seed treatments on Conservation Areas of Missouri.

## 2. Methods

### 2.1. Study area

We conducted fieldwork at four Missouri Department of Conservation Areas (CA) situated in north-central Missouri. Our initial site selection was limited to areas situated in two Major Land Resource Areas found in northern Missouri, US: the Central Claypan and the Iowa and Missouri Heavy Till Plain. Missouri CA are managed public lands, which include areas under agricultural production to provide food resources and habitat cover for wildlife. The state's dominant agricultural row crops include maize and soybeans, the majority of which are typically planted using a neonicotinoid seed treatment. Missouri experiences a humid continental climate with strong seasonality. Temperature variation across the state can be substantial with mild to cold winters (e.g., mean low, January:  $-1^{\circ}\text{C}$ ) and warm to hot and humid summers (e.g., mean maximum, July:  $31^{\circ}\text{C}$ ). Mean annual precipitation comes in the form of showers or thunderstorms and varies across the state from a low of 864 mm in the northwest to a high of 1270 mm in the southeast (Decker, 2018).

### 2.2. Study design

We collected field data from early April through late September of 2016 at 24 agricultural fields (mean  $\pm$  SE: 1.87 ha  $\pm$  0.27) located across four CA which included: Indian Hills ( $40.33749^{\circ}\text{N}$ ,  $92.2492^{\circ}\text{W}$ ), Fountain Grove ( $39.72338^{\circ}\text{N}$ ,  $93.31714^{\circ}\text{W}$ ), Atlanta ( $39.88961^{\circ}\text{N}$ ,  $92.49373^{\circ}\text{W}$ ), and Whetstone Creek ( $38.96766^{\circ}\text{N}$ ,  $91.71155^{\circ}\text{W}$ ). Each CA contained six study fields arranged in a modified random block design (treated,  $n = 4$ , untreated,  $n = 2$ ) as we were limited by availability of untreated fields. Although several of our reference fields were > 1 km from treated study fields, some untreated fields were

within 500 m of other agricultural fields unrelated to our study. We controlled for treatment type by selecting fields with a known crop rotation history. Treated fields ( $n = 15$ ) had previously received at least one neonicotinoid seed treatment within the past five years. Reference fields (=untreated;  $n = 9$ ) included areas that had never received a neonicotinoid seed treatment (e.g., hayfields) or had not been planted using neonicotinoid-treated seed in  $> 10$  years. During the year of data collection all treated fields were planted to either clothianidin-treated maize ( $n = 11$ ; Poncho® Bayer CropScience) or imidacloprid-treated soybeans ( $n = 4$ ; Gaucho® Bayer CropScience). It is important to note that the nine untreated fields remained in their current state of use (e.g., hayfield, idle fields = uncultivated). As we were using data reflecting baseline conditions for this ‘observational’ study, we did not seek to directly compare treated and untreated agricultural row crops. We acknowledge this may have confounded our study results; however, it is not uncommon for row crops to be situated near uncultivated fields on Conservation Areas or other agricultural areas of the Midwestern US. Field margins surrounding both treated and reference fields were a mix of herbaceous and woody material and the proportion of surrounding natural habitat was  $> 60\%$  for 21 of 24 fields (USDA, 2016). Because  $\sim 70\%$  of native bees in North America nest in the ground (Holm, 2014), we collected soil, crop, and wildflowers from all study fields and their associated margins to determine neonicotinoid and fungicide concentrations on Conservation Areas. These media may act as potential routes of exposure for native pollinators.

### 2.3. Soil sampling

Soil sampling occurred at four discrete periods throughout the agricultural growing season: pre-seeding (mid-April), two weeks post-seeding (mid-June), mid-growing (July), and the harvest period (September). We collected soil from cultivated fields using a JMC Backsaver Handle with a 46 cm dry sampling tube. All samples were collected by walking across the field in an M pattern (Schaafsma et al., 2015) with  $\sim 15$  subsamples randomly taken from the top 10 cm of soil (Botías et al., 2015). Samples were consistently collected between rows to minimize collection of soil from directly treated seed area. Similarly, we created composite samples ( $\sim 1.5$  kg) of uncultivated margin soil by randomly collecting  $\sim 15$  subsamples from the surrounding field margins. All margin soil samples were taken  $\sim 2$  m from the edge of the cultivated field (Botías et al., 2015) to minimize direct contamination from treated seeds. For both field and margins, subsamples were each  $\sim 100$  g and all subsamples were pooled in new Ziploc® polyethylene freezer bags before being placed into coolers for transport to the laboratory. Upon returning from the study sites, separate field and margin soil samples were thoroughly mixed and a composite for each zone was then stored in pre-cleaned 250 ml amber glass jars. All jars were stored in the freezer ( $-20^\circ\text{C}$ ) for approximately three months until analysis.

### 2.4. Plant collection

To quantify neonicotinoid concentrations in non-target vegetation (e.g., wildflowers, flowering shrubs), we harvested aboveground biomass from all field margins. Plant material was also collected from row crops and reference fields (see Supplemental Information). Beginning in June (post-seeding), margin plant samples were collected during three time periods mentioned above. At each field margin, we randomly selected collection locations based on both pollinator interactions with flowering plants and margin flower availability. All sampling locations were situated  $\sim 2$  m from the cultivated edge (Botías et al., 2015) or within 2 m of where the reference fields were surrounded by the presence of wildflowers and shrubs (i.e., margin). Using a 50 cm  $\times$  50 cm quadrat, we recorded plant cover (%), identified plant species, and then harvested all aboveground biomass within the quadrat. To harvest plant biomass, we used standard garden hedge shears with 23 cm serrated

blades to cut plant material as close to the ground surface as possible. This procedure was repeated in the margins at each site three times. All material collected from individual quadrats was mixed together to form a representative composite for the overall study field margin community. A subset of material was then placed in a new Ziploc® polyethylene freezer bag. All plant samples were kept in coolers during transport back to the laboratory where they were then stored in freezers at  $-20^\circ\text{C}$  until analysis. Plants were stored frozen for almost one year before any extractions were performed.

### 2.5. Native bee collection

During the growing season of 2016, native bees were sampled across all CAs in the field margins surrounding our treated row crops and uncultivated reference fields (e.g., hayfields, idle fields). Except for the pre-seeding period, pollinator sampling occurred during the same periods listed for soil and plant tissue collections (see above). We used SpringStar™ blue vane traps (SpringStar Inc., Woodinville, WA) to monitor bee communities at each site (Hladik et al., 2016). Blue vane traps (BVT) are highly effective and capture significantly greater numbers of species compared to other trap methods (Joshi et al., 2015). This trend is especially true for larger bees (e.g., *Bombus*, *Melissodes*) that may otherwise avoid pan traps (Geroff et al., 2014). We installed three traps in each study field margin using a linear pattern with  $\sim 30$  m between traps. Where possible, all traps were placed at vegetation height to better insure visibility. A more detailed description of BVT and their installation is found in Main et al. (2019). BVT were void of any euthanizing agent and typically deployed for a 24 h period beginning at  $\sim 700$  h Central Standard Time.

We further validated trap capture by conducting sweep-net samples to fully characterize local bee fauna (Roulston et al., 2007). Researchers walked the margin surrounding study fields for 40 min intervals at each site, for a total of 80 min sweep-net sampling per field. Net-caught bees were placed in jars containing ethyl acetate. Bees collected in traps and sweep-nets were placed in 50-mL Falcon centrifuge tubes and frozen at  $-20^\circ\text{C}$  until identification. All bees removed from public lands in this study were authorized for collection under a MDC Wildlife Collector's Permit (#16916).

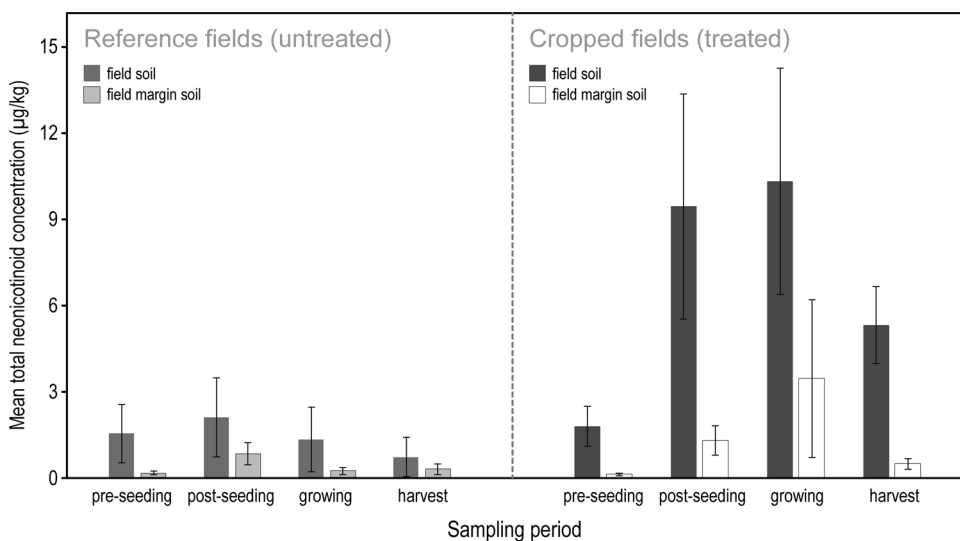
### 2.6. Bee identification

All previously frozen bees were processed in the laboratory. Bee specimens were identified using the Discover Life key (Ascher and Pickering, 2016) in tandem with a key of tallgrass prairie bees outlined in Arduser (2016). All bees were identified to species with the exception of genera *Ceratina* and *Lasioglossum* (*Dialictus*) spp., which were classified to morphospecies. A representative voucher collection is stored at the University of Missouri in Columbia, MO.

### 2.7. Pesticide analyses: soil and plant tissues

Field and field margin soils were analyzed at the University of Nebraska Water Sciences Laboratory (Lincoln, NE) following previously published methods (Satkowski et al., 2018). Concentrations of six neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) were determined by microwave-assisted solvent extraction followed by analysis. All analyses were conducted on a Waters Model 2695 high-performance liquid chromatography [HPLC] system combined with a Quattro Micro triple quadrupole mass spectrometer (LC-MS/MS). A detailed description of the analytical methods is presented in Satkowski et al. (2018).

Treated row crop, untreated reference field (e.g., clover in hayfields), and field margin plant tissues (e.g., wildflowers) were analyzed by the accredited USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories (Gastonia, NC). Samples were analyzed to determine concentrations of



**Fig. 1.** Mean total neonicotinoid concentrations in agricultural field and surrounding field margin soils ( $\mu\text{g}/\text{kg}$ ) across four sampling periods of 2016. Concentrations are measured in study fields that were designated as reference fields (untreated) or cropped fields (treated) located on Missouri Conservation Areas. Error bars indicate the standard error.

neonicotinoid and fungicide residues in target and non-target plant tissues. All plant samples were analyzed for the presence of 11 pesticides including neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam), fungicides (azoxystrobin, fluxapyroxad, metalaxyl, pyraclostrobin, trifloxystrobin) and the systemic insecticide flonicamid. The fungicides chosen for analyses are often included on treated seed and/or are widely used in our study region. Further details are presented in Supplemental Information.

## 2.8. Statistical analyses

For the statistical analyses mentioned herein, we estimated bee abundance by combining field margin BVT and timed margin sweeps = cumulative margin bee abundance. Species richness was further estimated to be cumulative across margin traps and sweeps due to the same sampling effort occurring in every study field. Here, we present the results of smaller native species who are more likely to be restricted in their foraging and/or maximum flight distance (Benjamin et al., 2014). Although we caught numerous large bees (e.g., bumble bees (*Bombus* spp.)), we chose to model them separately due to their potential to act as long-distant foragers whose flight distance can be greater than 1500 m (Benjamin et al., 2014). Results of models that include all bees caught during the study (including large species) are presented in Supplemental Information.

As we were primarily interested in potential impacts of neonicotinoid seed treatment on native bees, we included pesticide variables in each of our models. Pesticide metrics were summed as total field margin soil concentration (neonicotinoids) and total field margin plant fungicide concentrations. Bees are likely exposed to a range of pesticides in the natural environment (Hladik et al., 2016), which may promote additive or synergistic effects (Lundin et al., 2015). Concentration data were log-transformed prior to analysis to improve model fit.

### 2.8.1. Effect of pesticides and field variables on field margin bee abundance and richness

We analyzed effects of neonicotinoid seed treatment use and field-level variables on native bee abundance and richness in field margins using a series of generalized linear mixed-effects models (GLMER) in package “lme4” (Bates et al., 2015) in R 3.4.4 (R Core Team, 2018). We used a negative binomial distribution as our insect count data was zero-inflated. Model fixed effects included field margin soil concentration, fungicide concentrations in field margin vegetation, crop type, margin floral richness, and time. Crop type was included as a fixed effect to account for potential differences between maize, soybean, and

reference fields and also act as a proxy for physical disturbance. Time was defined as the sampling period (i.e., month of sample collection: June, July, and September). Field ID was nested in Conservation Area as a random effect to account for the block study design and to account for repeated sampling within fields. We developed a global model for each response variable (abundance, richness) that included the fixed effects listed above and two interactions including margin soil concentration  $\times$  time and margin soil concentration  $\times$  plant fungicide concentration. Finally, using package ‘MuMin’ (Bartoń, 2016), we conducted subset model selection where candidate models are ranked based on comparison of corrected Akaike information criteria (AICc) and model weights. Model fit was evaluated through the calculation of pseudo  $R^2$  values. The marginal  $R^2$  value (or the proportion of variance explained by fixed effects alone) is presented for the top abundance and richness models. Further statistical details are provided in the supplemental information.

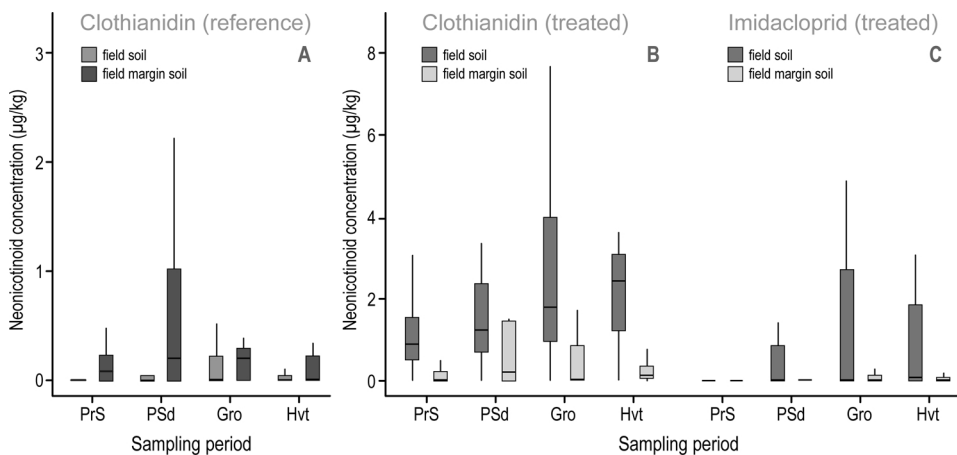
## 3. Results

### 3.1. Neonicotinoid concentrations in agricultural field and field margin soils

Neonicotinoids were detected in 87% (pre-seeding) to 100% (harvest) of soils collected from row-cropped fields where neonicotinoid seed treatments had been used. Field margins surrounding treated fields had detectable neonicotinoid residues in 53% (pre-seeding) to 93% (harvest) of margin soil samples (Table S1). By comparison, neonicotinoids were less frequently detected in untreated agricultural field soils (reference sites) and their associated field margins. Between 22% (pre-seeding) and 56% (harvest) of untreated field soil samples and 33% (post-seeding) to 56% (growing period) of adjacent field margin soils contained detectable residues. Mean total neonicotinoid concentrations in untreated fields and field margins remained relatively low throughout the study ( $< 3 \mu\text{g}/\text{kg}$ ; Fig. 1). Overall, clothianidin (62%) was the most frequently detected neonicotinoid followed by imidacloprid (24%) and thiamethoxam (3%). Concentrations varied across the study and clothianidin in soil collected from treated fields had the greatest mean and maximum values during all four sampling points (Table S1, Fig. 2).

### 3.2. Neonicotinoid and fungicide concentrations in field margin plant tissues

Neonicotinoids were rarely detected in field margin vegetation associated with treated or untreated fields. Neonicotinoid concentrations were greatest in wildflower plants (clothianidin, mean: 0.22, max: 9.8  $\mu\text{g}/\text{kg}$ ) harvested from a field margin associated with a treated field



**Fig. 2.** Boxplot comparison of differences between neonicotinoid active ingredient concentrations (µg/kg) detected in soil of (A) reference fields (untreated) and adjacent field margins; (B,C) row-cropped agricultural fields (treated) and adjacent field margins. Outliers are omitted from the figure. Only the most frequently detected neonicotinoid active ingredients are presented here. Sampling period codes on the x-axes are as follows: PrS (pre-seeding), PSd (post-seeding), Gro (growing), and Hvt (harvest).

(Table 1). Only the strobilurin fungicides were detected in wildflower plant tissues collected from field margins surrounding treated and untreated fields; however, maximum concentrations were greater in plants from field margins associated with untreated fields (azoxystrobin, mean: 0.52, max: 9 µg/kg).

### 3.3. Native bees collected in agricultural field margins

From June to September, we collected 1411 native bees from field margins surrounding treated and untreated study fields. Bees represented individuals from 22 distinct genera accounting for 55 species (excluding morphospecies) collected during the course of the study. In margins of reference fields, the number of individuals from different genera most frequently collected followed the order *Lasioglossum* (149) > *Bombus* (87) > *Melissodes* (72; Table 2). In contrast, the number of individuals from different genera most frequently collected in field margins surrounding treated fields followed the order *Melissodes* (183) > *Bombus* (162) > *Lasioglossum* (153). Species from the genera *Anthophora*, *Nomada* and *Osmia* were only collected in field margins associated with untreated fields; whereas, species from *Augochlorella* were caught only in field margins adjacent to treated fields.

### 3.4. Effects of pesticides and field variables on field margin bee abundance

Our best model indicated that sampling period (= Time) and margin floral richness explained 27% of the variation in field margin bee abundance (Table 3). The relationship between time and wild bee abundance was negative with fewer bees in field margins over the course of the study ( $\beta = -0.30 \pm 0.12$ ,  $z = -2.55$ ,  $P = 0.01$ ). Furthermore, the number of wildflower species, measured as floral richness, was positively associated with greater bee abundance in field margins ( $\beta = 0.21 \pm 0.09$ ,  $z = 2.26$ ,  $P = 0.02$ ). In contrast, neither measured pesticide concentrations nor differences in crop type were retained in our top model. We found a similar pattern when large bees were included in our abundance model (Table S5).

**Table 1**

Summary of detections and neonicotinoid concentrations (µg/kg) in plant tissues collected from field margins surrounding reference fields (untreated;  $n = 9$ ) and cultivated row crop fields (treated;  $n = 15$ ) located on Missouri Conservation Areas in 2016. ND indicates no detection. The limit of detection (LOD) for each neonicotinoid active ingredient is indicated.

Margin Plants	Reference field margins			Treated field margins			
	Detections (%)	Mean	Max	Detections (%)	Mean	Max	LOD (ppb)
Clothianidin	3.7	0.05	1.3	2.2	0.22	9.8	6
Imidacloprid	0	ND	ND	0	ND	ND	2
Thiamethoxam	3.7	0.07	2	6.7	0.07	1	1

**Table 2**

Abundance of native bees collected in 24 field margins surrounding reference (untreated;  $n = 9$ ) and treated fields ( $n = 15$ ) located on Missouri Conservation Areas in year 2016. The total number of bees (1411) caught in field margins by Blue Vane Traps (BVT) and margin sweep-nets (Sweep) are differentiated. The number of individual bee species by genera are indicated in parentheses.

Family	Genera ( $n$ spp.)	Reference		Treated		
		BVT	Sweep	BVT	Sweep	
Andrenidae	<i>Andrena</i> spp. (2)	3	0	0	4	
Apidae	<i>Anthophora</i> sp. (1)	0	1	0	0	
	<i>Bombus</i> spp. (6)*	43	44	81	81	
	<i>Ceratina</i> spp.	4	6	15	9	
	<i>Eucera</i> spp. (2)	11	19	23	11	
	<i>Melissodes</i> spp. (9)	44	28	122	61	
	<i>Nomada</i> spp. (2)	0	1	0	0	
	<i>Ptilothrix</i> sp. (1)	1	0	7	3	
	<i>Svastra</i> sp. (1)	5	3	6	1	
	<i>Triepeolus</i> spp. (4)	0	2	1	5	
	<i>Xylocopa</i> sp. (1)*	2	2	13	11	
	Colletidae	<i>Hylaeus</i> sp. (1)	9	4	1	3
	Halictidae	<i>Agapostemon</i> spp. (4)	8	3	19	5
		<i>Augochlora</i> sp. (1)	7	16	83	33
<i>Augochlorella</i> sp. (1)		0	0	3	2	
<i>Augochloropsis</i> sp. (1)		12	11	13	12	
<i>Halictus</i> spp. (5)		44	12	72	14	
Megachilidae	<i>Lasioglossum</i> spp.	86	63	47	106	
	<i>Coelioxys</i> sp. (1)	0	1	0	1	
	<i>Heriades</i> sp. (1)	1	0	1	1	
	<i>Megachile</i> spp. (9)	8	5	10	20	
	<i>Osmia</i> spp. (2)	1	1	0	0	
Overall total		289	222	517	383	

\* Indicate genera considered long-distance foragers that were modeled separately.

### 3.5. Effects of pesticides and field variables on field margin bee richness

Our second model evaluating bee richness retained time, neonicotinoid soil concentration, and total fungicide concentrations in field

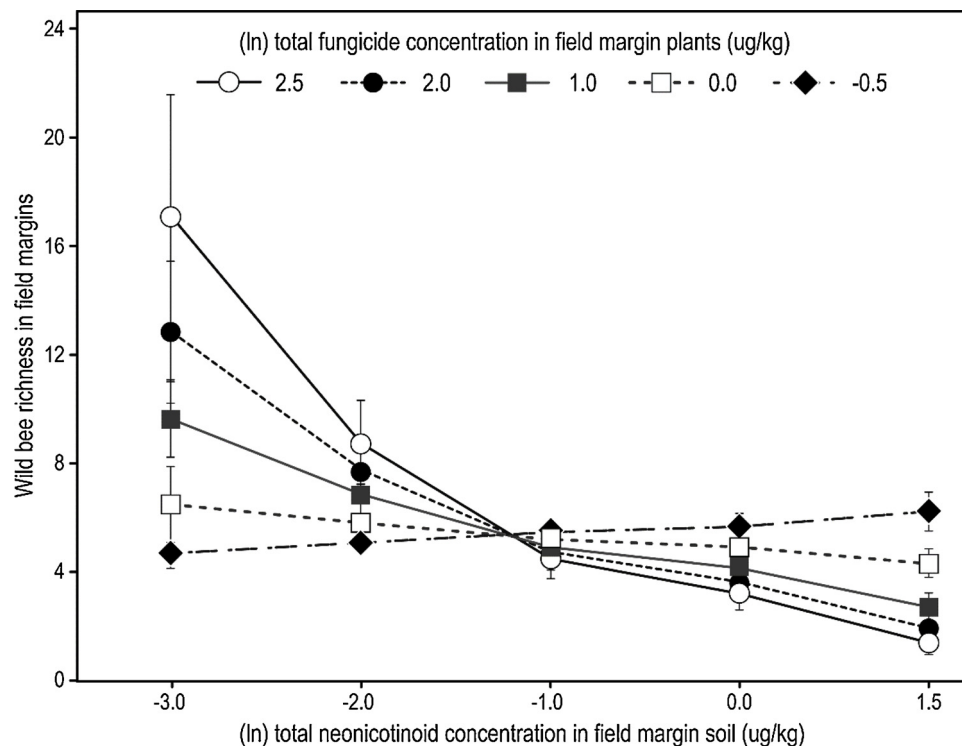
**Table 3**

The AICc model selection table for the analysis of native bee abundance and richness in Conservation Area agricultural field margins using pesticide and field-level variables (2016). AICc values from generalized linear mixed models are shown and include change in AICc ( $\Delta$  AICc), model weights (AIC<sub>WT</sub>), and marginal R<sup>2</sup> values for the best model. Only the top five models and the null model are presented with the best model indicated in bold text.

Top model set: Abundance <sup>1</sup>	AICc	$\Delta$ AICc	AIC <sub>WT</sub>	Marginal R <sup>2</sup>
<b>Time + Floral Richness</b>	<b>1112.5</b>	<b>0.0</b>	<b>0.22</b>	<b>0.27</b>
Time + Floral Richness + Plant Fung.	1113.8	1.3	0.12	
Time + Plant Fung.	1114.5	2.0	0.08	
Time + Floral Richness + MSoil Neonic.	1114.7	2.1	0.08	
Time + Floral Richness + MSoil Neonic. x Plant Fung.	1115.1	2.6	0.06	
Null	1118.9	6.4	–	
Top model set: Richness <sup>1</sup>	AICc	$\Delta$ AICc	AIC <sub>WT</sub>	Marginal R <sup>2</sup>
<b>MSoil Neonic. x Time + MSoil Neonic. x Plant Fung.</b>	<b>944.5</b>	<b>0.0</b>	<b>0.59</b>	<b>0.24</b>
Crop + MSoil Neonic. x Time + MSoil Neonic. x Plant Fung.	946.2	1.7	0.25	
Time + MSoil Neonic. x Plant Fung.	947.9	3.4	0.11	
Time + Crop + MSoil Neonic. x Plant Fung.	949.4	4.9	0.05	
MSoil Neonic. x Plant Fung.	969.1	24.6	0.00	
Null	983.0	38.5	–	

<sup>1</sup>Explanatory variables in the global model for both abundance and richness include: total neonicotinoid concentration in margin soils (MSoil Neonic.), total fungicide concentrations in margin plants (Plant Fung.), Time (sampling period), Crop (maize, reference, soybeans), and floral richness in field margins.

– indicate model weight was not calculated for null models.



**Fig. 3.** Relationship between bee richness and the interaction effects of total neonicotinoid concentration in field margin soils and total fungicide concentration in non-target margin plants. The neonicotinoid and fungicide data are presented on a natural log scale (ln) and error bars represent the standard error.

margin vegetation (marginal R<sup>2</sup> = 24%; Table 3). In contrast to wild bee abundance, there was a significant negative relationship of neonicotinoid concentrations in margin soils on wild bee richness ( $\beta = -0.21 \pm 0.09$ ,  $z = -2.51$ ,  $P = 0.01$ ). A significant interaction between insecticides and fungicides further impacted field margin bee richness ( $P < 0.001$ ). Field margins with greater neonicotinoid concentrations in soil and greater fungicide concentrations in plants contained less rich bee communities ( $\beta = -0.21 \pm 0.04$ ,  $z = -5.41$ ,  $P < 0.001$ ; Fig. 3). In contrast, margins that contained low neonicotinoid levels in soil, despite having high fungicide levels in wildflowers, had greater numbers of bee species indicating a potential synergistic

relationship between fungicides and neonicotinoid insecticides. Although the main effect of neonicotinoid soil concentrations was negative, when interacting with time, this became a slightly positive relationship ( $P < 0.001$ ). There were greater numbers of bee species during the harvest sampling period regardless of soil concentrations ( $\beta = 0.10 \pm 0.04$ ,  $z = -2.36$ ,  $P = 0.02$ ).

Crop type was weakly correlated to field margin soil concentration ( $r = -0.32$ ; Table S6) and neonicotinoids were frequently detected in up to 56% of margin soils surrounding untreated fields. Although crop type was accounted for in our global model, it was only retained in the best model for bee species richness that included all bees, regardless of

size. However, crop type was not a significant covariate in the best model (Table S5). Similar to the small bee richness model, there was a negative relationship of bee richness (all bees) in field margins with greater neonicotinoid concentrations in soil and greater fungicide concentrations in wildflowers ( $\beta = -0.13 \pm 0.03$ ,  $z = -3.83$ ,  $P < 0.001$ ; Table S5).

#### 4. Discussion

Neonicotinoid insecticides are shown to affect honeybee populations and some native bee species (e.g., *Bombus terrestris*, *Osmia bicornis*) through reductions in reproductive success, shifts in foraging behavior, interference with navigation, and potential for increased mortality (Blacqui re et al., 2012; Main et al., 2018; Sandrock et al., 2014; Whitehorn et al., 2012). As the majority of wild bee species nest in the ground, contamination of soil resources may be an overlooked route of pesticide exposure for non-target pollinators. In this study, up to 93% of field margins surrounding study fields contained neonicotinoids in soil, including margins adjacent to fields that had never received a neonicotinoid treatment. Although we had hypothesized that non-target margin plant communities may be a route of neonicotinoid exposure, we found < 7% of harvested plant material contained any neonicotinoid active ingredient. However, a number of fungicides were detected in field margin wildflowers. Here, we provide evidence that native bee richness was significantly less in field margins with increasing concentrations of neonicotinoids in soil in tandem with high levels of fungicides in non-target plants. By comparison, changes in wild bee abundance was not impacted by pesticides, but rather was more closely associated with the presence of more diverse margin floral communities adjacent to our study fields. Collectively, these results provide new evidence that agricultural field margins containing neonicotinoids in non-target soils and fungicides in non-target plants may experience reductions in overall native bee species richness despite maintaining abundant bee communities.

##### 4.1. Neonicotinoid concentrations in soils and non-target plants: potential exposure profile

In this study, we evaluated two potential routes of exposure for non-target native bee communities: (1) contamination of field margin soils (Bonmatin et al., 2015; Woodcock et al., 2016); and, (2) exposure via non-target margin plants (Bonmatin et al., 2015; Bot as et al., 2015). In Missouri, neonicotinoid concentrations in study fields and field margins appeared to persist from previous agricultural activities (e.g., pre-seeding concentrations). Similar results have been found across the ‘‘corn belt’’ (e.g., Iowa, Indiana; de Perre et al., 2015; Hladik et al., 2017) and southern US (e.g., Tennessee; Stewart et al., 2014) where neonicotinoid concentrations in pre-seeding soils ranged from ND to 36  $\mu\text{g}/\text{kg}$ . Although some data indicate these insecticides likely accumulate in soils with annual seed treatment use (Goulson, 2013), other studies suggest that accumulated neonicotinoid concentrations plateau within five years (Schaafsma et al., 2016). However, data on environmental levels in field margin (i.e., non-target) soils are less readily available because most studies have focused on evaluating neonicotinoid concentrations in production field soils. In this study, field margin concentrations of the most frequently detected neonicotinoid, clothianidin, remained relatively minimal across all sampling periods ranging from 0.11 (pre-seeding) to 3.34  $\mu\text{g}/\text{kg}$  (growing) near treated fields and 0.14 (pre-seeding) to 0.74  $\mu\text{g}/\text{kg}$  (post-seeding) near untreated, reference fields. By comparison, greater neonicotinoid residues were measured in footslope soils of Iowa (range: ND to 28  $\mu\text{g}/\text{kg}$ ; Hladik et al., 2017) and field margins of the United Kingdom (range:  $\leq 0.04$  to 19.12  $\mu\text{g}/\text{kg}$ ; Bot as et al., 2015). Although our results indicate field margin soil concentrations across CAs are likely to remain minimal, soil from one margin surrounding a clothianidin-treated maize field peaked at 41.7  $\mu\text{g}/\text{kg}$  during the growing season. Importantly, up to 56% of

field margins surrounding untreated fields contained quantifiable levels of neonicotinoids ranging from 0 to 9.33  $\mu\text{g}/\text{kg}$ . It is possible that these concentrations were a result of surficial runoff and/or leaching (Jones et al., 2014) from neighboring agricultural fields planted with neonicotinoid-treated seed. Neonicotinoids will also bind to and persist in soils with higher content of organic matter and mineral clay (Bonmatin et al., 2015; Satowski et al., 2018). Alternatively, other studies have indicated that dust from planter exhaust and seed spillage could contribute to contamination of untreated fields and field margins (Limay-Rios et al., 2016; Schaafsma et al., 2015). As many agricultural fields are in close proximity to neighboring fields that may or may not be in production, it is possible that contamination of untreated sites may readily occur. Our findings suggest that changing planting practices from using neonicotinoid-treated to untreated seed may not eliminate potential outside sources of contamination; however, this was not directly measured in our study.

In some regions, bees are likely to be more exposed to neonicotinoids via contamination of non-target plants and potentially for a longer duration of time once crops are no longer in bloom (e.g., canola/oilseed rape; Bot as et al., 2015; Bredeson and Lundgren, 2019). Numerous studies have found detectable neonicotinoid residues ranging from LOD to 106  $\mu\text{g}/\text{kg}$  in non-target plants including wildflowers and woody vegetation (Bot as et al., 2016, 2015; Stewart et al., 2014). In the southeastern US, neonicotinoids were detected at > 1  $\mu\text{g}/\text{kg}$  (mean: 10  $\mu\text{g}/\text{kg}$ ) in 23% of wildflower samples surrounding recently planted cotton, maize and soybean fields (Stewart et al., 2014). In our study, neonicotinoid residues were infrequently detected (< 7%, overall) in plants collected from surrounding field margins with a maximum concentration of 9.8  $\mu\text{g}/\text{kg}$  (clothianidin) detected in a composite of *Eriogon strigosus* (daisy fleabane). As we created composite plant samples and our analytical LODs were between 1 (thiamethoxam) to 6  $\mu\text{g}/\text{kg}$  (clothianidin), this may have precluded our ability to detect potential neonicotinoid residues in a greater range of samples. To date, most studies have evaluated neonicotinoid levels in pollen, nectar, or flowers (oral exposure); however, wild bees may be exposed to neonicotinoids via direct physical contact, moving through vegetation, or using contaminated nest materials (e.g., family Megachilidae).

##### 4.2. Impacts of pesticides and field variables on native bee richness and abundance in field margins

Currently, most data evaluating insecticide effects on wild pollinator communities are at the species level with fewer data available on community level effects (Brittain and Potts, 2011; Goulson et al., 2015). This trend is especially true of neonicotinoids where numerous studies have evaluated lethal and sub-lethal effects on individual species of bumblebees (e.g., *Bombus terrestris*, *B. lucorum*, *B. impatiens*), solitary bees such as mason bees (e.g., *Osmia bicornis*, *O. cornuta*), and leafcutter bees [e.g., *Megachile rotundata*; (Blacqui re et al., 2012; Pisa et al., 2015)]. Negative impacts of neonicotinoids on reproductive success, feeding inhibition, foraging behavior, nesting success, and colony development are well documented (Pisa et al., 2015; Rundl of et al., 2015; Wood and Goulson, 2017) which likely translate to losses at the community level. Various life-history traits such as smaller body size, flight season, floral specialization, and nesting location may make some bees more susceptible to effects of insecticides (Brittain and Potts, 2011).

Independently, neonicotinoids in the margin soils studied here were negatively associated with native bee richness as fewer small species were collected in field margins with greater soil concentrations. This pattern was observed regardless of whether the adjacent field was cultivated to a treated row crop or a reference field. However, in models that included bumble bees and carpenter bees, soil concentration was weakly associated with an increased richness in field margins, though this was not significant. Occupancy analyses of UK bee populations indicated that increased population extinction rates were associated with neonicotinoid seed treatment use, particularly on oilseed rape

foragers; sub-lethal effects of neonicotinoids could cause losses in overall bee biodiversity (Woodcock et al., 2016). Studies of other pesticides (e.g., organophosphates, carbamates) are also shown to negatively impact wild bee richness in vineyards and blueberry fields after multiple applications and/or increasingly intensive chemical management (Brittain et al., 2010; Tuell and Isaacs, 2010). As neonicotinoid concentrations in field margin soils were associated with a reduction of small bee species richness across sites, we hypothesize those bees that nest in soil – a relatively unexplored route of exposure – may be more sensitive to chronic exposure via neonicotinoids. This explanation could also be why inclusion of bumble bees and carpenter bees, many of which nest above ground, lessened the relative impact of soil concentrations on overall bee richness. Further, we speculate that some bee species may also be preferentially nesting in cultivated field soil (Julier and Roulston, 2009; Wuellner, 1999) where, based on our results, soil concentrations are likely to be greater. If bees are excavating new nests in contaminated fields or field margins, they are most likely to be affected by pesticides via both oral and contact exposure. Ground-nesting bees may be exposed to insecticide residues in soil through nest excavation, ingestion, cleaning body parts, or walking/landing on contaminated soil surfaces (Kopit and Pitts-Singer, 2018). Further evaluation of soil as a route of neonicotinoid exposure to non-target native bees is important to determine the potential for cumulative exposure throughout the annual life cycle of many soil-nesting species.

In our models, we found a negative interaction of neonicotinoid concentration in field margin soils and fungicide concentrations in margin plants such that as the combined pesticide concentrations increased, a stronger decline in bee richness was observed. However, those field margins that had greater fungicide concentrations in wildflowers, but low neonicotinoid levels in soil, maintained species rich bee communities. Therefore, the negative relationship of greater neonicotinoid soil concentration with bee richness was weakened when only fungicide concentrations are greater. Importantly, regulatory decisions are often based on single pesticide toxicity studies; whereas, native bees, especially in agroecosystems, are likely to be chronically exposed to numerous pesticides or mixtures (including herbicides, fungicides, and insecticides) at any given time (Hladik et al., 2016). As evidenced by our findings, these pesticides may act synergistically rather than additively, leading to greater declines in wild pollinators (Goulson et al., 2015; Gill et al., 2012). While fungicides are generally non-toxic to bees, *Bombus impatiens* colonies produced fewer workers when exposed to flowers treated with fungicides (chlorothalonil; Bernauer et al., 2015). Commonly applied fungicides such as pyraclostrobin are known to disrupt nesting behavior of two species of solitary bee; *Osmia lignaria* and *Megachile rotundata* (Artz and Pitts-Singer, 2015).

Although we hypothesized that native bees would be less abundant in field margins surrounding treated fields, our best model retained no pesticide related variables. Native bee abundance in field margins was positively associated with margin floral richness, regardless of treatment. The diverse vegetative strata provided by field margin plant communities is likely to benefit pollinators through habitat, pollen, and nectar resources (Kennedy et al., 2013; Main et al., 2019). Conversely, agricultural activity may positively impact pollinator communities by creating microhabitats, promoting availability of resources, and creating opportunity for patchy resource availability to which some bees can adapt (Potts et al., 2010). Conservation Area fields likely present a vastly different condition than many large-scale agricultural production areas, which may have influenced our findings. In Missouri, CA cultivated field sizes are often < 2 ha in size and managers typically maintain surrounding margin vegetation communities (e.g., shrubs, trees, herbaceous plants). Some bees may have been far more abundant on CAs based on availability of suitable habitat which potentially buffered impacts of pesticide use. Indeed, pesticide impacts on wild bees have been shown to be buffered by increasing proportions of natural habitat in the landscape surrounding pesticide-treated orchards (Park

et al., 2015).

The amount of cropland in production, increasing use of agrochemicals such as herbicides and insecticides, and the amount of fallow land adjacent to cultivated fields can lead to shifts in bee abundance and diversity (Brittain et al., 2010; Holzschuh et al., 2008; Potts et al., 2010). We acknowledge that our study design, specifically the comparison of uncultivated reference fields to treated crop fields, may have confounded our findings. Irrespective of physical disturbance, the current study was used as baseline data to evaluate wild bee communities on CA and determine whether neonicotinoids were persisting in soils or being accumulated by non-target vegetation. To more clearly identify the potential for neonicotinoid-treated seed to influence wild bee abundance and richness, future experiments could assess pollinators collected from fields using similar crops under differing treatments. Despite crop type not significantly influencing bee richness or abundance, it is possible that lower bee richness may have resulted from bees leaving the field margin to forage on any portions of the field crop in bloom. In Sweden, however, wild bee density in clothianidin-treated oilseed rape fields was significantly reduced compared to untreated controls regardless of positive effects associated with increased canola flower cover (Rundlöf et al., 2015).

## 5. Conclusions

Native bees provide numerous ecosystem services around the globe, yet their declines continue to be documented (Garibaldi et al., 2013; Potts et al., 2010). Despite few detectable insecticide concentrations in field margin vegetation, neonicotinoid concentrations in field margin soils were associated with a reduction in overall species richness of non-target native bees. Acknowledging our study limitations, results of this study indicate that neonicotinoid presence in field margin soils may be detrimental for non-target organisms. Many studies have evaluated neonicotinoid levels in other media (e.g., crops, wildflowers, field soils); however, soil has remained a relatively understudied route of exposure. The reproductive success of wild bees has been negatively correlated with neonicotinoid residues indicating bee species may have a reduced capacity to establish viable populations one year post-exposure (Woodcock et al., 2017). As the data presented here were baseline data collected over a single agricultural growing season, we are unaware of whether the pollinator communities assessed were already a reflection of an altered system. Many of our study fields were annually planted to neonicotinoid treated seeds and field margins surrounding untreated, reference fields further show evidence of neonicotinoid contamination. Therefore, further experimental studies will be important to explicitly test the relationships we documented here. To our knowledge, this is the first field study to provide evidence of reduced richness of small native bees associated with soil neonicotinoid concentrations in agroecosystems.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.agee.2019.106693>.

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