EVALUATING EFFECTS OF NEONICOTINOID SEED TREATMENTS AND AGROECOSYSTEM LAND MANAGEMENT PRACTICES ON SOLITARY BEES IN MIDWESTERN AGROECOSYSTEMS

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by
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EVALUATING EFFECTS OF NEONICOTINOID SEED TREATMENTS AND AGROECOSYSTEM LAND MANAGEMENT PRACTICES ON SOLITARY BEES IN MIDWESTERN AGROECOSYSTEMS

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DEDICATION

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THESIS ABSTRACT

Bees are declining due to multiple factors including habitat loss and non-target exposure to pesticides resulting from agricultural intensification. Solitary bees are often overlooked as important pollinators, but they are valuable contributors to both the commercial success and ecological diversity of agroecosystems. Yet, solitary bee communities in agroecosystems may be adversely impacted by exposure to pesticides such as neonicotinoid insecticides, and it is unclear if bee habitat adjacent to agricultural fields can buffer against the potential negative impacts of neonicotinoids. Laboratory studies indicate lethal and sublethal effects of neonicotinoids on bees, and previous field studies have demonstrated reduced abundance, richness, and reproductive success of solitary bees in agroecosystems where neonicotinoids are present. Thus, we proposed two field studies of wild, solitary bee communities in agricultural field margins to determine the impacts of neonicotinoid insecticides and site-specific habitat variables (e.g., floral richness) on bees in Missouri agroecosystems. First, in 2019, we surveyed bee abundance and richness in field margins adjacent to 29 soybean fields located on public conservation areas (n = 18) and private land enrolled in the Conservation Reserve Program (n = 11). Public study fields were planted to one of three experimental treatments: untreated (no insecticide), treated (imidacloprid), or previously-treated (untreated, but neonicotinoid use prior to 2017). Privately-owned study fields were planted to one of two experimental treatments: treated (neonicotinoid) or previously-treated (untreated, but neonicotinoid use in 2018). Second, in 2018 and 2019, we placed solitary bee nest boxes in field margins of soybean fields (2018, n = 18; 2019, n = 24). In 2018, all fields were located on public conservation areas and, in 2019, privately-owned fields were added to the study. The experimental treatments for public and privately-owned study fields were the same as described for the first study. In the first study, we found wild, solitary bee abundance and richness was best explained by field treatment, with fewer bees and lower bee diversity observed in margins of previously-treated and treated fields compared to margins of untreated fields. We also found that bare soil and floral richness in field margins positively influenced bee abundance and richness, but it remains unclear if the positive influences of habitat can outweigh the negative impact of neonicotinoid seed treatments on bee abundance and richness. In the second study, we found field treatment had no negative impact on nesting effort of cavity-nesting bees. However, field treatment significantly reduced resin bee nesting success, with a lower percentage of resin bees emerging from nests collected from margins of previously-treated public (15%) and treated public (7%) fields compared to untreated fields (46%). We also found that the amount of woody vegetation within 800 m of nesting sites may impact nesting effort of resin bees, with more nest cells produced and cavities used in margins of fields with a greater amount of woody vegetation within 800 m. Reducing or eliminating neonicotinoid use in areas managed for wildlife may be beneficial to the conservation of wild bee populations.

THESIS FORMAT

The chapters of this thesis are written as independent manuscripts. Therefore, there may be some overlap in their content. Literature cited, tables, and figures follow each chapter. I have used plural pronouns throughout the thesis to represent the important contributions of co-authors.

CHAPTER 1 – EVALUATING SOLITARY BEE ABUNDANCE AND RICHNESS IN FIELD MARGINS SURROUNDING NEONICOTINOID-TREATED SOYBEAN FIELDS

ABSTRACT

Wild, solitary bees are important contributors to both the commercial success and ecological diversity of agroecosystems, providing valuable pollination services to both agricultural crops and wildflowers in agricultural field margins. Yet, wild bee communities in agroecosystems may be adversely impacted by exposure to pesticides such as neonicotinoid insecticides. Laboratory studies indicate lethal and sublethal effects of neonicotinoids on bee species, and previous field studies have demonstrated reduced abundance and richness of wild bee nesting and foraging guilds in response to neonicotinoid presence in agricultural field soils. Here, we conducted an experimental field study to assess whether neonicotinoid seed treatment or presence in environmental media (e.g., agricultural dust) influenced wild, solitary bee abundance and richness in agricultural field margins in the Midwestern United States. Our secondary objective was determining if site-specific habitat variables (e.g., margin floral richness or available bare soil) buffer against the potential impacts of neonicotinoids. In 2019, we planted 29 Missouri agricultural fields to soybeans (*Glycine max*) using one of three seed treatments: untreated (no insecticide), treated (imidacloprid), or previously-treated [untreated, but either 1 year (private land) or 3 years (public land) since last neonicotinoid use]. We used visual survey methods to quantify wild bee abundance and richness in field margins from May – September and collected soil, wildflowers, and agricultural dust samples that were analyzed for neonicotinoid residues. We detected neonicotinoids in margin wildflowers (21%), field soils (32%), and agricultural dust (100%). Neonicotinoid detections in environmental media were not significant predictors of bee abundance or richness. Wild bee abundance was best described by field treatment, with fewer bees observed in margins of previously-treated (one year since neonicotinoid use) and treated fields compared to margins of untreated fields. However, bee abundance did not differ between margins of untreated fields and margins of previously-treated fields where neonicotinoids had not been used in three years. Wild bee richness was also best described by field treatment, with lower bee richness observed in margins of previously-treated (both 1-year and 3-years since neonicotinoid use) and treated fields compared to margins of untreated fields. Bare soil and floral richness in field margins were positive predictors of bee abundance and richness, but it remains unclear whether these habitat variables can buffer against the impact of neonicotinoid seed treatments. Our findings indicate that consistent use of neonicotinoid seed treatments may reduce wild bee abundance and richness in agricultural field margins but that bee abundance may recover within three years of discontinuing neonicotinoid application to fields. Reducing or eliminating neonicotinoid use in areas managed for wildlife may be beneficial to the conservation of wild bee populations.

INTRODUCTION

The recent worldwide decline in many insect populations is well documented (Potts *et al*., 2010; Goulson *et al*., 2015; Hallmann *et al*., 2017) and given the numerous ecosystem services provided by insects, this decline has led to broad concern among scientists, natural resources agencies, and some members of the general public (Kluser and Peduzzi 2007; Tylianakis 2013; Althaus *et al*., 2021). Pollination services provided by insects are valued at $3.07 billion annually in the United States (US) alone (Losey and Vaughan 2006). Insects are consummate pollinators, facilitating reproduction for 80% of wild plant species and increasing agricultural yields of 75% of crops worldwide (Potts *et al*., 2010). These vital pollination services make safeguarding pollinating insect abundance and diversity a crucial conservation and agricultural priority.

Bees are among the most important insect pollinators (Klein *et al*., 2007; Garibaldi *et al*., 2013). Due to their commercial use and the publicity surrounding Colony Collapse Disorder (CCD), European honeybees (*Apis mellifera*) have received the bulk of attention regarding the loss of insect pollinators (Blacquiere *et al*., 2012; Colla & MacIvor 2017). However, wild bees also provide critical and often underappreciated pollination services (Garibaldi *et al*., 2013). For example, the presence of wild pollinators (in addition to honeybees) can increase soybean yield by 6% (Milfont *et al*., 2013). More diverse pollinator communities offer a direct benefit to growers by improving yields in many crop species (Hoehn *et al*., 2008), and pollination services to crops decrease with isolation from pollinator friendly habitats (Garibaldi *et al*., 2011). Predicted global shortages in pollination services over the next few decades (Lautenbach *et al*., 2012) make it critical to protect wild bees to ensure sustainable food production (Park *et al*., 2015), as wild bees have the potential to increase resilience of pollination services to reductions in honeybee populations (Winfree *et al*., 2007).

In agroecosystems, wild bees are threatened by multiple stressors including habitat loss, pesticide use, and parasites (Goulson *et al*., 2015). Conventional agricultural practices rely extensively on the use of pesticides, such as insecticides and herbicides, to meet the increasing demands for agricultural commodities from an ever-growing global population (Douglas and Tooker 2015). Pesticides are the most contentious potential contributor to bee declines and neonicotinoid insecticides are the class most strongly implicated (Goulson 2013; Pisa *et al*., 2015). Neonicotinoids are water soluble, systemic insecticides, which means the chemical is taken up by the target plant during the initial growth stage and spreads throughout its tissues, including leaves, pollen, and nectar (David *et al.,* 2016). Neonicotinoids primarily protect crops against piercing-sucking insects (e.g., aphids or leafhoppers), but are also effective against chewing insects (e.g., soybean leaf beetle) and soil insects (e.g., corn root-worm) (Tomizawa and Casida 2005).

Since their introduction in the early 2000's, neonicotinoids have become the most widely used class of insecticides in the world, representing ~80% of all seed treatment sales and ~30% of the total market share of insecticides (Jeschke and Nauen 2008; Jeschke *et al*., 2011; Ihara and Matsuda 2018). Large-scale use of seed treatments has driven rapid increase in neonicotinoid use and preemptive pest management in U.S. field crops (Douglas and Tooker 2015). Neonicotinoids seed treatments are nearly ubiquitous in their use in the 'Corn Belt' of the Midwestern U.S., as they are applied to almost 100% of corn (*Zea mays*) and ≥ 40% of soybeans (*Glycine max*) in the U.S. (Douglas and Tooker 2015; Hladik *et al*., 2018). However, uptake of neonicotinoid active ingredients by target crops is estimated to be <10%, leaving ~90% of active ingredient available to either degrade, sorb to soil particles, or leach to aquatic systems (Goulson 2013). Neonicotinoids have been detected in agricultural field margin plants and soil, where they can persist and accumulate over time (Krupke *et al*., 2012; Goulson 2013, Jones *et al*., 2014; David *et al*., 2016), potentially providing season-long routes of exposure for nontarget organisms such as ground-nesting bees (Long and Krupke 2016; Main *et al.*, 2019).

It is increasingly clear that bees may be exposed to neonicotinoid seed treatment active ingredients through a variety of routes including planter dust, contaminated soil, and/or contaminated pollen/nectar (Goulson 2013; Nuyttens *et al*., 2013; Godfray *et al*., 2014; Krupke *et al*., 2017; Kopit and Pitts-Singer 2018). Overall, the majority of laboratory and semi-field studies have demonstrated that neonicotinoids can be deleterious to bees (Main *et al*., 2018), and research suggests that chronic sub-lethal effects are more prevalent than mortality through acute toxicity (Hladik *et al*., 2018). Yet relatively few studies have assessed neonicotinoid impacts on pollinators in field settings (Cutler and Scott-Dupree 2014; Rundlof *et al*., 2015; Peters *et al*., 2016) and even fewer of these studies have investigated impacts on solitary bees (Pisa *et al*., 2015), so the evidence is inconclusive. Further, most neonicotinoid research on bees has investigated effects of these insecticides on honeybees or bumblebees (*Bombus spp.)*, whereas effects on solitary bees remain poorly understood (Lundin *et al*., 2015). Studies have linked neonicotinoids to several negative effects on honeybees including impaired foraging (Ramirez-Romero *et al*., 2005), altered learning and memory (Decourtye *et al*., 2004), and increased mortality (Biddinger *et al*., 2013). However, Biddinger *et al*. (2013) indicated their results showing responses of European honeybees to neonicotinoids could not be extrapolated to responses of horn-faced mason bees (*Osmia cornifrons)*, suggesting that honeybees may be poor bioindicators for neonicotinoid effects on other bee species and highlighting the need to study multiple species to gain a clearer picture of the effects of neonicotinoids on all bees.

In addition to pesticides, two primary drivers that can influence wild bee communities in agroecosystems include local agricultural management practices and the composition and structure of the surrounding landscape (Kremen *et al*., 2007). A global quantitative synthesis of local and landscape habitat effects on wild bee pollinators in agroecosystems suggested that a 10% increase in the amount of high-quality bee habitats in a landscape resulted in a 37% increase on average of wild bee abundance and richness (Kennedy *et al*., 2013). In Europe, wildflower plantings in agroecosystems benefited bee communities at a local scale, with greater species richness observed in managed field margins or meadows compared to unmanaged control areas (Knop *et al*., 2006). However, it is unclear if these benefits extend to the larger landscape (e.g., > 1 km) for solitary bee species, as another study found that pollinator-friendly management may increase honeybee and bumblebee abundance but does not increase the diversity of other farmland bees and wasps (Wood *et al*., 2015). If natural areas support enough wild pollinators or provide refuge from pesticides, they could potentially buffer bee communities against negative effects of pesticides (Klaus *et al*., 2021). Loss of natural areas in the landscape and increased pesticide use may negatively affect wild pollinators and their ecosystem services, yet little is known about how the interaction between pesticide exposure and local habitat conditions may influence native bee communities (Park *et al*., 2015). Understanding whether landscape composition and structure (e.g., amount of cultivated land in the surrounding landscape) might mitigate impacts of pesticides on native bee communities can inform decisions surrounding management of agroecosystems.

Study Objectives

In this study we evaluated the impacts of neonicotinoid seed treatment use and local habitat characteristics on wild, solitary bee abundance and richness in agroecosystems. Our first objective was to evaluate the impacts of annual neonicotinoid seed treatment use on wild bee communities in field margins. Our second objective was to evaluate the effectiveness of land management practices, such as maintaining wildflowers in field margins, on wild bee abundance and richness and test for interactions between neonicotinoid seed treatment use and local habitat characteristics. Our third objective was to explore possible routes of neonicotinoid exposure for wild bees by quantifying pesticide concentrations in environmental media (e.g., soil and wildflowers) with which bees interact. Our central hypothesis was that multi-year use of neonicotinoid treated seeds would reduce wild bee abundance and richness. With these objectives, we hoped to inform agricultural practices and land management decisions that may be influencing wild bee populations in Missouri agroecosystems.

METHODS

Experimental Design

We sampled bee communities in margins surrounding publicly and privately owned agricultural fields across north-central Missouri. Our experimental unit was an agricultural field and the surrounding field margins (n = 29), as this represented field conditions at a scale where land management practices and pesticide exposure could interact to potentially impact native bees. We defined a field margin as an uncultivated area adjacent to an agricultural field, typically comprised of a mix of wildflowers and grassy vegetation. We selected fields with herbaceous margins containing wildflowers over fields with woody margins. We located study fields on both publicly and privately managed agroecosystems to evaluate potential interactions between neonicotinoid seed treatment use and local habitat characteristics,

This study built on an experimental design established by a previous experimental field study (2017 – 2018) that evaluated the impacts of multi-year planting of neonicotinoid seed treatments on abundance and richness of wild bee nesting and diet guilds in agricultural field margins (Main *et al*., 2021). We used publicly managed fields (n = 18) that were selected and experimentally manipulated during the previous study. These public fields were located across three state managed conservation areas (CA) in north-central Missouri, U.S. (six fields per CA; Figure 1.1) that are managed for wildlife. The three CA included Atlanta (39.88961°N, 92.49373°W), Thomas Hill Reservoir (39.599477°N, 92.620690°W), and Whetstone Creek (38.96766°N, 91.71155°W). 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. Study fields were situated in a surrounding landscape matrix of woodland, grassland, and cropland that varied among CA. We used the same study fields as the previous study and continued the experimental treatments established by Main *et al*. (2021) for those fields.

In the spring of each study year (2017 – 2018, previous study; 2019, current study), all public study fields were planted with soybeans (*Glycine max*) and seed treatment type (treated or untreated) was manipulated. Treated refers to fields planted with seeds coated with both a neonicotinoid insecticide (imidacloprid) and fungicides, whereas untreated refers to fields planted with seeds treated with fungicides only. Although the untreated category did not represent a completely untreated situation (i.e., naked seeds), it is rare to find seeds treated with only a neonicotinoid and not also treated with fungicides, thus, we included fungicides in the control state (i.e., untreated fields). Further, fungicides are generally considered to be less toxic to bees (Tesoriero *et al.*, 2003). At each CA, the six agricultural fields (mean field area: 1.3 ha) were organized in a random block design. Each field was planted to one of three treatments: untreated (*n* = 6), previously-treated (*n* = 6), or treated (*n* = 6). Untreated fields had no known prior neonicotinoid use (UT fields) and were planted with untreated seeds from 2017 - 2019, previously-treated fields were those where neonicotinoid treated seed was last used in 2016 (i.e., thus termed PT3 fields) and were planted with untreated seeds from 2017 - 2019, and treated fields (treated public fields) were continuously cropped using neonicotinoid treated seeds for > 5 years.

In addition to the public fields from the previous study, we selected privately owned fields (n = 11) with the assistance of Missouri Department of Conservation private land conservationists from a candidate set of fields enrolled in the United States Department of Agriculture's (USDA) Farm Services Agency Conservation Reserve Program (CRP) and owned by landowners willing to participate in the study. Specifically, all private fields were enrolled in the CP-33 program, which is designed for upland bird conservation (Zimmel and Carpenter 2007). Enrollment in the CP-33 program requires habitat buffers adjacent to agricultural fields that are 9 - 37 meters wide. Buffers may be allowed to regrow to natural cover or can be planted with a mixture of warm season grasses and forbs. We selected private fields based on the similarity of field margin habitat (e.g., margin area and representative plant species) to public land field margins, and all fields were surrounded by an existing margin that contained a mix of grasses and wildflowers. We selected privately-owned fields that were located 2 - 20 km from each

CA (Figure 1.1). These distance parameters ensured that bee populations on private land would not be influenced by bee populations on study CAs (i.e., source-sink dynamics), but would still experience similar environmental and climatic conditions (precipitation, daily temperature, etc.).

All private fields were planted with neonicotinoid-treated corn in the previous year, thus, no fields in the untreated treatment category (no prior neonicotinoid use) were located on private land. Fields on private land were either planted with treated soybean seeds (treated private fields) or untreated soybean seeds (previously-treated fields) in 2019. As private fields planted with untreated seed in the study year had been planted with neonicotinoid treated seed in the previous year, we termed this treatment category PT1 fields. The lack of UT fields on private land represents a realistic scenario because private fields in a corn-soybean rotation generally involve a neonicotinoid seed treatment at least every few years, as nearly 100% of corn seeds and ≥ 40% of soybean seeds are coated with neonicotinoids (Hladik *et al.*, 2018; Douglas and Tooker 2015). We were unable to locate established soybean fields on private land that have not been treated with a neonicotinoid within the past few years.

Wild Bee Surveys

In 2019, we quantified wild bee abundance and richness for each field using two non-lethal, visual sampling methods: transect sampling and timed observations (Morandin *et al.*, 2007; Gibson *et al*. 2011). Transect sampling consisted of one observer walking four 25-m transects in field margins on each visit to a field and recording all observed bees that contacted the reproductive parts of flowers within 1 m of either side of the transect line (Montgomery *et al*., 2021). Timed observation involved one observer watching an approximately semi-circular area (radius ~ 1 m) containing at least one individual of a single plant species, for 10 min, and documenting any insect that contacted the reproductive parts of the plant during the observation period (Gibson *et al.*, 2011). Whenever possible, we repeated timed observations for four different plant species per field (to maximize the potential of observing a diverse suite of bee species), and we kept the plant species consistent among fields by only observing pollinators on common plant taxa (e.g., *Erigeron spp.* in June or *Solidago spp.* in August). To adequately capture within-field variability, we conducted one timed observation and one transect sample in each of the four field margins, for a total of eight sampling efforts per study field per observation period. All visual bee surveys were conducted by the same observer to control for potential bias introduced by multiple observers with differential identification skills (Krahner *et al*., 2021; Packer and Darla-West, 2021). We identified all bees to genus (e.g., *Osmia*) as it is challenging to identify most bees to a species level ‘on-the-wing’ (Morandin *et al*., 2007). Individual bees that we were unable to visually identify to genus, such as bees that quickly dispersed, were counted in an unidentified category and only included in total abundance metrics. We surveyed bee communities during eight distinct time periods from May – September 2019, with two sampling periods/month in June – August and one sampling period/month in May and September.

Time of day, temperature, humidity, wind speed, and weather patterns (e.g., rainfall and cloud cover) can influence bee foraging activity and, thus, bee detections in survey efforts (Polatto *et al*., 2014). Consequently, we only conducted visual surveys when the temperature was between 20 - 37 ° C, wind was between 0 km/h and 20 km/h, and we did not conduct surveys on rainy or extremely overcast days (Templ *et al*., 2019).

All surveys took place between 7 AM and 4 PM. We randomized the time of day that we surveyed each field between sampling periods, to prevent any field from consistently being sampled earlier in the morning or later in the afternoon, when bees are less active (Morales and Aizen 2005; Olesen *et al*., 2008). Finally, to ensure independence among fields, the margins surveyed for each field were a minimum of 500 meters apart, as solitary bees reportedly have a small foraging range (<600 m; Gathmann and Tscharntke, 2002) with many species foraging less than 300 m from their nest (Zurbuchen *et al*., 2010).

Field-Level Habitat Characteristics

To assess environmental variability among study sites, during each sampling period, one observer recorded vegetation characteristics using a 50 cm x 50 cm quadrat at twelve haphazardly-placed locations along herbaceous margins surrounding each field (Main *et al*., 2019). The observer walked all herbaceous field margins and randomly tossed the quadrat, making sure to space the twelve locations across all field margins to adequately capture any within-field variability in habitat characteristics. Within each quadrat, we quantified habitat characteristics that may impact bee populations including blooming plant cover (%), bare soil (%), vegetation height (cm), number of blooming plants, number of dead stems, and floral richness. Apart from floral richness, we averaged all collected quadrat variables for each field for each sampling period. We summarized floral richness by counting the total number of unique flowering plant species recorded across all twelve quadrats.

Soil, Vegetation, and Agricultural Dust Sampling

To quantify pesticide concentrations in materials with which nesting and foraging bees interact, we collected soil and flower biomass from study fields and adjacent margins. Soil sampling occurred at three discrete time periods throughout the agricultural growing season: pre-seeding (May), mid-growing (July), and pre-harvest (September). We collected field soils between soybean rows at a depth of 10 cm with ~15 random locations sampled throughout each study field (Main *et al*., 2021). Similarly, we collected margin soils in a zigzag pattern at ≥ 2 m from the field edge. We composited all subsamples from each field or margin (~1.5 kg) in a clean polyethylene bag before storing them in coolers for transport to the laboratory. We thoroughly mixed individual study field and margin soil samples in the laboratory, transferred them to 50 mL centrifuge tubes, and froze them at -20°C until chemical analysis.

To determine pesticide concentrations in margin wildflowers, we collected floral reproductive parts (pistils and stamens) from a random sample of wildflowers in field margins. We collected wildflower samples monthly at five discrete time periods throughout the agricultural growing season: pre-seeding (May), post-seeding (June), mid-growing (July), soybean-flowering (August), and pre-harvest (September). One observer randomly walked the margin, collecting from plants that were in bloom. We collected only reproductive parts of the flowers instead of entire flower heads, as this is the part of plants with which bees interact the most and either ingest (nectar) or collect on their bodies (pollen) (David *et al*., 2016). A notable exception is leafcutter bees (e.g., *Megachile spp.*) that may ingest parts of leaves or flower petals during the process of constructing their nest out of these materials (Kopit and Pitts-Singer 2018). We placed floral parts in polyethylene bags and stored in coolers for transport to the laboratory. We stored flower samples similarly to the soil samples (as described above) until chemical analysis. We composited all collected flower biomass per study field across sampling periods to obtain an overall sample for the study year.

To obtain a sample of agricultural dust (produced by planters during the sowing of seeds) that settled in field margins for pesticide residue analysis, we placed three dust collection stations in the four margins surrounding each field (12 stations per field). Each dust collection station consisted of collection slides [standard microscope slides (surface area = 19.5 cm^2) sprayed with Tanglefoot® Tangle-Trap® Sticky Coating] attached to posts and placed approximately 5-meters from the field edge, evenly spaced across the entire margin. Modifying a design used in previous studies (Xue *et al.,* 2015; Krupke *et al.,* 2017), we placed eight dust collection slides on a post at heights of 50 cm and 100 cm above ground level. We designed and located collection stations to provide an estimate of field-realistic levels of pesticide residues that foraging bees might encounter in flight or on margin plants of similar heights during planting season. We twice replaced the collection slides on each post to collect dust that settled during three distinct time periods: one-week prior to planting, during study field planting, and one-week post planting. After removing slides from collection posts, we placed them in slide storage boxes (unique for each field and sampling period). Slide storage boxes were contained in coolers until they could be transported to the lab, where they were stored at -20°C until chemical analysis. Dust sampling took place only on public fields. We were unable to collect dust samples from private fields due to the challenges of aligning planting and slide collection with many different farmers and because of some resistance to this aspect of the study from landowners.

Pesticide Residue Analyses

Soil, flower biomass, and dust samples collected from study fields and the surrounding margins were analyzed by the accredited USDA AMS National Science Laboratory in Gastonia, NC, USA. Samples were extracted for analysis of agricultural pesticides using the QuEChERS method (Lehotay *et al*., 2005). 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. The pesticides included for analyses were neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiamethoxam), fungicides (azoxystrobin, fluxapyroxad, metalaxyl, pyraclostrobin, tebuconazole, trifloxystrobin), a pyridine insecticide (flonicamid), and a pyrethroid insecticide (bifenthrin). The limit of detection (LOD) ranged from 1 to 6 ppb for the neonicotinoids, 0.4 to 1 ppb for the fungicides, 3 ppb for flonicamid, and 1 ppb for bifenthrin.

Data Analysis

Due to the challenges and potential errors associated with visually identifying bees to genus without collecting them, we focused on total abundance and total richness as our dependent variables, as opposed to looking at abundance or richness of specific nesting guilds (e.g., cavity-nester abundance). It is, however, possible that certain nesting guilds are differentially impacted by neonicotinoids due to variances in how different nesting guilds interact with environmental media (e.g., soil). This lack of taxonomic resolution represents a limitation of our bee sampling method and is a tradeoff of not capturing and euthanizing bees. All bee sampling methods (e.g., sweep nets) have unique bias and tradeoffs. Due to our small sample size of 29 fields, we estimated bee abundance for each field by summing all observed bees across all visual survey efforts within a sampling period. Similarly, we calculated genera richness by summing all within-field observations, making sure each observed genus was only counted once in the total richness metric for each sampling period. We excluded European honeybees, bumblebees, and Eastern carpenter bees (*Xylocopa virginica*) from our analysis due to their comparatively large foraging distance compared to most solitary bees (Straub *et al.*, 2015).

We conducted two separate analyses, one including all fields (public and private, n = 29) and one containing only public fields (n = 18). We analyzed public fields in a separate analysis because we were only able to collect agricultural dust on public lands and, additionally, we had soil neonicotinoid detection data from preceding years (2017 and 2018) due to a previous study conducted on the same fields (Main *et al.*, 2021). We did not have neonicotinoid soil detection data for private land in previous years. The inclusion of dust and previous year's soil data as variables provided the opportunity to compare models containing treatment categories as predictors to models containing neonicotinoid detection data in environmental media (soil, flowers, and agricultural dust). In both analyses, each model contained two random effects: (1) 'sampling period' to account for seasonal changes in bee communities and (2) 'individual field (Field.ID)' nested in 'Site' to account for spatial autocorrelation and the repeated sampling of the same fields over time. As our abundance data were zero-inflated, we used a negative binomial distribution to reduce overdispersion in our models. For total richness, we used a generalized Poisson distribution. We assessed model fit through visualization of model residuals and overdispersion parameter estimates.

To properly use concentration data as a predictor variable, it needed to be log-transformed, subsequently resulting in a high level of censored data (i.e., non-detects and trace detections). Therefore, we only included a categorical variable (detect/non-detect) for environmental media variables included in the models containing only public field data. We composited flower samples collected throughout the field season before chemical analysis, so they represented cumulative neonicotinoid detections for the entire field season. Despite having soil tested at three time intervals, neonicotinoid presence in field soil was, similarly, based on the entire field season in our models. That is, if in any time period a field had soil with neonicotinoid concentrations > LOD, we listed this field as a 'detect' and any field with all concentrations < LOD (including trace detections) we listed as 'non-detect'. As every public field had neonicotinoids detected in agricultural dust (leaving only one category), based on the mean concentration (0.26 ng/cm^2), we split neonicotinoid detections in agricultural dust into two categories high ($\geq$ 0.26 ng/cm^2) and low ($\leq$ 0.26 ng/cm^2).

For the analysis including all fields (public and private), we fit a single generalized linear mixed effect model (GLMM) in R (package 'glmmTMB'; Brooks *et al.*, 2017) to evaluate the impacts of treatment, neonicotinoid detections in field soil, floral richness, and bare soil % on wild bee abundance and richness (Table 1.1). We standardized all continuous variables to a mean of zero and screened for correlations. We tested for interactions between floral richness and treatment and floral richness and neonicotinoid detections, but because all interactions were not significant, we included only main effects in our models.

In the analysis of only public fields, to compare models containing treatment to models containing neonicotinoid detections in different environmental media, we used a model selection approach and corrected Akaike's Information Criterion (AICc) (Bedrick and Tsai 1994) to evaluate candidate GLMM models (packages 'glmmTMB and 'AICcmodavg' in R) that examined the effects of treatment or neonicotinoid detections (in agricultural dust, wildflowers, and/or field soil) and site-level habitat variables (floral richness and bare soil %). We considered candidate models with a ΔAICc ≤ 2 to be competitive. We considered parameter estimates in competitive models to be significant if $P < 0.05$ and marginally significant if $P < 0.1$. We assessed model fit through visualization of model residuals and overdispersion parameter estimates.

RESULTS

Pesticide Concentrations in Soils, Flowers, and Agricultural Dust

Imidacloprid was the most detected neonicotinoid compound in soils, found above trace amounts in 32% of field soils (range: ND to 41 μg/kg) and in 2% of field margin soils (range: ND to 3 μg/kg). We only detected clothianidin in trace amounts in 17% of field soil, and clothianidin was not detected in any field margin soils. We detected thiamethoxam above trace amounts in 5% of field soils (range: ND to 12 μg/kg), and thiamethoxam was not detected in field margin soils. We did not detect acetamiprid and dinotefuran in field or margin soils. When summed across neonicotinoid compounds, average neonicotinoid concentrations in field soils were greatest during the pre-seeding period of the agricultural growing season (mean concentration: 6 μg/kg). We detected neonicotinoids in 61% of treated fields on both public and private lands (Table 1.2). Average neonicotinoid concentrations were greatest in treated public fields (7.78 μg/kg), and the greatest individual field soil concentration (41μg/kg) was detected in a treated public field. We did not detect neonicotinoids in soil from untreated (UT) fields, and we only detected neonicotinoids in two field soil samples from fields planted with neonicotinoid-treated seed three years prior (PT3). Azoxystrobin and metalaxyl were the only fungicides detected above trace amounts and combined were detected in 8% of field soils (range: ND to 11 μg/kg for azoxystrobin and ND to 2 μg/kg for metalaxyl). We only detected the fungicide fluxapyroxad in trace amounts in field and margin soils. We did not detect the pyridine insecticide flonicamid in any soils. We detected the pyrethroid insecticide bifenthrin in a single field soil sample collected in September (151 μg/kg).

Imidacloprid was the only neonicotinoid detected in margin flowers, found in 21% of flower samples (range: ND to 7 ng/g). Four of the six flower samples with imidacloprid detections were from UT and PT3 fields. We detected the fungicide azoxystrobin above trace amounts in 10% of flower samples (range: ND to 1 ng/g). We detected the fungicides pyraclostrobin and trifloxystrobin in <3% of margin flowers. We did not detect the insecticides flonicamid or bifenthrin in margin flowers.

We detected imidacloprid in 100% of dust samples collected in public field margins. Average imidacloprid concentrations in dust samples were greatest in treated public fields (0.24 ng/cm^2) and were lowest in UT fields (0.03 ng/cm^2) (Table 1.2). Imidacloprid concentrations were greatest for dust collected during field planting (range: 0.002 to 1.86 ng/cm^2; mean: 0.4 ng/cm^2; SD: 0.64 ng/cm^2). Imidacloprid concentrations ranged from 0.007 to 0.072 ng/cm^2 (mean: 0.04 ng/cm^2; SD: 0.01 ng/cm2) for dust collected prior to and after field planting. We only detected clothianidin in dust in trace amounts, and we detected thiamethoxam in 14% of dust samples (range: 0.001 to 0.002 ng/cm^2). We detected no other insecticides in agricultural dust samples. We detected the fungicide metalaxyl in 57% of dust samples, with concentrations ranging from 0.001 to 0.1 ng/cm^2. The fungicides azoxystrobin, pyraclostrobin, and tebuconazole were less frequently detected than metalaxyl. Dust collected on slides attached to the planter during the planting of neonicotinoid-treated seeds yielded concentrations of imidacloprid as high as 43 ng/cm^2.

Bee Communities

We observed a total of 4,182 solitary bees across all fields and sampling periods, representing at least 22 genera (Table 1.3). The most abundant genera/tribes we observed were *Ceratina* (20%), *Megachile* (16%), *Mellisodes* (13%), and *Augochlorini* (12%). Bees that we were unable to be identify to genus ‘on-the-wing’ represented 2% of all observed abundance. The greatest abundance detected for an individual field during a single sampling period was 69 bees, and the greatest genera richness was 12. The average genera richness (across all sampling periods and all study fields) was five. Average richness was lowest in the September sampling period (four) and greatest during the second sampling period in July (seven).

Model Results

Analysis of All Fields

The interactions between field soil neonicotinoid detections and floral richness (*P* = 0.41) and between any field treatment and floral richness (*P* = 0.26 – 0.85) were not significant predictors of total bee abundance, so we proceeded to test only for main effects. Field treatment had a significant effect on bee abundance. Bee abundance was greater in margins of untreated fields compared to margins surrounding treated private fields (β = -0.58 ± 0.17, *z* = -3.39, *P* = 0.001), treated public fields (β = -0.39 ± 0.16, *z* = -2.43, *P* = 0.02), and PT1 fields (β = -0.50 ± 0.18, *z* = -2.72, *P* = 0.006; Table 1.4; Figures 1.2 and 1.3). However, total bee abundance did not differ between untreated fields and PT3 fields (β = -0.16 ± 0.12, *z* = -1.40, *P* = 0.16; Table 1.4; Figures 1.2 and 1.3). Bee abundance exhibited a positive response to wildflower richness in field margins (β = 0.14 ± 0.04, *z* = 3.55, *P* < 0.001) and a greater percentage of bare soil (β = 0.10 ± 0.04, *z* = 2.49, *P* = 0.01; Table 1.4; Figure 1.3). Neonicotinoid detections in field soil were not a significant predictor of total bee abundance (β = 0.07 ± 0.13, *z* = 0.52, *P* = 0.6; Table 1.4; Figure 1.3).

The interaction between field neonicotinoid detections and floral richness was not a significant predictor of total bee richness (*P* = 0.54) and the interaction between field treatment and floral richness did not converge, so we tested only for main effects. Field treatment had a significant effect on bee genera richness, with bee richness in margins surrounding untreated fields greater than that observed in margins of treated private fields (β = -0.38 ± 0.12, *z* = -3.21, *P* = 0.001), treated public fields (β = -0.26 ± 0.11, *z* = -2.31, *P* = 0.02), PT3 fields (β = -0.21 ± 0.08, *z* = -2.52, *P* = 0.01), and PT1 fields (β = -0.35 ± 0.13, *z* = -2.74, *P* = 0.006; Table 1.4; Figures 1.3 and 1.4). Margin wildflower richness had a significant positive effect on bee richness (β = 0.06 ± 0.03, *z* = 2.18, *P* = 0.03;

Table 1.4; Figure 1.3). Bare soil percentage was not a significant predictor of total richness ($\beta = 0.04 \pm 0.03$, $z = 1.59$, $P = 0.11$; Table 1.4; Figure 1.3). Neonicotinoid detections in field soils were not a significant predictor of total bee richness ($\beta = 0.06 \pm 0.09$, $z = 0.63$, $P = 0.53$; Table 1.4; Figure 1.3).

Analysis of Public Fields Only

Ranked by AICc, our top abundance (AICc Weight = 0.73) and richness models (AICc Weight = 0.77) both contained the predictor variables field treatment, floral richness, and bare soil percentage (Table 1.5). No candidate models that included neonicotinoid detection in soils, flowers, and/or agricultural dust had a ΔAICc ≤ 2 compared to the top models (Table 1.5).

In the top total bee abundance model, field treatment had a significant effect on bee abundance, with bee abundance greater in margins surrounding untreated fields compared to margins of treated public fields ($\beta = -0.34 \pm 0.12$, $z = -2.95$, $P = 0.003$; Table 1.6; Figure 1.5). However, total bee abundance in margins surrounding PT3 fields ($\beta = -0.13 \pm 0.11$, $z = -1.13$, $P = 0.26$) was not significantly different than untreated fields (Table 1.6; Figure 1.5). Floral richness had a significant positive effect on total abundance ($\beta = 0.18 \pm 0.04$, $z = 4.17$, $P < 0.001$) in public field margins (Table 1.6; Figure 1.5). Percent of bare soil was a marginally significant predictor for total abundance ($\beta = 0.09 \pm 0.05$, $z = 1.80$, $P = 0.07$; Table 1.6; Figure 1.5).

In the top total bee richness model, field treatment had a significant effect on total bee richness, which was greater in margins surrounding untreated fields compared to PT3 fields ($\beta = -0.19 \pm 0.08$, $z = -2.45$, $P = 0.01$) and treated field margins ($\beta = -0.21 \pm 0.08$, z

= -2.65, *P* = 0.008; Table 1.6; Figure 1.5). Floral richness had a significant positive effect on total richness ($\beta = 0.07 \pm 0.03$, $z = 2.31$, $P = 0.02$) and percent of bare soil was not a significant predictor for total richness ($\beta = 0.04 \pm 0.03$, $z = 1.27$, $P = 0.2$; Table 1.6; Figure 1.5)

DISCUSSION

Solitary bees provide valuable pollination services to many crops and maintain most wildflower species, making them vital components of agroecosystems (Losey and Vaughan 2006; Potts *et al*., 2010). In support of our initial hypothesis, our results indicate that consistent, annual planting of neonicotinoid-treated seed is linked to field-measured declines in wild, solitary bee communities in agricultural field margins. In our study, both bee abundance and bee richness were significantly greater in margins adjacent to untreated fields. In comparison with untreated fields, all other field treatment categories showed significant reductions in total bee richness (genera) and all but one treatment type (i.e., PT3) showed significant reductions in total bee abundance. Our results support the conclusions of Rundlof *et al.* (2015), which also identified neonicotinoid seed treatment as a significant factor in reductions in wild bee density in uncultivated field margins of flowering oilseed rape fields.

The time required for bee communities to recover following disturbance (e.g., pesticide use) or habitat restoration is variable (M'Gonigle *et al*., 2015; Griffin *et al*., 2017; Purvis *et al*., 2020). In our study, PT1 fields showed reductions in both bee abundance and richness, providing no clear evidence that native bee communities recover after one year of discontinuing treated seed use. However, our results indicate total bee abundance may recover within three years of discontinuing treated seed use, as bee abundance in PT3 fields was not significantly different from UT fields. Conversely, bee species richness may take longer than three years without neonicotinoid seed treatment use to recover, as PT3 fields showed significantly lower richness compared to UT fields.

The relationship between time since neonicotinoid use and bee community recovery is not completely clear. Main *et al*. (2021) found that the presence of neonicotinoids (i.e., detections) negatively impacted the abundance and richness of some bee nesting and foraging guilds. However, they did not find significant differences between bee communities in untreated field margins and field margins adjacent to fields that had not been planted with a neonicotinoid in two years. Our study differed from Main *et al*. (2021) in several ways that may have impacted the ability or inability to detect an effect of field treatment including bee community survey method, length of study, taxonomic resolution of response variables, number of sampling periods, and the variability of land use among study sites.

One possible explanation for bee richness not recovering three years post neonicotinoid use is that less abundant, more rare bee species (e.g., foraging specialists) could be extirpated from agroecosystems where pesticides are continuously utilized (Kosior *et al*., 2007; Bommarco *et al*., 2012), although our data were of insufficient taxonomic resolution to test this hypothesis. In Great Britain, an overall decline in the diversity of wild bees in recent decades was driven, at least in part, by significant range contractions for specialist species that are associated with natural or semi-natural habitat or have narrow forage requirements (Vanbergen *et al*., 2014). Extirpated specialist species may be replaced by diet generalist bees (e.g., *Halictus spp*., *Augochlora spp*.) that are typically more abundant and can populate more heavily disturbed sites such as agricultural areas (Kim *et al*., 2006; Sardiñas *et al*., 2016; Antoine and Forrest, 2020). However, the shift in pollinator communities to include fewer specialists and more generalists could have downstream impacts on wildflowers and crops that are dependent on specialist bee pollinators, including a reduction in pollination services (Steffan-Dewenter *et al*., 2006; Mathiasson and Rehan, 2020).

Several studies have found that high floral richness was associated with a more abundant and diverse wild bee community (Morrison *et al*., 2017; Main *et al*., 2019; Rollin *et al*., 2019). Similarly, our results indicate that maintaining floral richness in field margins may increase both native bee abundance and richness. However, we were unable to detect significant interactions between neonicotinoid seed treatment use and floral richness, and it remains unclear if the benefits of increased floral richness can overcome or outweigh the negative impacts of continuous neonicotinoid use and exposure. In our models, the negative impact of neonicotinoid seed treatment on bee abundance and bee richness was greater than the positive impact afforded by increased floral richness. A recent study of bee community diversity in prairie restorations along an agricultural landscape gradient found that local bee diversity increased with increasing local floral richness independent of the surrounding landscape (Lane *et al*., 2020). Relatedly, enhancing plant diversity in agricultural landscapes was found to promote both bees of conservation concern (rare and specialist bees) and dominant crop-pollinating bees, including managed honeybee populations (Sutter *et al*., 2017). The results of these studies suggest that landscapes dominated by agricultural production should not be overlooked as sites for habitat management designed to benefit bee communities (Morrison *et al*., 2017).

Bare ground availability has been shown to benefit bee communities (Potts *et al*., 2005), including those in agricultural areas (Ballare *et al*., 2019). The results of our study indicate that access to bare soil in field margins may increase bee abundance in agroecosystems, possibly by providing an alternative nesting location to the field (Purvis *et al*., 2020). We had relatively low detections of neonicotinoids in margin soils (2%), suggesting bees nesting in margins would be exposed to fewer pesticides than those nesting in more contaminated field soils (32% neonicotinoid detections). Some soil-nesting bee species (e.g., *Peponapis pruinosa, Melissodes agilis*) have been documented to preferentially nest in agricultural field soils (Julier and Roulston, 2009; Sardiñas *et al*., 2016), but it is unclear if this preference is the result of a lack of proper nesting habitat available elsewhere in the landscape. Much of the nesting habitat previously available to ground-nesting bees in the Great Plains of the midwestern U.S. has been converted to row crops or pasture (Olimb and Robinson, 2019; Sanchez-Bayo and Wyckhuys, 2019; Olynyk *et al*., 2021), often giving bees no alternatives to agricultural field soil, which may chronically expose them to neonicotinoids (Krupke *et al*., 2012; Chan *et al*., 2019; Main *et al*., 2020). In a recent laboratory study, neonicotinoids concentrations in soil at <15 ppb were associated with reduced bee longevity and development (Anderson and Harmon-Threatt, 2019). As the developing progeny of most solitary bees remain in the ground for most of their life cycle (Antoine and Forrest, 2020), limiting ground nesting bee exposure to neonicotinoids in field soil by providing nesting habitat (i.e., bare soil) in less contaminated areas on the landscape, such as field margins, could have positive impacts on wild bee communities in agricultural areas.

Several studies have found neonicotinoid residues in pollen and nectar samples (Botias *et al*., 2015; David *et al*., 2016) and whole flowers (Stewart *et al*., 2014) collected from agricultural field margins, even after a five-year moratorium on neonicotinoid use (Wintermantel *et al*., 2020). Neonicotinoid residue detection rates range from 23 % (Stewart *et al*., 2014) to 63% (David *et al*., 2016) of samples, at concentrations up to 257 ng/g (Stewart et al., 2014), with average concentrations sometimes greater than average concentrations found in pollen collected from flowering crops (Botias *et al*., 2015). In our study, we detected neonicotinoids in 21% of field margin flower components with 7 ng/g as the greatest concentration we observed. However, neonicotinoid residues in margin wildflowers are likely dependent on plant species and seasonality (Mogren and Lundgren, 2016), as well as heterogeneity in soil properties and environmental factors in field margins (Bonmatin *et al*., 2015). By compositing floral biomass for each field across sampling periods, we may have reduced our ability to detect neonicotinoids in margin plants. Further, our results were also potentially limited by our analytical LOD for neonicotinoids (1 to 6 ppb), particularly for low concentrations. It is notable that most of our neonicotinoid detections in field margin plants were in UT and PT3 fields where neonicotinoids had not been planted in ≥ 3 years, suggesting lateral movement of neonicotinoids (and subsequent uptake by margin plants), potentially from nearby agricultural sources not associated with our study (Krupke *et al*., 2012; Krupke *et al.*, 2017; Hladik *et al*., 2018). These results indicate that exposure to neonicotinoids via interactions with margin wildflowers should not be overlooked as a potential route of neonicotinoid exposure for solitary bees.

Krupke *et al*. (2012) investigated the possible exposure of honeybee hives to neonicotinoids via planter exhaust contamination of wildflowers and found plants visited by foraging bees growing near fields contained neonicotinoids, possibly from deposition of neonicotinoids on the flowers. Another study showed that off-target movement of neonicotinoids occurred while planting treated corn seed (Greatti *et al*., 2006). Our results indicate that contact with neonicotinoids via dust produced during planting activities may be an overlooked route of exposure for bees foraging in field margins. We detected neonicotinoids in 100% of dust slide samples, including those that were placed in UT fields and those that were placed prior-to and after field planting, suggesting that neonicotinoids are being transported through the air (possibly across great distances) and settling on field margin plants. Limay-Rios *et al.* (2016) found that wind erosion of surface soil in fields with a history of seed treatment use can cause particulate matter containing pesticide residues to land on field margin plants. Further, neonicotinoids at concentrations >1.4 $\mu g/m^2$ were detected up to 100 m from the edge of corn fields in Indiana following planting activities (Krupke *et al*., 2017). Bees (particularly early-season bees that forage during planting season) may be exposed to neonicotinoids in agricultural dust during flight or while foraging in agricultural field margins (Krupke *et al*., 2012).

Study Limitations

All bee community survey methods have their own, unique limitations (e.g., euthanizing bees or a bias towards bees of certain sizes) (Gibson *et al*., 2011). Our study was no exception, and our results and interpretation were potentially limited by using a visual survey method. Using visual survey methods made it appropriate to only assess total bee abundance and richness, as opposed to abundance and richness of specific nesting or foraging guilds (e.g., ground-nester abundance, specialist forager richness) (Main *et al*., 2021). This reduced our ability to determine if certain nesting or foraging guilds are differentially impacted by neonicotinoids (Main *et al*., 2021) or local habitat characteristics (Potts *et al*., 2005). For example, ground-nesters may be more heavily impacted by neonicotinoids than other bee nesting guilds due to exposure to neonicotinoids in soil (Pisa *et al*., 2015; Main *et al*., 2021), which is the environmental media where we detected the greatest neonicotinoid concentrations (up to 41 μg/kg in treated fields).

Main *et al*. (2021) found that neonicotinoid detections in field soils were linked to reductions in the richness of nesting and diet guilds, including aboveground and ground nesting bees as well as diet generalist bees, while finding no effect of field treatment. Although we did find field treatment to have a significant impact on bee abundance and richness, we were unable to directly link reductions in bee abundance and richness to neonicotinoid detections in any environmental media. We propose that field treatment (as defined in our study) likely best represents chronic exposure to neonicotinoids, whereas pesticide residue data in environmental media (e.g., soil, agricultural dust) could represent only a specific snapshot in time, making it harder to link directly to bee communities. In our study, concentrations and detections of neonicotinoids in environmental media were infrequent and fluctuated throughout the season, making it challenging to specifically test the impacts of neonicotinoid residues in environmental media on wild bee communities.

Using existing field margins and including both private and public land in our study may have produced too much variation in the data, limiting our statistical power, especially regarding potential interactions between neonicotinoid use and habitat characteristics such as floral richness. Also, the lack of completely untreated fields on private land may have further limited our ability to draw conclusions about potential interactions between neonicotinoid use and local habitat characteristics. Further, as all our fields on private land were enrolled as CRP acreage, they likely represent a 'best-case-scenario' for bee habitat on private land (similar to fields on CAs likely representing a 'best-case-scenario'; Main *et al*., 2021), which further limited our scope of inference.

Conclusions

Our results indicate that continuous (annual) planting of neonicotinoid-treated seed is linked to field-measured declines in bee communities in agricultural field margins on both public and private land. Such declines could be detrimental to maintaining abundant and diverse bee communities in Midwestern row crop agroecosystems. Bee communities in agricultural field margins are likely to be chronically exposed to neonicotinoids, regardless of whether treated seeds are directly planted in the adjacent field (Krupke *et al*., 2017; Wintermantel *et al*., 2020). The presence of adequate foraging and nesting resources (e.g., floral richness and access to bare soil in field margins) provides benefits to bees, but it remains unclear if the benefits of local habitat characteristics can offset the negative impacts of neonicotinoid seed treatment use (Mogren and Lundgren, 2016). Thus, eliminating the use of neonicotinoids on areas managed for conservation is likely to reduce negative impacts of neonicotinoid seed treatments on wild bee communities.

MANAGEMENT IMPLICATIONS

Landowners and managers, especially those in more conservation-focused and less production-centric agroecosystems (e.g., public lands managed for wildlife), should consider that at least a three-year moratorium on neonicotinoid use may be required for bee abundance to recover, and bee richness may require an even longer time period to recover. To benefit bee communities, neonicotinoid use in an agricultural field should occur no more than every three years. Alternatively, untreated seed could be used, especially in areas where pest pressures are lower or when the goal of the crop planting is to provide food and cover for wildlife (i.e., not maximum production).

Bee habitat consists of two main components: forage and nest sites (Black *et al*., 2011). Our results indicate that bees may be actively using agricultural field margins with a rich suite of wildflower species and/or exposed bare soil (70% of bee species nest in the ground). Providing a rich suite of wildflowers and/or exposed bare soil in areas where the risk of neonicotinoid exposure is lower (e.g., adjacent to fields where neonicotinoids are not used or in areas more isolated from agricultural activities) may be crucial for bee populations in agroecosystems.

Appropriate wildflower richness and bare soil conditions can be obtained using management activities that reset plant succession, expose soil, and promote beneficial plant species, such as controlled burns and targeted mowing (Black *et al*., 2011). However, careful consideration should be given to the timing, location, and extent of management activities, as these factors can determine whether management activities have a positive or negative effect on bee populations. For example, timing of burns is crucial, and burns should not be conducted when pollinators are in a critical foraging stage (Black *et al*., 2011). Early spring burns can be especially problematic because they can severely limit foraging options by destroying many of the wildflowers that early emerging bees are dependent upon for survival and reproduction. An autumn or winter burn that avoids crucial foraging stages would be more beneficial for bees. In addition, habitat patches should not be burned completely. A mosaic of burned and unburned areas is ideal to protect nesting habitat for above-ground nesting bees, such as *Ceratina spp.* that nest in standing plant stems (Nooten and Rehan 2019). As a fire moves through an area it may leave small patches unburned and leaving these patches intact allow them to serve as potential refuges that are important for recolonization. A program of rotational burning where small sections are burned every few years will likely ensure adequate colonization potential for pollinators, and fires should not burn more than one-third of habitat in any given year.

Mowing can create uniform vegetation height and remove flowering resources. However, mowing practices can be adapted to the maximum benefit of pollinators. Mowing at higher and/or varied heights can protect topographical features, such as grass tussocks and standing plant stems, that provide structural diversity to the habitat and offer potential nesting sites for pollinators. In addition, when management objectives allow, it would benefit pollinators if land managers adopted a targeted mowing approach that avoids blooming flowers.

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TABLES: CHAPTER 1

Table 1.1: Description of variables used in models to evaluate effects of neonicotinoid seed treatment on abundance and richness of wild, solitary bees in agroecosystems located in north-central Missouri, USA during May through September 2019.

Variable Name	Variable Type	Variable Description	How we calculated the variable	Models in which variable was included
Bare Soil %	Continuous	Estimate of the amount of bare soil available for soil-nesting bees to utilize in each field's margins during each sampling period in 2019.	We averaged (for each sampling period) the percentage of bare soil from 12 vegetative quadrat samples conducted in the margins of each field.	**All fields analysis** - both total abundance and total richness (genera) models. **Public fields analysis** - included in candidate model sets including the top models for abundance and richness (see Table 1.5).
Floral Richness	Continuous	Estimate of the total floral richness in each field's margins during each sampling period in 2019.	We summed (for each sampling period) the total number of unique floral species from 12 vegetative quadrat samples conducted in the margins of each field.	**All fields analysis** - both total abundance and total richness (genera) models. **Public fields analysis** - included in candidate model sets including the top models for abundance and richness (see Table 1.5).
Treatment	Categorical	The treatment category of the study field in 2019. Treatment categories encompass both the historical and study-year use of neonicotinoid seed treatments, as well as field management status (public or private).	We manipulated neonicotinoid seed treatment type (treated or untreated) in 2019, and we gathered information on the past use (or lack thereof) of neonicotinoids in previous years. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.	**All fields analysis** - both total abundance and total richness (genera) models. **Public fields analysis** - included in candidate model sets including the top models for abundance and richness (see Table 1.5).

Variable Name	Variable Type	Variable Description	How we calculated the variable	Models in which variable was included
Neonicotinoid Detection Agricultural Dust (2019)	Categorical	Detection of high (1) or low (0) concentrations of neonicotinoids in agricultural dust collected in each public field's margins in 2019.	We split neonicotinoid detections in agricultural dust into two categories: high ($\geq$ 0.26 ng/cm^2) and low ($\leq$ 0.26 ng/cm^2) based on the mean concentration found in all agricultural dust samples.	**Public fields analysis** - included in candidate model sets, but not the top models for abundance and richness (see Table 1.5).
Neonicotinoid Detection Field Soil (2017)	Categorical	Detection [Yes (1) / No (0)] of neonicotinoids in field soil collected from each field in 2017.	Neonicotinoid presence in field soil was based on the entire field season. That is, if in any time period a field had soil with neonicotinoid concentrations >LOD, we listed this field as a 'detect' and any field with all concentrations <LOD (including trace detections) we listed as 'non-detect'.	**Public fields analysis** - included in candidate model sets, but not the top models for total abundance and richness (see Table 1.5).
Neonicotinoid Detection Field Soil (2018)	Categorical	Detection [Yes (1) / No (0)] of neonicotinoids in field soil collected from each field in 2018.	Neonicotinoid presence in field soil was based on the entire field season. That is, if in any time period a field had soil with neonicotinoid concentrations >LOD, we listed this field as a 'detect' and any field with all concentrations <LOD (including trace detections) we listed as 'non-detect'.	**Public fields analysis** - included in candidate model sets, but not the top models for total abundance and richness (see Table 1.5).

Variable Name	Variable Type	Variable Description	How we calculated the variable	Models in which variable was included
Neonicotinoid Detection Field Soil (2019)	Categorical	Detection [Yes (1) / No (0)] of neonicotinoids in field soil collected from each field in 2019.	Neonicotinoid presence in field soil was based on the entire field season. That is, if in any time period a field had soil with neonicotinoid concentrations >LOD, we listed this field as a ‘detect’ and any field with all concentrations <LOD (including trace detections) we listed as ‘non-detect’.	**All fields analysis** - both total abundance and total richness (genera) models. **Public fields analysis** - included in candidate model sets, but not the top models for abundance and richness (see Table 1.5).
Neonicotinoid Detection Wildflowers (2019)	Categorical	Detection [Yes (1) / No (0)] of neonicotinoids in wildflowers collected from each field's margins in 2019.	We composited (for each field) all wildflower samples collected throughout 2019. Sample were chemically analyzed, and we counted any field with a neonicotinoid concentration above trace amounts as a ‘detect’.	**Public field analysis** - included in candidate model sets, but not the top model for total abundance or richness (see Table 1.5).

Table 1.2: Detection frequency, mean and maximum neonicotinoid concentrations in field soil (μg/kg), wildflowers (μg/kg), and agricultural dust samples (ng/cm^2). We collected field soil and wildflower samples in May – September 2019. We collected agricultural dust samples during field planting as well as one week prior to and one week after field planting in May – June 2019. We summed all detected neonicotinoid active ingredients together for each sample, and we exclude trace detections. NS = not sampled.

	Total Neonicotinoid Concentration								
	Field Soil			**Wildflowers**			**Agricultural Dust**		
Treatment[1]	Detection Frequency (%)	Mean (μg/kg)	Max (μg/kg)	Detection Frequency (%)	Mean (μg/kg)	Max (μg/kg)	Detection Frequency (%)	Mean (ng/cm^2)	Max (ng/cm^2)
PT1 (n = 4)	19	1.86	17	0	0	0	NS	NS	NS
PT3 (n = 6)	11	0.67	9	33	1.67	7	100	0.21	1.86
Treated Public (n = 6)	61	7.78	41	17	0.83	5	100	0.24	1.1
Treated Private (n = 7)	61	6.39	22	17	0.33	2	NS	NS	NS
Untreated (n = 6)	0	0	0	33	0.67	2	100	0.03	0.06

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

Table 1.3: Abundance of wild bees by genera/tribe observed in field margins surrounding study fields. Numbers presented here represent the overall capture for each field treatment category in 2019.

	Treatment[1]					
Genera/Tribe	**PT1 (n=4)**	**PT3 (n=6)**	**Tpriv (n=7)**	**Tpub (n=6)**	**UT (n=6)**	**Total**
Agapostemon spp	14	24	18	25	20	101
Andrena spp.	21	19	44	36	49	169
Anthophora spp.	3	3	3	1	3	13
Augochlorini	85	105	61	133	118	502
Calliopsis spp.	0	1	1	0	0	2
Ceratina spp.	76	215	196	179	164	830
Coelioxys spp.	0	6	2	8	12	28
Eucera spp.	2	12	12	19	21	66
Florilegus spp.	0	7	1	4	5	17
Halictus spp.	52	49	85	64	80	330
Heriades spp.	4	12	8	5	24	53
Hoplitis spp.	6	1	4	2	2	15
Hylaeus spp.	1	20	8	10	7	46
Lasioglossum spp.	39	60	59	65	51	274
Megachile spp.	94	156	97	113	228	688
Melissodes spp.	43	153	84	102	167	549
Melitoma spp.	6	1	0	9	3	19
Nomada spp.	1	2	3	0	3	9
Nomia spp.	0	0	2	0	0	2
Osmia spp.	1	1	0	4	18	24
Ptilothrix spp.	0	0	0	0	5	5
Sphecodes spp.	0	1	2	2	1	6
Svastra spp.	0	1	2	0	6	9
Triepeolus spp.	4	18	11	16	41	90
Unidentified	15	16	16	32	28	107
Total	**503**	**934**	**761**	**885**	**1099**	**4182**

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Tpub – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Tpriv – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

Table 1.4: Results of mixed-effects model evaluating treatment, neonicotinoid detections in field soil, and site-specific environmental variables (floral richness and % bare soil in field margins) on wild (solitary) bee abundance and richness in public and private agricultural field margins. Models had a random intercept term for Field.ID nested within Site and a random intercept term for sampling period.

	Response					
	Total Abundance			**Total Richness**		
Fixed Effects Variables	β	± SE	*P*	β	± SE	*P*
Treatment: PT1[1]	-0.50	0.18	0.006**	-0.35	0.13	0.006**
Treatment: PT3[1]	-0.17	0.12	0.16	-0.21	0.08	0.01*
Treatment: Treated Private[1]	-0.58	0.17	0.001***	-0.38	0.12	0.001**
Treatment: Treated Public[1]	-0.39	0.16	0.02*	-0.26	0.11	0.02*
Floral Richness	0.14	0.04	<0.001***	0.06	0.03	0.03*
Bare Soil %	0.10	0.04	0.01*	0.04	0.03	0.11
Neonicotinoid Detection Field Soil	0.07	0.13	0.60	0.06	0.09	0.53

Model: Response ~ Treatment + FloralRich + BareSoil + Neo_Det_Field_Soil + (1|Sample_Period) + (1|Site/Field_ID)

Significance Codes: '***' <= 0.001 '**' = 0.01 '*' = 0.05 '.' = 0.1

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed three years prior to study period and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

Table 1.5: Candidate model sets for dependent variables total bee abundance and richness (genera) in public field margins in 2019 ranked by AICc. All models had a random nested intercept term for Field.ID nested within Conservation Area and a random intercept term for sampling period.

Dependent Variable	Candidate Model Sets	K	AICc	Δ_AICc	AICc_Wt	Cum_Wt	LL
Total Abundance	Treat + FloRich + BareSoil	9	1027.17	0	0.73	0.73	-503.87
	Treat + FloRich + BareSoil + Dust + Flower	11	1031.65	4.47	0.08	0.8	-503.75
	Treat + 17-19Soil + FloRich + BareSoil	12	1031.72	4.55	0.07	0.88	-502.58
	17-19Soil + FloRich + BareSoil	10	1031.83	4.66	0.07	0.95	-505.03
	Treat + 19Soil + Dust + Flower + FloRich + BareSoil	12	1033.66	6.49	0.03	0.98	-503.55
	19Soil + Dust + Flower + FloRich + BareSoil	10	1035.24	8.06	0.01	0.99	-506.73
	17-19Soil + Dust + Flower + FloRich +BareSoil	12	1035.97	8.8	0.01	1	-504.71
	17-19Soil	8	1039.63	12.45	0	1	-511.24
	17-19Soil + Dust +Flower	10	1043.52	16.35	0	1	-510.87
	19Soil + Dust + Flower	9	1043.87	16.69	0	1	-512.21
	Treat + 19Soil + Dust + Flower	10	1044.06	16.89	0	1	-511.14
Total Richness	Treat + FloRich + BareSoil	9	601.06	0	0.77	0.77	-290.81
	Treat + FloRich + BareSoil + Dust + Flower	11	605.5	4.44	0.08	0.86	-290.68
	17-19Soil + FloRich + BareSoil	10	607.68	6.63	0.03	0.89	-292.95
	Treat + 19Soil + Dust + Flower + FloRich + BareSoil	12	607.74	6.69	0.03	0.91	-290.59
	Treat + 17-19Soil + FloRich + BareSoil	12	607.98	6.92	0.02	0.94	-290.71
	17-19Soil	8	608.48	7.42	0.02	0.96	-295.67
	19Soil + Dust + Flower + FloRich + BareSoil	10	608.78	7.72	0.02	0.97	-293.5
	Treat + 19Soil + Dust + Flower	10	609.42	8.36	0.01	0.99	-293.82
	19Soil + Dust + Flower	8	610.5	9.45	0.01	0.99	-296.68
	17-19Soil + Dust + Flower + FloRich + BareSoil	12	611.24	10.18	0	1	-292.34
	17-19Soil + Dust + Flower	10	612.26	11.21	0	1	-295.24

Explanation of variables: Treat = Treatment; FlorRich = Floral richness in field margin; BareSoil = Bare soil % in field margin; Dust = Neonicotinoid detection in agricultural dust (low or high); Flower = Neonicotinoid detection in margin wildflowers; 17-19Soil = Neonicotinoid detections in field soil for three years were included (2017 – 2019); 19Soil = Only study year (2019) neonicotinoid detection in field soil were included.

Table 1.6: Results of the top mixed-effect model for the analysis of wild (solitary) bee abundance and richness in public field margins. Fixed effects include treatment and site-specific environmental variables (floral richness and % bare soil in field margins). Model had a random nested intercept term for Field.ID nested within Conservation Area and a random intercept term for sampling period.

	Response					
	Total Abundance			**Total Richness**		
Fixed Effects Variables	β	± SE	*P*	β	± SE	*P*
Treatment: PT3[1]	-0.13	0.11	0.26	-0.19	0.08	0.01*
Treatment: Treated Public[1]	-0.34	0.12	0.003**	-0.21	0.08	0.008**
Floral Richness	0.18	0.04	<0.001***	0.07	0.03	0.02*
Bare Soil %	0.09	0.05	0.07.	0.04	0.03	0.20

Model: Response ~ Treatment + FloralRich + BareSoil + (1|Sample_Period) + (1|Site/Field_ID)

Significance Codes: '***' <= 0.001 '**' = 0.01 '*' = 0.05 '.' = 0.1

[1] **Treatment Categories:** PT3 – Fields last planted with neonicotinoid-treated seed in 2016; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior.

FIGURES: CHAPTER 1

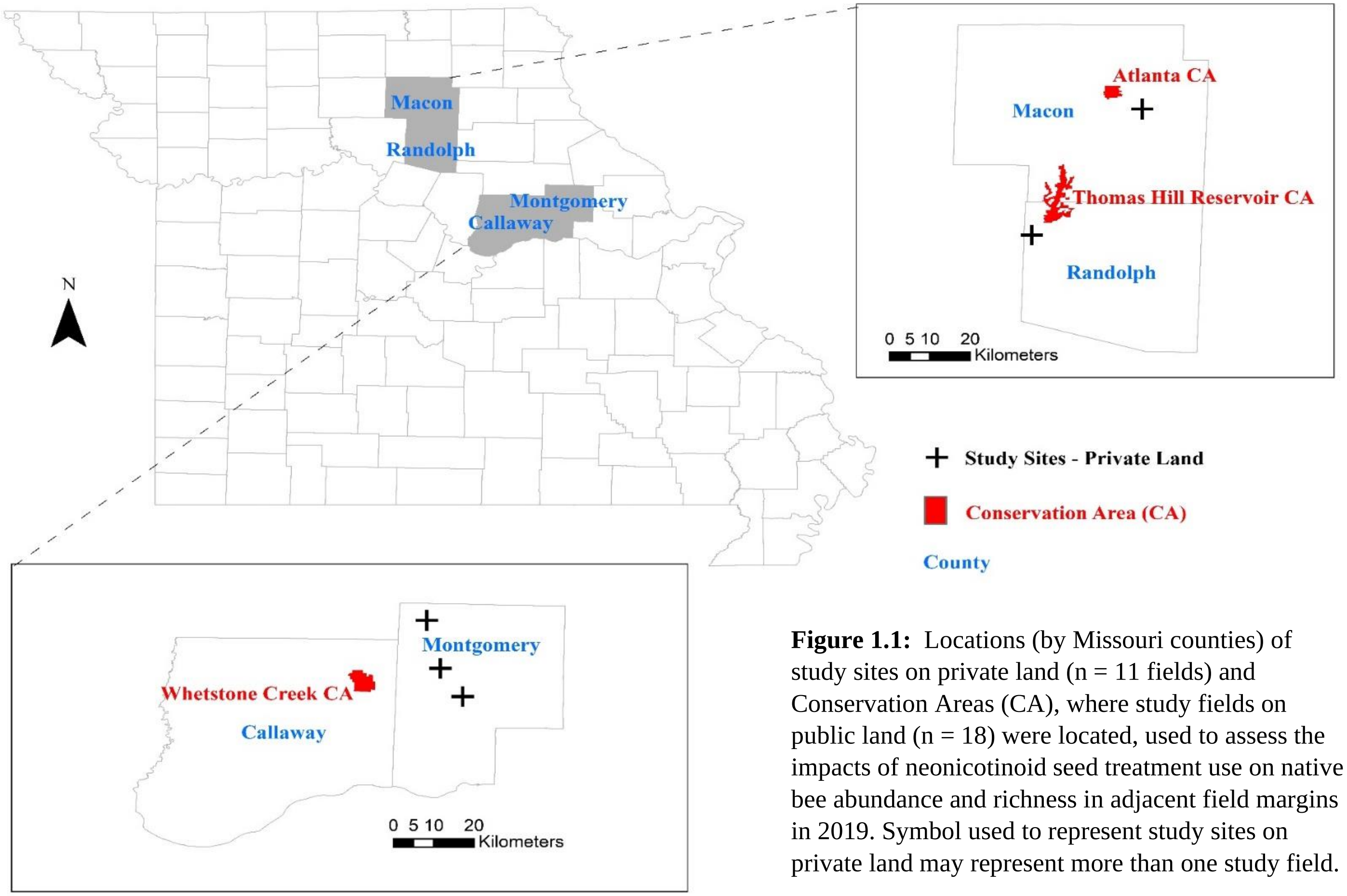

Figure 1.1: Locations (by Missouri counties) of study sites on private land (n = 11 fields) and Conservation Areas (CA), where study fields on public land (n = 18) were located, used to assess the impacts of neonicotinoid seed treatment use on native bee abundance and richness in adjacent field margins in 2019. Symbol used to represent study sites on private land may represent more than one study field.

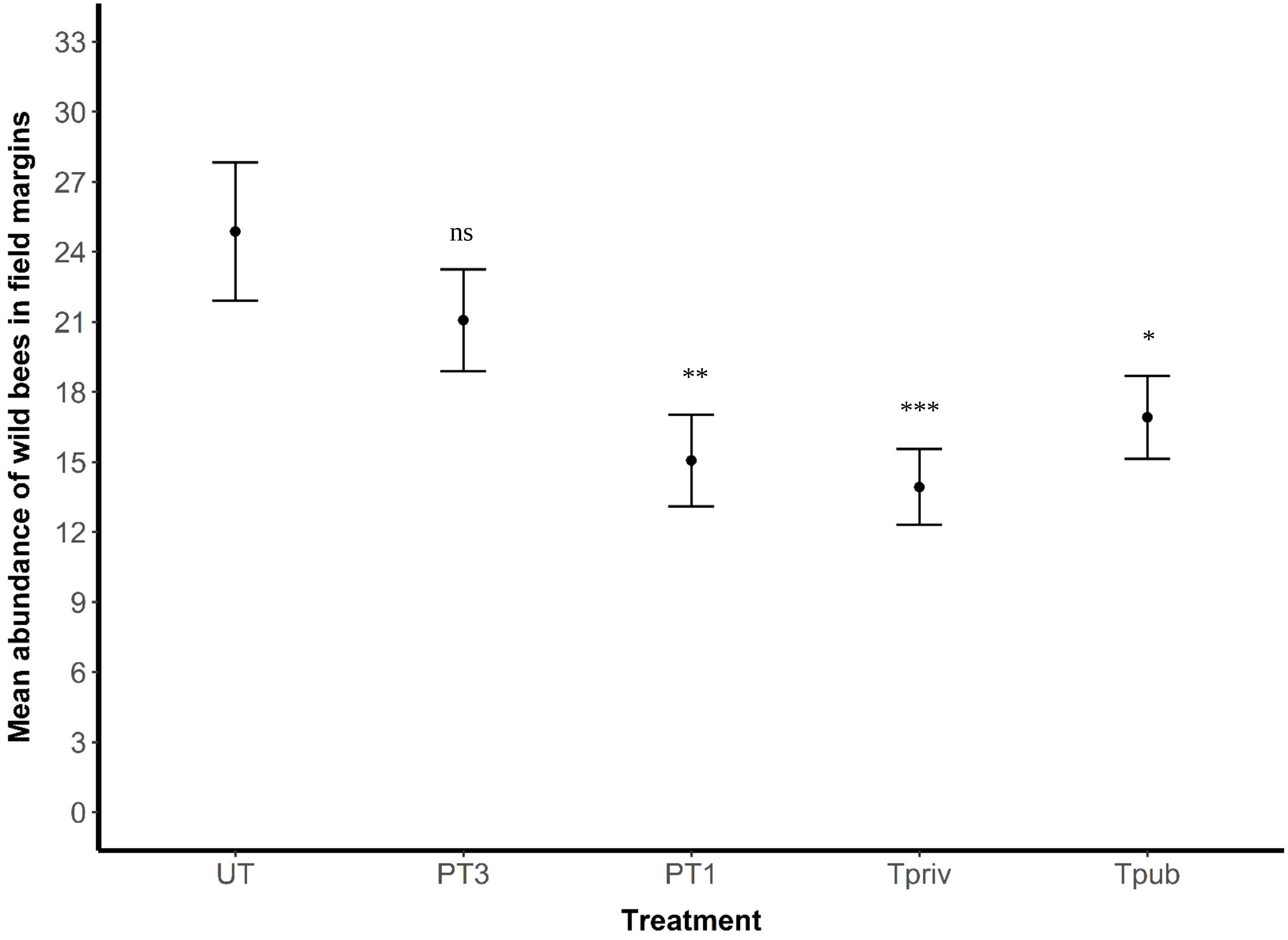

Figure 1.2: Point plot of mean bee abundance in field margins by field treatment. Significance codes indicate differences in relation to untreated (UT) fields. Error bars represent the standard error. Treatment Categories: UT – fields not planted with neonicotinoid-treated seed in the study year or any year prior and

located on public land; PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Tpub – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Tpriv – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land.

Significance Codes: ‘***’ <= 0.001 ‘**’ = 0.01 ‘*’ = 0.05 ‘ns’ = not significant

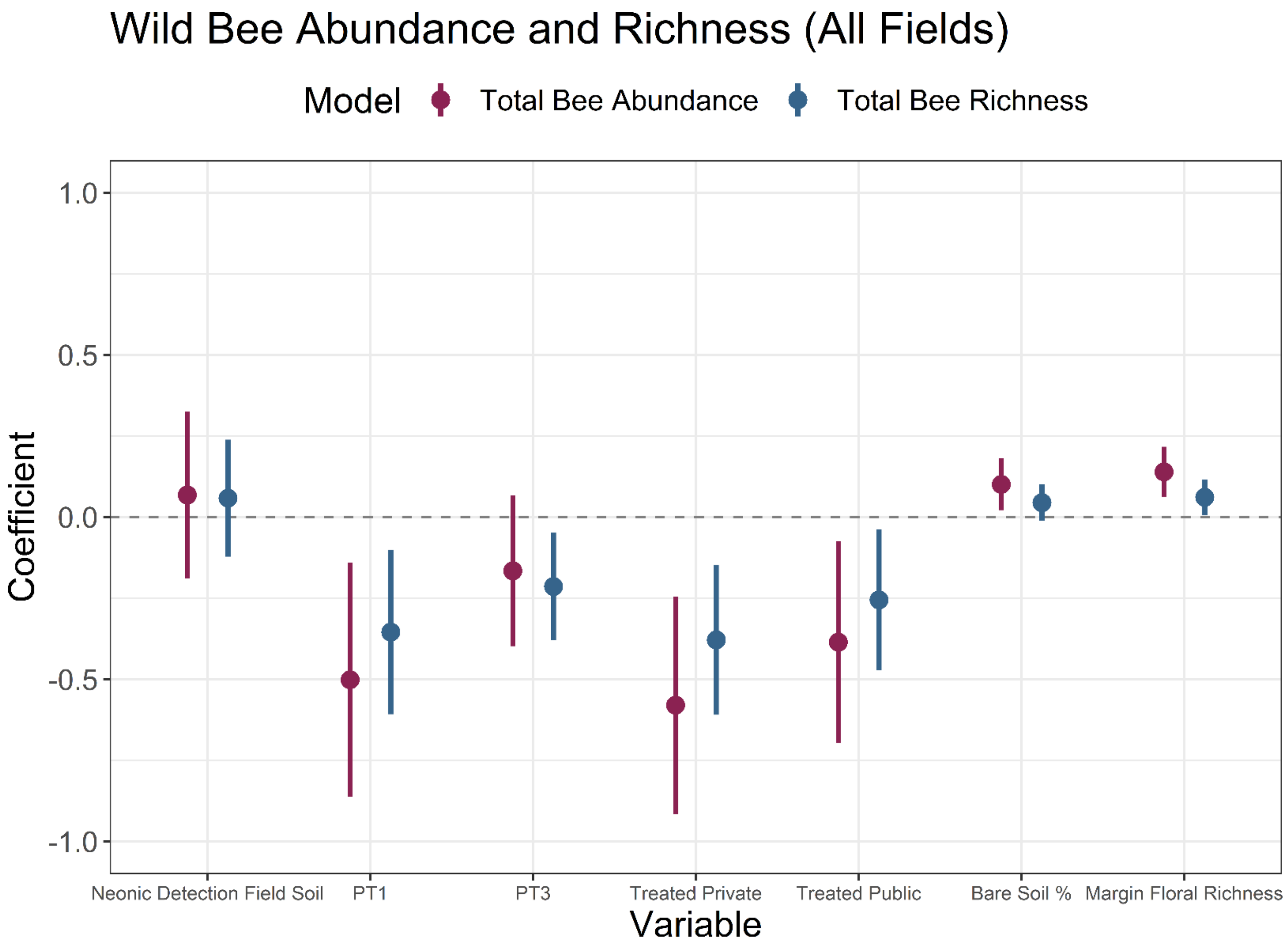

Figure 1.3: Coefficients ± 1.96 * standard error from models evaluating total solitary bee abundance and richness in all study fields (public and private). Model variables include neonic detection in fields soils collected in 2019, treatment, as well as the site-level habitat variables bare soil % and floral richness in field

margins. Treatment Categories: PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

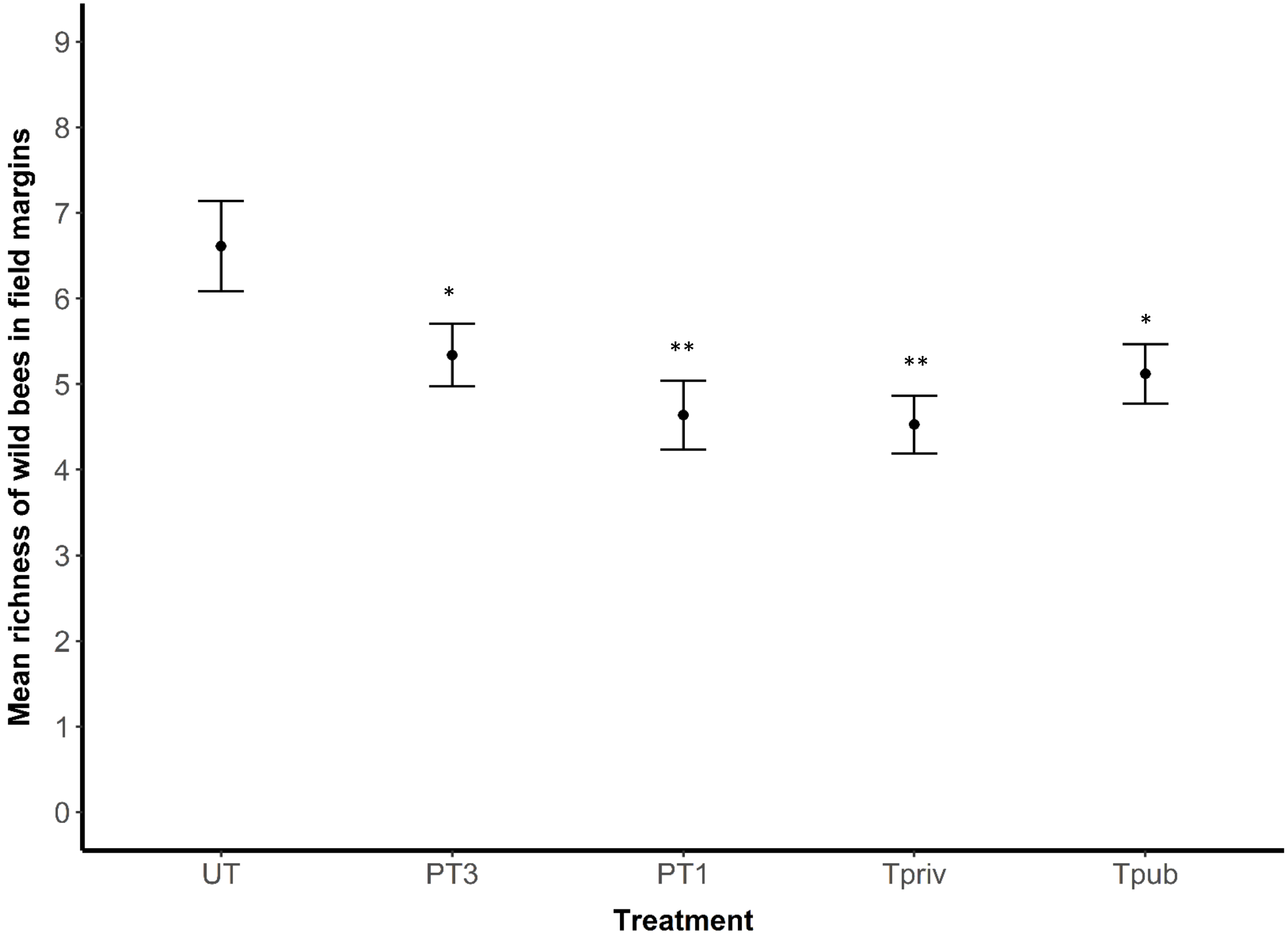

Figure 1.4: Point plot of mean bee richness (genera) in field margins by field treatment. Significance codes indicate differences in relation to untreated (UT) fields. Error bars represent the standard error. Error bars represent the standard error. Treatment Categories: UT – fields not planted with neonicotinoid-treated seed in the study year or any year prior and located on public land; PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land;

PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Tpub – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Tpriv – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land.

Significance Codes: ‘***’ <= 0.001 ‘**’ = 0.01 ‘*’ = 0.05

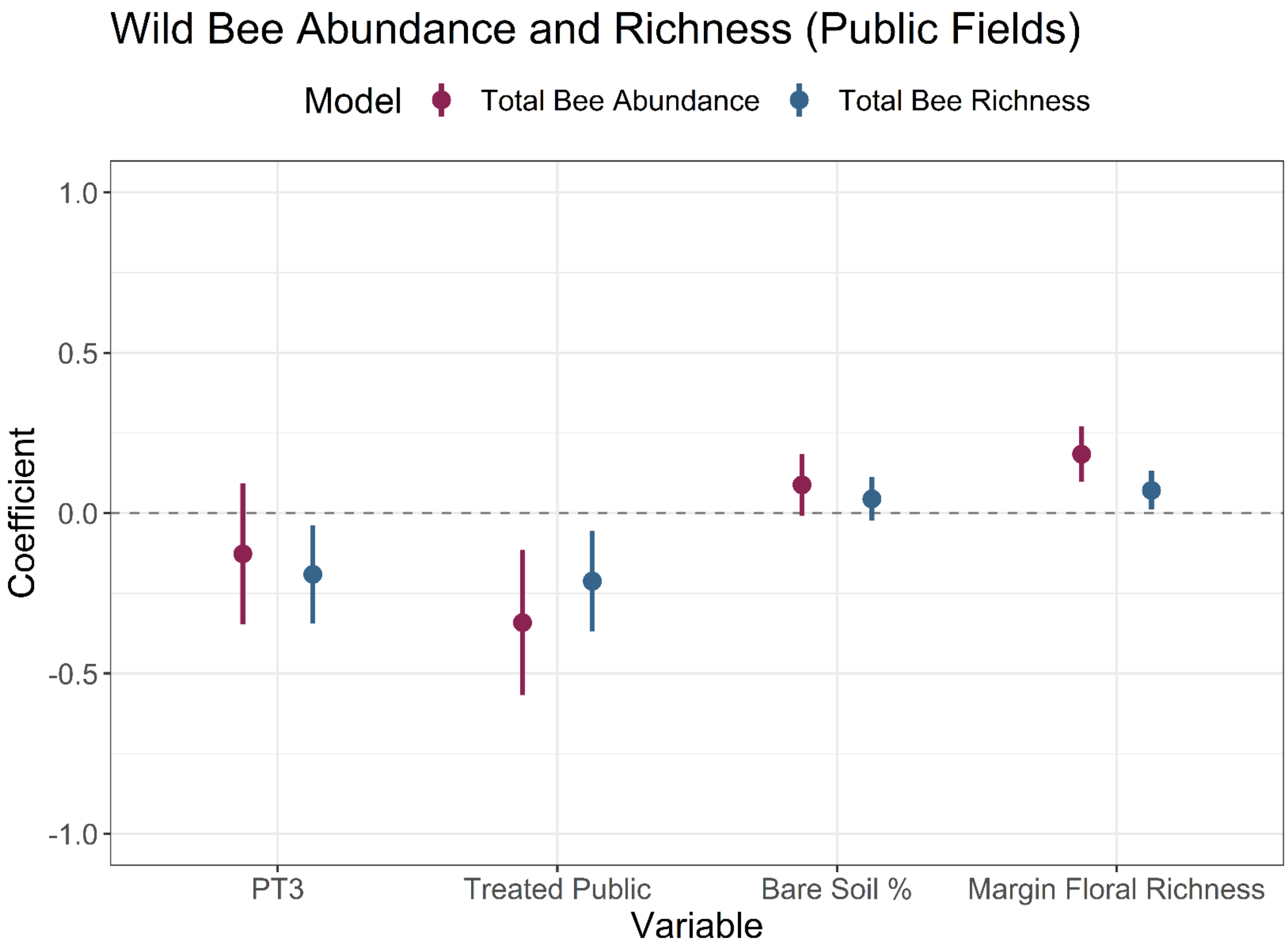

Figure 1.5: Coefficients ± 1.96 * standard error from top models evaluating total solitary bee abundance and richness in public study fields. Model variables include treatment as well as the site-level habitat variables bare soil % and floral richness in field margins. Treatment Categories: PT3 – Fields last planted with

neonicotinoid-treated seed in 2016; T Pub – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land.

Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior.

CHAPTER 2 – EVALUATING CAVITY-NESTING BEE REPRODUCTIVE EFFORT AND SUCCESS IN FIELD MARGINS SURROUNDING NEONICOTINOID-TREATED SOYBEAN FIELDS

ABSTRACT

Wild, solitary bees provide critical and underappreciated pollination services, yet little is known about the impacts of neonicotinoid insecticides on solitary bee reproduction in agroecosystems. Here, we conducted a two-year experimental field study to evaluate whether neonicotinoid seed treatment influenced cavity-nesting bee reproductive effort and success in agricultural field margins in the midwestern United States. Our secondary objective was to determine whether site-specific habitat variables such as margin floral richness or the percentage of cultivated land surrounding the field impacted nesting effort or success. Finally, we sought to quantify pesticide concentrations in nest material collected by wild, cavity-nesting bees. In the springs of 2018 and 2019, we placed solitary bee nest boxes in field margins of Missouri soybean (*Glycine max*) fields (2018, n = 18; 2019, n = 24). In 2018, all fields were located on public conservation areas and were planted to one of three experimental treatments: untreated (no insecticide), treated (imidacloprid), or previously-treated (untreated, but neonicotinoid use prior to 2017). In 2019, privately-owned fields enrolled in the Conservation Reserve Program were added to the study. Fields on private land were planted to one of two experimental treatments: treated (neonicotinoid) or previously-treated (untreated, but neonicotinoid use in 2018). We removed nest boxes in the fall of each year and quantified the number of nest cells, number of cavities used, and the percentage of nests producing an emerged adult (% emergence) for both resin and leafcutter bees. Field treatment had no negative impact on nest cell production or cavity use of either resin or leafcutter bees. However, continuous planting of neonicotinoid seed treatments reduced resin bee nest success, with a lower percentage of resin bees emerging from nests collected from margins of previously-treated public (15%) and treated public (7%) fields compared to untreated fields (46%). We also found that the amount of woody vegetation near nesting sites impacted nesting effort of resin bees, with more nest cells produced and cavities used in margins of fields with a greater amount of woody vegetation within 800 m. Finally, neonicotinoids were infrequently detected in nest material (7% of total samples), but 15 other pesticide residues were detected, and all 29 nest material samples had at least one pesticide detection. Our results indicate that neonicotinoid seed treatments may reduce resin bee reproductive success in agricultural field margins, potentially creating an ecological trap. Reducing the overall use of pesticides on areas managed for wildlife may facilitate successful bee reproduction.

INTRODUCTION

Neonicotinoids are a relatively new class of neuro-active insecticides that bind to nicotinic acetylcholine receptors in the central nervous system of insects (Ihara and Matsuda 2018). High doses of neonicotinoids overstimulate and block the receptors, causing insect paralysis and death, while low to moderate doses activate the receptors causing nerve stimulation. Globally, neonicotinoids represent ~80% of all seed treatment sales and ~30% of the total market share of insecticides (Ihara and Matsuda 2018). Since their commercial registration in the 1990s, neonicotinoids have become the most widely used class of insecticides in the world (Goulson 2013), especially as the popularity of seed treatments (~60% of all neonicotinoid applications) has increased in recent decades (Jeschke and Nauen 2008; Jeschke *et al*., 2011). Neonicotinoids are applied to almost 100% of corn (*Zea* mays) and ≥ 40% of soybean (*Glycine* *max*) seeds in the United States (US) (Hladik *et al.,* 2018; Douglas and Tooker 2015), leading to the near ubiquitous presence of neonicotinoids in Midwestern agroecosystems dominated by corn-soybean rotations (Durant and Otto 2019).

Neonicotinoids are systemic, water-soluble insecticides, which means that following planting of treated seed, the chemical is taken up by the target plant during the initial growth stage and spreads throughout its tissues, including leaves, pollen, and nectar (David *et al.,* 2016). However, uptake of neonicotinoid active ingredients by target crops is estimated to be <10%, leaving ~90% of active ingredient available to either degrade, sorb to soil particles, or leach/spread to non-target environmental media (Goulson 2013). Neonicotinoids have been detected in plants and soil of agricultural field margins, where they can persist and accumulate over extensive time periods (Krupke *et al.,* 2012; Goulson 2013, Jones *et al.,* 2014; David *et al.,* 2016), potentially providing season-long routes of exposure for non-target organisms such as bees (Long and Krupke 2016).

Field-realistic neonicotinoid exposure for bees is often disputed in the literature (Wood and Goulson 2017), but evidence suggests bees are consistently exposed to neonicotinoids, and it is increasingly clear that bees may be exposed to seed treatment neonicotinoid active ingredients through a variety of routes including planter dust (Nuyttens *et al.,* 2013; Krupke *et al.*, 2017), contaminated soil (Main *et al*., 2021), and/or contaminated pollen/nectar (Goulson 2013; Godfray *et al.,* 2014). Most of the neonicotinoid research on bees has investigated effects of these insecticides on honeybees or bumblebees, whereas effects on solitary bees remain poorly understood. Research suggests that chronic sub-lethal effects are more prevalent than mortality through acute toxicity (Hladik *et al.,* 2018). Studies using honeybees have linked neonicotinoids to several negative sublethal effects including impaired foraging (Ramirez-Romero *et al*., 2005) and altered learning and memory (Decourtye *et al*., 2004). However, Biddinger *et al*. (2013) reported honeybee response to neonicotinoids could not be extrapolated to horn-faced mason bees (*Osmia cornifrons*) and suggested that honeybees may be poor bioindicators for neonicotinoid effects on other bee species. Indeed, the study highlighted the need to study multiple species to gain a clearer picture of the effects of neonicotinoids on all bees (Biddinger *et al*., 2013). For example, it is possible that the large colony size of honeybees mediates the deleterious effects of neonicotinoids (Wood and Goulson 2017), thus complicating inferences to native, solitary nesting bee species.

Relatively few studies have assessed neonicotinoid impacts on pollinators in field settings (but see Cutler and Scott-Dupree 2014; Rundlof *et al.*, 2015; Peters *et al.,* 2016; Main *et al*. 2020, Main *et al*., 2021). Nonetheless, it is increasingly clear that bees may be exposed to seed treatment neonicotinoid active ingredients through a variety of routes including contaminated nest material and/or nest provisions (Kopit and Pitts-Singer 2018). However, thus far, no published studies have quantified the amount of neonicotinoid residues that may exist on or in nest-building materials for direct or indirect cavity-nesting bee exposure (Kopit and Pitts-Singer 2018), and field studies evaluating impacts of neonicotinoids on solitary bee nest success are lacking from the literature (Klaus *et al*., 2021).

Solitary bees have two primary needs for successful reproduction: forage (e.g., wildflowers) and nesting habitat (e.g., available nesting material resources). Among solitary bee species, an individual female builds and provisions her nest with pollen and nectar for her larvae. Approximately 65% of solitary bees nest in holes in the ground excavated by the female (Wilson and Carril 2015). In comparison, ~30% of solitary bees are part of a guild known as cavity-nesters (Batra 1984), which rely on pre-existing cavities for nesting structures including abandoned insect burrows, hollow twigs, indentations in rocks, or cavities in trees. However, some of these species also nest in anthropogenic material such as bricks, holes drilled in wooden blocks, wood planks, or hollow reeds bundled together allowing for an easy provision of additional habitat for these species (MacIvor 2017). Within cavities, nests contain multiple cells, individually sealed by the female with a single egg and pollen provision inside.

Reduction in reproductive success is one key mechanism by which sub-lethal neonicotinoid exposure may negatively impact pollinator populations. A meta-analysis of effects of neonicotinoids on beneficial arthropods reported that pollinators exposed to neonicotinoids had ~27% reduction in reproductive success compared to controls (Main *et al.*, 2018). Canola seeds treated with clothianidin were reported to reduce native bee density and solitary bee nesting effort in Swedish agricultural fields (Rundlof *et al.*, 2015). In a large field study across multiple European countries, buff-tailed bumblebee (*Bombus terrestris)* and red mason bee (*Osmia bicornis)* reproduction was negatively correlated with neonicotinoid residues, suggesting a reduced capacity of bee species to establish new populations in the year following exposure (Woodcock *et al.*, 2017). Lab studies have also shown that buff-tailed bumblebees and red mason bees may experience reduced fecundity and reproductive success when exposed to neonicotinoids (Elston *et al.*, 2013; Sandrock *et al*., 2014), and neonicotinoids may impact sex ratios and male fertility of the European orchard bee (*Osmia cornuta)* (Strobl *et al*., 2019; Strobl *et al*., 2021). Similarly, lab studies have demonstrated contraceptive effects of neonicotinoids on male honeybees (Straub *et al*., 2016). Nonetheless, the evidence on neonicotinoid effects on bee reproduction remains inconclusive. Larval exposure to field-realistic neonicotinoid concentrations had no effect on development rate or over-winter survival of red mason bees (Nicholls *et al*., 2017; Strobl *et al*., 2021). It remains unclear how neonicotinoids may be affecting reproductive success of other bee species and whether effects are consistent across multiple ecoregions and exposure regimes.

Study Objectives

In this study, we evaluated the impacts of neonicotinoid seed treatment use and local habitat quality on wild, cavity-nesting bee species in Midwestern row crop agroecosystems. Our first objective was to evaluate the impacts of continuous neonicotinoid seed treatment use on cavity-nesting bee reproductive effort and success in agricultural field margins. Our second objective was to evaluate the effects of land management practices, such as maintaining wildflowers in field margins or reducing the overall acreage of cultivated land in the surrounding landscape, on cavity-nesting bee reproductive effort and success in agricultural field margins. Our third objective was to quantify pesticide residue concentrations in nesting material gathered by cavity-nesting bees in agroecosystems. Our central hypothesis was that multi-year use of neonicotinoid treated seeds would reduce both cavity-nesting bee reproductive effort and success. Our secondary hypothesis was that maintaining floral richness in field margins may buffer against the potential deleterious effects of neonicotinoid seed treatment use. With these objectives, we hoped to inform agricultural practices and land management decisions that may be influencing cavity-nesting bee reproduction in Midwestern row crop agroecosystems where neonicotinoid use is ubiquitous (i.e., corn-soybean rotations).

METHODS

Experimental Design

To assess the effects of seed treatment neonicotinoids and agroecosystem land management practices on reproductive effort and success among cavity-nesting bees in agroecosystems, we placed solitary bee nest boxes in field margins of soybean fields in spring 2018 (n = 18) and spring 2019 (n = 24). Our experimental unit was an agricultural field and the surrounding field margins, as this represented field conditions at a scale where land management practices, local habitat variables, and pesticide exposure could interact to potentially affect native bee reproduction. We defined a field margin as an uncultivated area (adjacent to an agricultural field) typically comprised of a mix of wildflowers and grassy vegetation. We selected fields with herbaceous margins containing wildflowers over fields with woody margins.

This study built on the experimental design established by a previous experimental field study (2017 – 2018) that evaluated the impacts of multi-year planting of neonicotinoid seed treatments on abundance and richness of wild bee nesting and diet guilds in agricultural field margins (Main *et al*., 2021). We used publicly managed fields (2018, n = 18; 2019, n = 14) that were selected and experimentally manipulated during the previous study. These public fields were located across three state managed conservation areas (CA) in north-central Missouri, U.S. (Figure 2.1) that are managed for wildlife. The three CA included Atlanta (39.88961°N, 92.49373°W), Thomas Hill Reservoir (39.599477°N, 92.620690°W), and Whetstone Creek (38.96766°N, 91.71155°W). 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. Study fields were situated in a surrounding landscape matrix of woodland, grassland, and cropland that varied among CA. We used the same study fields as the previous study and continued the experimental treatments established by Main *et al*. (2021) for those fields.

Each spring (2017 – 2019), all public study fields were planted with soybeans [*Glycine max* (L.) Merr.] and seed treatment type (treated or untreated) was manipulated. Treated refers to fields planted with seeds coated with both a neonicotinoid insecticide (imidacloprid) and fungicides, whereas untreated refers to fields planted with seeds treated with fungicides only. Although the untreated category did not represent a completely untreated condition (i.e., naked seeds), it is rare to find seeds treated with only a neonicotinoid and not also treated with fungicides, thus, we included fungicides in the control group (i.e., untreated fields). Further, fungicides are generally considered to be less toxic to bees compared to neonicotinoids (Tesoriero *et al.,* 2003). At each CA, fields were planted to one of three treatments: untreated (2018 and 2019, *n* = 6), previously-treated (2018, *n* = 6; 2019, n = 4), or treated (2018, *n* = 6; 2019, n = 4). Untreated fields had no known prior neonicotinoid use (UT fields) and were planted with untreated seeds from 2017 - 2019, previously-treated fields were those where neonicotinoid treated seed was last used in 2016 and were planted with untreated seeds from 2017 - 2019 (PT public fields), and treated fields (treated public fields) were continuously cropped using neonicotinoid treated seeds for > 5 years. The number of previously-treated and treated fields on public land differed between 2018 and 2019.

In order to evaluate potential interactions between neonicotinoid seed treatment use and site-specific habitat characteristics, in 2019, in addition to the public fields from Main *et al*.'s (2021) study, we selected private fields (n = 10) with the assistance of Missouri Department of Conservation private land conservationists from a candidate set of fields that were enrolled in the United States Department of Agriculture's (USDA) Farm Services Agency Conservation Reserve Program (CRP) and owned by landowners willing to participate in the study. Specifically, all private fields were enrolled in the CP-33 program. We selected private fields based on the similarity of field margin habitat (e.g., margin area and representative plant species) to public land field margins and all fields were surrounded by an existing margin that contained a mix of grasses and wildflowers. We selected privately-owned fields that were located between 2 - 20 km from each CA. These distance parameters ensured that bee populations on private land would not be influenced by bee populations on study CAs (i.e., source-sink dynamics), but would still experience similar environmental and climatic conditions (precipitation, daily temperature, etc.).

All private fields were planted with neonicotinoid-treated corn in the previous year, thus, no fields in the untreated treatment category (no prior neonicotinoid use) were located on private land. Fields on private land were either planted with treated soybean seed (treated private fields) or untreated soybean seed (previously-treated private fields) in 2019. As private fields planted with untreated seed in 2019 had been planted with neonicotinoid treated seed in the previous year, we termed this treatment category PT1 fields. The lack of UT fields on private land represents a realistic scenario because private fields in a corn-soybean rotation generally involve a neonicotinoid seed treatment at least every few years, as nearly 100% of corn seeds and ~50% of soybean seeds are coated with neonicotinoids (Hladik *et al.*, 2018; Douglas and Tooker 2015). We were unable to locate established soybean fields on private land that have not been treated with a neonicotinoid within the past few years.

Nest Box Placement

Solitary bee nest boxes are designed to attract a range of solitary cavity-nesting bee species and have a long history of use in reproductive studies for cavity-nesting bees (MacIvor 2017). We purchased wooden (cedar) nest boxes online from Crown Bees© (Item #: 110305). Nest boxes contained a stack of wooden (pine) blocks with a series of 8 mm holes (48 holes per box) that provided nest sites for various cavity-nesting bee species. Wooden blocks were not reused between 2018 and 2019. We placed three nest boxes in each field margin and spaced them ~ 50 m apart to capture within-field variability. Nest boxes were placed in field margins prior to seeding events (i.e., early May of each year). We placed boxes approximately 1-meter off the ground with their openings facing south/southeast and in direct sunlight, to take full advantage of the early morning sunshine and encourage nesting (MacIvor 2017). All nest boxes were located within a field margin along one side of the field. We selected margins with floral resources over margins dominated by grasses and herbaceous margins with wildflowers were selected over woody margins. To control for the possibility that field area affects nesting success, we placed nest boxes along a pre-determined length of the field (~ 100 m), even if the entire field margin length was greater. To ensure independence among fields, the margins where nest boxes were placed in each field were a minimum of 500 meters apart. Solitary bees reportedly have a small foraging range (<600 m; Gathmann and Tscharntke, 2002) with many species foraging less than 300 m from their nest (Zurbuchen et al., 2010).

Field-Level and Landscape-Level Habitat Characteristics

To assess environmental variability among field margins, at each field we recorded vegetation characteristics using a 50 cm x 50 cm quadrat at twelve haphazardly-placed locations along herbaceous margins surrounding the field during eight distinct sampling periods from May – September (Main *et al*., 2019). The observer walked all herbaceous field margins and randomly tossed the quadrat, taking care to space the twelve locations adequately enough to capture any within-field variability in habitat characteristics. Within each quadrat, we quantified blooming plant cover (%), bare soil (%), vegetation height (cm), number of blooms (estimated), number of blooming plants, number of dead stems, dominant color, and overall floral richness. Seasonal margin floral richness was estimated for each study field margin by calculating the mean of the total floral richness observed (using vegetative quadrats) over eight distinct sampling periods spread throughout the nesting season (May – September). We quantified habitat categories in the landscape surrounding each field by calculating the percentage of cultivated land, woodlands/shrublands, and grassland/pasture within 800 m of each field using the USDA National Agricultural Statistics Service Cropland Data Layer (USDA 2019), FRAGSTATS software (v4; McGarigal *et al*., 2012), and ArcGIS software (ESRI 2018). The distance of 800 m was selected to fully incorporate the average flight distance of most solitary bees (500 m) (Gathmann and Tscharntke 2002; Zurbuchen *et al.*, 2010).

Overwintering and Extraction of Bee Nests

We removed nest boxes from field margins in October 2018 and 2019 and stored nest box trays containing bee nests in a controlled setting (4 to 5°C) from October – January, to mimic overwintering conditions (Dr. Adrian Carper, University of Colorado Boulder, personal communication, September 20, 2018). In February of each year, we carefully removed individual leafcutter cocoons and stored each in a sealed centrifuge tube (Morphew 2017). Centrifuge tubes from each box were grouped together and stored in a polyethylene bag. Due to the difficulty of removing the larvae/cocoon from resin bee nests without causing damage, we placed (in its entirety) any wooden tray that contained resin nests inside a polyethylene bag, as opposed to storing individuals in separate tubes as was possible with the leafcutter bee nests. During the extraction of nests, we counted the total number of cavities used and the total number of individual nest cells for both resin and leafcutter bees in each box. In March of 2019 and 2020, we placed centrifuge tubes and polyethylene bags containing nests outside in dark storage bins to mimic environmental conditions that stimulate seasonal emergence (Sandrock *et al*., 2014). From March to September of 2018 and 2019, we monitored the nests and counted the number of bees emerging (i.e., successful nests).

Nest Material Pesticide Residue Analysis

In October of 2019, after all bees had been given the appropriate time to emerge, we froze all nest material at -20°C to preserve it for chemical analysis. To obtain the requisite mass for samples to be tested, we combined nest material from fields under the same treatment regime but kept material from nests with an emerged bee separate from nests with an un-emerged bee, so they could be analyzed separately. Resin and leafcutter bee nest material was also kept separate. Thus, we had four nest material categories tested: un-emerged leafcutter nest material (n = 10), un-emerged resin nest material (n = 7), emerged leafcutter nest material (n = 6), and emerged resin nest material (n =6). Due to high nest parasitism and low emergence, we did not have a sample representing each field treatment type for every nest material category. Nest material (n = 29) was tested for a total of 172 insecticide, fungicide, herbicide, and degradate residues at the United States Geological Survey (USGS) California Water Science Center in Sacramento, CA following previously established methods (Hladik *et al.*, 2016). Sample masses ranged from 0.06 – 2.92 g and LODs ranged from 1 to 2 ng/g.

Data Analysis

We subset our data into two groups because 1) we had two years of data for study fields on public land (2018 and 2019) and only one year of data for study fields on private land (2019) and 2) we observed large differences in the percentage of parasitized nests between study years (for resin nests: 3% in 2018 and 34% in 2019; for leafcutter nests: 15% in 2018 and 56% in 2019). Thus, one analysis contained data from only public fields (across both study years) and the other analysis included data from all study fields (public and private) but for 2019 only. In addition, we further subset the data by nesting group. That is, response variables for cavity-nesting bees using resin to construct nest cells (resin bees, e.g., *Megachile campanulae, Heriades* *leavitti*) and cavity-nesting bees using leaf material and/or flower petals to construct nests (leafcutter bees, e.g., *Megachile brevis*, *Megachile petulans*) were analyzed separately due to the ecologically distinct way in which each group constructs their nests and, thus, interacts with the agroecosystem environment and pesticides. For both resin and leafcutter bees, we analyzed two dependent variables representing nesting effort: total number of cavities used and total number of nests (individual brood cells), and one dependent variable representing nest success: the percentage of nest cells that produced an emerging adult bee (i.e., % emergence). Each of the three boxes in a field was treated as an independent data point in the analysis (i.e., nesting variables were not averaged or summed across the boxes in a field). When calculating % emergence, we did not include nests that did not produce an emerging adult bee due to parasitism, as the goal of the study was to determine effects of neonicotinoid seed treatments on reproductive success.

For each subset of data, we constructed a single generalized linear mixed effect model (GLMM) in R (package ‘glmmTMB’, Brooks *et al*., 2017) for the three response variables; total number of nest cells, total number of cavities used, and % emergence for each of the two bee nesting groups. In the analysis containing only public field data from both study years, models contained two random effects: (1) ‘year’ to account for annual variation in cavity-nesting bee reproductive effort and success and (2) ‘individual field (Field.ID)’ nested in ‘Site’ to account for spatial autocorrelation and the repeated sampling of the same fields over time (i.e., three nest boxes per field). In the analysis containing only data from 2019, only the ‘Field.ID’ nested in ‘Site’ random effect was included, as it was not necessary to account for annual variation. Models for resin bee nesting effort metrics contained the explanatory variables treatment, margin floral richness, and % woody vegetation within 800 meters of the study field (Table 2.1). Nesting success models for resin bees contained the independent variables treatment and margin floral richness. Woody vegetation was hypothesized to only impact the ability of resin bees to construct nests and was not hypothesized to impact nest success, thus, % woody vegetation was not included as an explanatory variable in resin bee nesting success models. PT1 fields were excluded from any resin bee models because we did not have any resin bee nests in these fields; thus, treatment categories for this analysis include untreated, treated public, treated private, and PT public. In the analysis containing only data from 2019, models for both leafcutter bee nesting effort and success response variables contained the explanatory variables treatment, margin floral richness, and % cultivated land within 800 m of the study field. The % of cultivated land within 800 m of the study field was not included in the data analysis containing only public fields due to a lack of variation in this parameter on public land (mean: 4%; SD: 2%).

Nesting effort (total number of nests and total number of cavities used) data were zero-inflated, thus, we used a negative binomial distribution to reduce overdispersion in our models. For the nesting success metric (i.e., % emergence), we used a beta distribution as these data (when converted to a proportion) were bounded between zero and one. We assessed model fit through visualization of model residuals and overdispersion parameter estimates. We standardized all continuous variables to a mean of zero and screened for correlations. We considered parameter estimates to be significant if $P < 0.05$ and marginally significant if $P < 0.1$. We tested for interactions between floral richness and treatment, % cultivated land and treatment, and % woody vegetation and treatment, but because all interactions were not significant, we only included main effects in our models.

RESULTS

Pesticide Concentrations in Bee Nest Material

Of the total 172 pesticides and pesticide degradates for which we tested, 16 were detected in bee nest material samples (Table 2.2). We detected the neonicotinoid imidacloprid in two un-emerged leafcutter bee nest material samples (7% of total samples), one from a treated public field (3.3 ng/g) and one from a PT public field (3.5 ng/g). Neonicotinoids were not detected in any other nest material samples. We had more frequent detections of pyrethroid insecticides (45% of total samples) at concentrations ranging from 1.3 to 1206.9 ng/g, and multiple samples contained residues of more than one pyrethroid insecticide (e.g., bifenthrin and cyhalothrin). We also detected: p,p'-DDE (a metabolite of DDT); pentachloroanisole (a metabolite of pentachlorophenol); the pyridine insecticide permethrin; the herbicides 2, 4-Dichlorophenoxyacetic acid, atrazine, and metolachlor; and the fungicides azoxystrobin, propiconazole, pyraclostrobin, and trifloxystrobin (Table 2.2). All 29 nest material samples had a detection of at least one pesticide.

Nesting Effort and Success

In 2018, mean resin bee nest cell production ranged among field treatments from 29 (treated public) to 54 (PT public) nests per field, and in 2019 ranged from 0 (PT1) to 115 (PT public) nests per field (Table 2.3). In 2019, mean cavity use by resin bees ranged from 0 (PT1) to 13 (PT public) cavities per field (Table 2.3). In 2018, mean resin bee % emergence ranged among field treatments from 7% (PT public) to 14% (untreated) per field, and in 2019 ranged from 0 (treated public) to 41% (untreated) per field (Table 2.3).

In 2018, mean leafcutter bee nest cell production ranged among field treatments from 49 (PT public) to 77 (treated public) nests per field, and in 2019 ranged from 2 (treated public) to 17 (treated private) nests per field (Table 2.3). In 2018, mean cavity use by leafcutter bees ranged among field treatments from 10 (treated public and PT public) and 12 (untreated) cavities per field, and in 2019 ranged from 1 (treated public and PT public) to 4 (treated private) cavities per field (Table 2.3). In 2018, mean leafcutter bee % emergence ranged among field treatments from 12 (treated public) to 38% (PT public) per field, and in 2019 ranged from 6 (treated public) to 60% (untreated) per field (Table 2.3).

Models Results

2019 Public and Private Fields

Total number of nest box cavities used by resin bees in 2019 was best explained by field treatment category. Resin bee cavity use was greater in margins of PT public fields (β = 1.88 ± 0.59, z = 3.21, P = 0.001) and treated public fields (β = 1.33 ± 0.66, z = 2.03, P = 0.04) compared to untreated fields (Table 2.4; Figure 2.2). However, resin bee cavity use did not differ between margins surrounding untreated and treated private fields (β = 1.08 ± 1.11, z = 0.97, P = 0.33; Table 2.4; Figure 2.2). Neither margin floral richness (β = 0.04 ± 0.32, z = 0.15, P = 0.88) or the % of woody vegetation within 800 m (β = -0.08 ± 0.45, z = -0.17, P = 0.86) had a significant effect on the number of nest box cavities used by resin bees in 2019 (Table 2.4; Figure 2.2).

Total number of resin bee nest cells in 2019 was also best explained by field treatment category. Resin bee nest cell production was greater in margins of PT public (β = 2.00 ± 0.60, z = 3.31, P < 0.001) and treated public (β = 1.13 ± 0.67, z = 1.68, P = 0.09) fields compared to margins surrounding untreated fields (Table 2.4; Figure 2.2). However, resin bee nest cell production did not differ between margins surrounding untreated and treated private fields (β = 0.84 ± 1.15, z = 0.74, P = 0.46; Table 2.4; Figure 2.2). Neither margin floral richness (β = 0.26 ±

0.35, z = 0.73, *P* = 0.46) or the % woody vegetation within 800 m (*β* = -0.16 ± 0.49, z = -0.33, *P* = 0.74) had a significant effect on resin bee nest cell production in 2019 (Table 2.4; Figure 2.2).

Field treatment category had a significant impact on the % emergence of resin bees in 2019. A lower percentage of resin bees emerged from nests collected from margins of PT public fields (*β* = -1.58 ± 0.79, z = -2.01, *P* = 0.04) and treated public fields (*β* = -2.46 ± 0.91, z = -2.71, *P* = 0.007) compared to untreated fields (Table 2.4; Figures 2.2 and 2.3). However, % emergence did not differ between resin bee nests collected from margins surrounding untreated public and treated private fields (*β* = -1.2 ± 0.8, z = -1.50, *P* = 0.13; Table 2.4; Figures 2.2 and 2.3). Resin bee % emergence responded positively to floral species richness within field margins, and the relationship was marginally significant (*β* = 0.51 ± 0.30, z = 1.69, *P* = 0.09; Table 2.4; Figure 2.2).

We did not detect any significant effects of field treatment or % cultivated land on leafcutter bee nesting effort (i.e., cavity use or nest cell production) or nesting success (i.e., % emergence) in 2019 (Table 2.4; Figure 2.4). Margin floral richness had a marginally significant negative effect on leafcutter bee cavity use (*β* = -0.58 ± 0.34, z = -1.72, *P* = 0.09) and nest cell production (*β* = -0.57 ± 0.34, z = -1.69, *P* = 0.09) in 2019 (Table 2.4; Figure 2.4).

Public Fields Only (2018 and 2019)

Field treatment category had a significant impact on the total number of cavities used by resin bees in public field margins. Resin bee cavity use in margins of untreated fields was lower than margins of PT public fields (*β* = 1.22 ± 0.38, z = 3.25, *P* = 0.001), but did not differ for margins surrounding treated public fields (*β* = 0.06 ± 0.45, z = 0.14, *P* = 0.89; Table 2.5; Figure 2.5). Margin floral richness did not have a significant effect on resin bee nest box cavity use in public field margins (β = -0.45 ± 0.37, z = -1.23, P = 0.22; Table 2.5; Figure 2.5); however, the % of woody vegetation within 800 m had a significant positive effect on the number of nest box cavities used by resin bees (β = 2.03 ± 0.85, z = 2.38, P = 0.02; Table 2.5; Figure 2.5).

Field treatment category also had a significant impact on resin bee nest cell production in public field margins. Resin bee nest cell production was greater in margins of PT Public fields (β = 1.11 ± 0.40, z = 2.75, P = 0.006) compared to margins surrounding untreated fields (Table 2.5; Figure 2.5). However, resin nest cell production did not differ among untreated fields and treated public fields (β = -0.23 ± 0.45, z = -0.50, P = 0.62; Table 2.5; Figure 2.5). Margin floral richness was not a significant predictor of resin bee nest cell production in public field margins (β = -0.14 ± 0.38, z = -0.37, P = 0.71; Table 2.5; Figure 2.5); however, resin bee nest cell production responded positively to % woody vegetation within 800 m (β = 1.81 ± 0.90, z = 2.02, P = 0.04; Table 2.5; Figure 2.5).

Field treatment category had a marginally significant impact on resin bee nest success in public field margins. Resin bee % emergence was lower in margins of treated public fields (β = -0.87 ± 0.49, z = -1.79, P = 0.07) compared to untreated fields (Table 2.5; Figure 2.5 and 2.6). However, resin bee % emergence did not differ between untreated and PT public fields (β = -0.39 ± 0.42, z = -0.92, P = 0.36; Table 2.5; Figures 2.5 and 2.6). Floral richness did not have a significant effect on % emergence of resin bees in public field margins (β = 0.23 ± 0.20, z = 1.16, P = 0.25; Table 2.4; Figure 2.5).

We did not detect any significant effects of field treatment or margin floral richness on leafcutter bee nesting effort (i.e., cavity use or nest cell production) or nesting success (i.e., % emergence) in public field margins (Table 2.5; Figure 2.7).

DISCUSSION

Wild, solitary bees maintain diverse wild plant communities and increase agricultural productivity, making their conservation of critical importance to global agroecosystems (Garibaldi *et al*., 2013; Deguines *et al*., 2014). Our results indicate that neonicotinoid seed treatments may be detrimental to cavity-nesting bee reproduction in Midwestern agroecosystems. Despite having no negative impacts on nesting effort, consistent use of neonicotinoid seed treatment in agricultural fields resulted in a ≥ 50% reduction in nest success for resin bees nesting in agricultural field margins.

Our study indicates that neonicotinoid seed treatment use did not negatively impact nesting effort of either resin or leafcutter bees in field margins of neonicotinoid treated soybean fields. In both analyses, nesting effort was similar among treatment categories for leafcutter bees. In comparison, in both analyses, our models indicated greater resin bee nesting effort for some treatment categories when compared to untreated fields. Studies in laboratory settings (Sandrock *et al*., 2014) and field settings (Rundlof *et al*., 2015; Woodcock *et al*., 2017) have demonstrated that solitary bee reproductive effort is reduced by neonicotinoid exposure. However, more recently, a semi-field study using mesocosms designed to mimic wildflower availability in agricultural field margins found that neonicotinoid treatment of flowering oilseed rape did not affect the offspring production of red mason bees when complementary floral resources were offered (Klaus *et al*., 2021). The results of Klaus *et al.* (2021) may explain the variation in resin bee nesting effort among field treatments in our study, as the field margins where we placed nest boxes provided complementary floral resources that may have positively impacted nesting effort.

Neonicotinoids have been shown to decrease the number of nest cells produced by solitary bees (Sandrock *et al*., 2014; Rundlof *et al*., 2015; Woodcock *et al*., 2017), however, studies evaluating effects on subsequent life stages and emergence are lacking (Klaus *et al*., 2021). Our results indicate that neonicotinoid seed treatment use may be negatively impacting reproductive success of cavity-nesting bees. Despite no significant reductions in nesting effort, we observed marginally significant reductions in % emergence for resin bees nesting in field margins adjacent to neonicotinoid treated fields in the analysis including both years of public fields data. Our results also indicated significant reductions in resin bee % emergence for the PT public and treated public treatment categories in the analysis containing public and private field data from 2019. Similar levels of nesting effort, but lowered nest success may indicate that herbaceous margins next to fields using neonicotinoid treated seed may act as an ecological trap (Schlaepfer *et al.*, 2002). Bees may be attracted to the field margins due to the habitat provided (or a lack of habitat elsewhere) but may be wasting their reproductive efforts due to neonicotinoid exposure resulting in reduced nest success. This potential ecological trap could have long-term effects on the abundance, diversity, and health of bee populations in Midwestern agroecosystems.

Contrary to our results of reduced reproductive success for resin bees nesting in field margins adjacent to neonicotinoid treated fields, Klaus *et al.* (2021) found that the number of emerged red mason bees was not impacted by neonicotinoid seed treatment. However, Klaus *et al.* (2021) used a single species of mason bee, and individuals were purchased for the purpose of the study and released into flight cages, limiting the field realism of these studies and the ability to extrapolate their results to other species of cavity-nesting bees. Conversely, our study assessed impacts of neonicotinoid seed treatments on reproduction of wild bee populations of bees and found neonicotinoid treatment negatively influenced nesting success but had no negative impact on nesting effort.

Successful solitary bee reproduction requires two main constituents: forage and nest sites (Black *et al*., 2011). However, floral or nesting resources that expose bees to pesticide residues may reduce reproductive success of solitary bees (Chan and Raine 2020). Our results indicate decreased reproductive success for resin bees nesting in herbaceous margins next to fields where neonicotinoid seed treatments are used. We present three possible explanations behind this pattern, which may warrant further experiments and investigation. First, due to previously documented deleterious sublethal effects of neonicotinoids on bee navigation (Jin *et al*., 2015), pollen collection (Feltham *et al*., 2014), and foraging rates (Stanley *et al.*, 2015), bees exposed to neonicotinoids may be bringing back less or lower quality pollen to their nests, thus, impacting the ability of the next generation of bees to successfully develop into and emerge as adults. Second, nest success in margins adjacent to fields where neonicotinoids are used may be impacted by direct mortality of bee larvae and/or pupae due to exposure to neonicotinoids in the nest. This exposure could occur via ingestion of contaminated pollen provisions or via contact with nest material containing neonicotinoid residues (Kopit and Pitts-Singer 2018). Finally, exposure to other pesticides (i.e., not neonicotinoids) could cause reductions in pollen provisions provided by female bees or direct mortality of larvae via ingestion or contact with other pesticides in the nest. We cannot rule out that the third explanation may have impacted our study due to the low level of neonicotinoids detected in nest material and the greater concentrations and detection frequencies for other pesticides present in nest material.

The risk of neonicotinoid exposure from nesting substrates and nesting materials are understudied (Sgolastra *et al*., 2019) and, to the best of our knowledge, no published studies have quantified the amount of neonicotinoid residues that may exist on or in nest-building materials for direct or indirect cavity-nesting bee exposure (Kopit and Pitts-Singer 2018). Based on our low detections of neonicotinoids in cavity-nesting bee nest material, the risk of neonicotinoid exposure from nesting material may be less of a threat to cavity-nesting bees than it is to soil-nesting bees (Sgolastra *et al*., 2019; Chan and Raine 2020; Main *et al*., 2021). In midwestern agroecosystems, neonicotinoids are typically applied as a seed treatment (Jeschke *et al*., 2011) and residues are, therefore, most common (and at higher concentrations) in soil compared to other nesting materials such as plant resin, leaves, or flowers used by many cavity-nesting bees (Botias *et al*., 2015; David *et al*., 2016). Among cavity-nesters, mason bees may be a better surrogate than resin or leafcutter bees for the risk of neonicotinoids in nesting material or nesting substrate to soil-nesting bees. Further, our lack of neonicotinoid residue detections in nest material may have been limited by our small sample size of nest material. We composited nest material by field treatment type before chemical analysis due to low sample weight. Despite few neonicotinoid detections in the nest material collected in our study, the risk of exposure to pesticides via nest material for cavity-nesting bees should not be overlooked, as we detected 15 non-neonicotinoid pesticides and degradates in nest material, many of which were not used in our study fields. Other pesticides were either transported into our study fields (Yoder *et al.*, 2001) or bees acquired these pesticides elsewhere in the environment while foraging or gathering nest material (Botias *et al*., 2017). A study which quantified pesticide residues in adult pollinators collected from the same study fields used in our study found that wild pollinators are potentially bioaccumulating a wide variety of pesticides in addition to neonicotinoids (Main *et al*., 2020). Further research is necessary to determine the impacts of the full range of pesticides that cavity-nesting bee species may be exposed to via nest materials and nest substrates

Many of the non-floral resources collected and used by cavity-nesting bees, including plant resin used to construct nests, are provided by woody vegetation (Bentrup *et al*., 2019;

Requier and Leonhardt 2020). Our results indicate that amount of woody vegetation within 800 m of nesting sites may be an important factor for resin bee nesting effort. In models containing only public field data, the % woody vegetation within 800m of study fields had a significant positive effect on both cavity use and nest cell production for resin bees nesting in field margins. Studies evaluating the impacts of woody vegetation on solitary bee reproduction are less common than those evaluating impacts of woody vegetation on solitary bee abundance and richness. One study found that reproductive output of red mason bees had a positive relationship with tree availability (Yourstone *et al*., 2021). However, red mason bees do not use resin to construct nests and the increase in reproductive output was likely due to trees being used as pollen sources or larger founding populations. Another study found greater wild bee abundance at study sites (including agricultural fields) with 5 to 15% woody vegetation within a 50 m radius compared to fields with < 5% woody vegetation (Templ *et al*., 2019). However, the impact of woody vegetation at larger scales is unclear, as another study found that native bee abundance and richness in oilseed field margins was not related to the proportion of woody vegetation at either 800 or 1200 m scales (Le Feon *et al*., 2013).

Most studies exploring connections between bees and floral resources have examined differences in bee abundance or richness across sites that vary in floral cover (Palladini and Maron 2014). Bee abundance generally increases with floral cover (Potts *et al*., 2003; Westphal *et al*., 2003; Hopwood 2008), however, it is unclear whether this pattern is driven by changes in bee reproduction (Palladini and Maron 2014). In our study the impact of floral richness on cavity-nesting bee reproductive effort and success is uncertain. A semi-field study designed to mimic agricultural field margins found that floral resource diversification can promote solitary bee reproduction and may offset insecticide effects (Klaus *et al*., 2021). The field nature of our study may have made it challenging to link floral richness with nest success. Unlike Klaus *et al.* (2021), we had multiple species nesting in boxes throughout the entire season, and these species vary in the variety and quantity of wildflowers that they require for successful nesting (Strickler 1979). Further, as we only collected nests at the end of the season, we had only one sampling event representing nesting metrics for each box and, thus, calculated only one number representing margin floral richness to include in our models even though we collected margin floral richness data at multiple time periods. We did not remove nests and replace the trays at multiple time periods; doing so would have given us the ability to more closely link floral richness at any time period to nesting effort and success. Future studies may want to consider collecting nests and replacing the trays at multiple time periods, to increase the ability to detect an effect of floral richness.

Study Limitations

Bee nest boxes in our study experienced high rates of parasitism in 2019, which restricted our sample size in that year and may have impacted the results, especially for leafcutter bees, potentially precluding our ability to detect significant effects of independent variables in our models. Parasites and pests were taxonomically diverse and included chalcid wasps, chrysidid wasps, various (unidentified) flies, and cleptoparisitic bees (e.g., *Coelioxys spp*.), with chalcid wasps being the most common. In addition, including private fields to our study in 2019 may have added too much data variation to the system and limited our ability to detect significant effects of independent variables in our models. Finally, in 2019, we only placed nest boxes in a subset of the previously-treated and treated fields on public land where nest boxes were placed in 2018, which limited our sample size in 2019 and may have impacted the results of our models that included data from public land from both study years.

MANAGEMENT IMPLICATIONS

Reducing neonicotinoid use in areas managed for conservation may sustain viable reproduction of some cavity-nesting bees. Land managers could consider alternatives to neonicotinoid use such as integrated pest management (IPM), especially in locations such as Missouri where the yield benefits of neonicotinoids are unclear (Pecenka *et al*., 2021). Managers could also consider prioritizing pollinator habitat management activities (e.g., target mowing that avoids blooming flowers) in areas removed from agricultural production, such as old fields or field margins. Emphasis on creating or enhancing pollinator habitat would not be incompatible with other management priorities such as brood-rearing and nesting cover for ground-nesting birds.

It is unclear if the CP-33 program, in conjunction with neonicotinoid use, benefits cavity-nesting bees, as the habitat provided by the program may attract nesting bees, but ultimately negatively impact their ability to successfully reproduce, thus, acting as an ecological trap. Potential changes to the program that could benefit native bees might include: 1) limiting neonicotinoid use 2) prioritizing entry into the program for land that increases habitat connectivity with areas removed from agricultural production 3) increasing financial incentives for wider buffer strips 4) planting a grass strip immediately adjacent to crop fields and locating forb plantings further away. The CP-42 CRP program, which is designed for pollinator conservation, may benefit native bee communities more than CP-33 practices, however, our study did not address this question.

Woody vegetation may be an important habitat component for nesting resin bees, and extensive clearing of trees for agriculture may reduce resin bee nesting effort. Maintaining a mosaic of habitat that includes woody species may benefit resin bees. Finally, cavity-nesting bees are exposed to a range of pesticides in their nesting material. Reducing the overall use of pesticides on areas managed for wildlife may facilitate successful cavity-nesting bee reproduction and sustain viable native bee communities in these ecosystems.

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TABLES: CHAPTER 2

Table 2.1: Description of variables used in models to evaluate effects of neonicotinoid seed treatment on resin and leafcutter bee nesting effort and success in agroecosystems located in north-central Missouri, USA in 2019.

Variable Name	Variable Type	Variable Description	How we calculated the variable	Models in which variable was included
% Woody Vegetation	Continuous	Estimate of the percentage of woody vegetation within an 800 m radius of each study field.	We quantified the percentage of woody vegetation within 800 m of each field using the USDA National Agricultural Statistics Service Cropland Data Layer, FRAGSTATS software (v4), and ArcGIS software.	Both 2019 and public fields-only resin bee nesting effort (total number of cavities utilized and total number of nest cells) models. Not included in any resin bee nesting success (% emergence) models.
% Agriculture	Continuous	Estimate of the percentage of cultivated land within an 800 m radius of each study field.	We quantified the percentage of cultivated land within 800 m of each field using the USDA National Agricultural Statistics Service Cropland Data Layer, FRAGSTATS software (v4), and ArcGIS software.	2019-only leafcutter bee nesting effort (total number of cavities utilized and total number of nest cells) and nesting success (% emergence) models.
Margin Floral Richness	Continuous	Estimate of the total floral richness in each field's margins during each study year (2018 - 2019).	We calculated the total number of unique floral species (observed in field margins using vegetative quadrats) for six (2018) and eight (2019) sampling periods. We then took the average across sampling periods to obtain one number representing each study field in each study year.	All models

Variable Name	Variable Type	Variable Description	How we calculated the variable	Models in which variable was included
Treatment	Categorical	The treatment category of the study field. Treatment categories encompass both the historical and study-year (2018-2019) use of neonicotinoid seed treatments, as well as field management status (public or private).	We manipulated neonicotinoid seed treatment type (treated or untreated) during the study years (2018-2019), and we gathered information on the past use (or lack thereof) of neonicotinoids in previous years. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.	All models

Table 2.2: Mean, minimum, and maximum concentrations (ng/g), as well as number of detections, for 16 pesticides and pesticide degradates in four categories of solitary bee nest material (as well as overall numbers) collected from solitary bee nest boxes placed in field margins in 2019. Boxes were placed in May and collected in October. The four nest material categories are: 1) Plant resin material from nests from which a bee did not emerge 2) Plant resin material from nests from which a bee did emerge 3) Plant biomass material (petals and leaves) from which a bee did not emerge 4) Plant biomass material (petals and leaves) from which a bee did emerge. Means were calculated using detections only.

		Nest Material Type											
		Resin Un-emerged (n = 6)				**Resin Emerged (n = 7)**				**Overall (n = 29)**			
Pesticide	Type[1]	Detects	Mean (ng/g)	Min (ng/g)	Max (ng/g)	Detects	Mean (ng/g)	Min (ng/g)	Max (ng/g)	Total Detects	Mean (ng/g)	Min (ng/g)	Max (ng/g)
2,4-D	H	1	18.2	18.2	18.2	0	NA	NA	NA	2	50.1	18.2	82.0
Atrazine	H	0	NA	NA	NA	2	14.7	6.4	23.0	2	14.7	6.4	23.0
Azoxystrobin	F	1	0.9	0.9	0.9	1	7.8	7.8	7.8	5	57.5	0.9	276.1
Bifenthrin	I	2	53.0	15.6	90.3	0	NA	NA	NA	9	18.6	1.3	90.3
Cyhalothrin	I	0	NA	NA	NA	0	NA	NA	NA	3	12.6	11.1	12.6
Imidacloprid	I	0	NA	NA	NA	0	NA	NA	NA	2	3.4	3.3	3.5
Metolachlor	H	6	30.1	0.6	109.2	7	1984	1.4	13825	16	922.5	0.6	13825
p,p'-DDE	D	0	NA	NA	NA	0	NA	NA	NA	6	9.1	1.5	23.3
Pentachloroanisole	D	2	17.4	6.6	28.2	1	14.5	14.5	14.5	7	42.7	1.9	154.3
Permethrin	I	0	NA	NA	NA	0	NA	NA	NA	7	83.0	11.1	275.5
Propiconazole	F	0	NA	NA	NA	0	NA	NA	NA	2	26.6	2.1	51.1
Pyraclostrobin	F	2	1.5	1.0	1.9	1	3.0	3.0	3.0	3	2.0	1.0	3.0
Tefluthrin	I	0	NA	NA	NA	0	NA	NA	NA	5	32.3	2.3	123.1
tau-Fluvalinate	I	0	NA	NA	NA	1	26.8	26.8	26.8	9	374.8	26.8	1207
Trifloxystrobin	F	1	2.8	2.8	2.8	0	NA	NA	NA	1	2.8	2.8	2.8
Trifluralin	I	0	NA	NA	NA	2	4.8	1.5	8.1	10	30.2	1.5	156.4

		Nest Material Type											
		Leafcutter Un-emerged (n = 10)				**Leafcutter Emerged (n = 6)**				**Overall (n = 29)**			
Agrochemical	Type[1]	Detects	Mean (ng/g)	Min (ng/g)	Max (ng/g)	Detects	Mean (ng/g)	Min (ng/g)	Max (ng/g)	Total Detects	Mean (ng/g)	Min (ng/g)	Max (ng/g)
2,4-D	H	1	82.0	82.0	82.0	0	NA	NA	NA	2	50.1	18.2	82.0
Atrazine	H	0	NA	NA	NA	0	NA	NA	NA	2	14.7	6.4	23.0
Azoxystrobin	F	1	276.1	276.1	276.1	2	1.4	1.0	1.7	5	57.5	0.9	276.1
Bifenthrin	I	5	4.9	1.3	17.8	2	18.5	12.0	25.0	9	18.6	1.3	90.3
Cyhalothrin	I	3	12.6	11.1	12.6	0	NA	NA	NA	3	12.6	11.1	12.6
Imidacloprid	I	2	3.4	3.3	3.5	0	NA	NA	NA	2	3.4	3.3	3.5
Metolachlor	H	2	346.3	0.9	691.6	1	1.9	1.9	1.9	16	922.5	0.6	13825
p,p'-DDE	D	4	2.4	1.5	2.9	2	22.5	21.7	23.3	6	9.1	1.5	23.3
Pentachloroanisole	D	4	62.4	1.9	154.3	0	NA	NA	NA	7	42.7	1.9	154.3
Permethrin	I	5	47.2	11.1	121.0	2	172.6	69.6	275.5	7	83.0	11.1	275.5
Propiconazole	F	2	26.6	2.1	51.1	0	NA	NA	NA	2	26.6	2.1	51.1
Pyraclostrobin	F	0	NA	NA	NA	0	NA	NA	NA	3	2.0	1.0	3.0
Tefluthrin	I	4	9.6	2.3	15.7	1	123.1	123.1	123.1	5	32.3	2.3	123.1
tau-Fluvalinate	I	6	230.6	55.7	351.2	2	981.4	755.8	1207	9	374.8	26.8	1207
Trifloxystrobin	F	0	NA	NA	NA	0	NA	NA	NA	1	2.8	2.8	2.8
Trifluralin	I	6	12.1	2.4	19.6	2	109.7	63.0	156.4	10	30.2	1.5	156.4

[1] **Pesticide Types:** H = Herbicide; F = Fungicide; I = Insecticide; D = Degradate

Table 2.3: Mean, minimum, and maximum number of cavities used, number of nest cells, and % emergence by treatment category and study year for both leafcutter and resin bees. Solitary bee nest boxes were placed in field margins in 2018 and 2019. Boxes were placed in May and collected in October of each study year, and bees were incubated to promote seasonal emergence in the spring of the following year.

			Cavities Used			**Nest Cells**			**% Emergence**		
Nest Type	Treatment	Year	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Leafcutter	PT1 (n = 4)	2019	3	0	7	9	0	25	55	9	100
	T Private (n = 6)	2019	4	0	10	17	0	64	18	0	38
	PT Public (n = 6)	2018	10	2	16	49	9	97	38	0	100
	PT Public (n = 4)	2019	1	0	1	5	0	8	17	17	17
	T Public (n = 6)	2018	10	0	27	77	0	262	12	0	43
	T Public (n = 4)	2019	1	0	4	2	0	9	6	0	12
	Untreated (n = 6)	2018	12	2	30	63	12	151	25	0	82
	Untreated (n = 6)	2019	2	0	4	15	0	47	60	0	100
Resin	PT1 (n = 4)	2019	0	0	0	0	0	0	NA	NA	NA
	T Private (n = 6)	2019	4	1	8	22	1	50	9	0	33
	PT Public (n = 6)	2018	4	0	15	54	0	120	10	0	54
	PT Public (n = 4)	2019	13	0	24	115	8	219	7	0	44
	T Public (n = 6)	2018	4	0	18	29	0	145	7	0	40
	T Public (n = 4)	2019	7	0	15	39	0	85	0	0	0
	Untreated (n = 6)	2018	4	0	11	30	0	94	14	0	56
	Untreated (n = 6)	2019	1	0	3	17	0	44	41	0	100

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; T Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land ; PT Public – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; T Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Untreated – fields not planted with neonicotinoid-treated seed in the study year or any year prior and located on public land.

Table 2.4: Results of mixed-effects models evaluating treatment and site-specific habitat variables (margin floral richness, % agriculture, and % woody vegetation) on resin and leafcutter bee nesting effort and success in 2019. Models had a random intercept term for Field.ID nested within Site.

		Response								
		Nest Cells			**Cavities Used**			**% Emergence**		
Nest Type	**Fixed Effects Variables**	β	± SE	*P*	β	± SE	*P*	β	± SE	*P*
Resin	Treatment: PT Public[1]	2.00	0.6	<0.001***	1.88	0.59	0.001**	-1.58	0.79	0.04*
	Treatment: Treated Private[1]	0.84	1.15	0.46	1.08	1.11	0.33	-1.20	0.80	0.13
	Treatment: Treated Public[1]	1.13	0.67	0.09.	1.33	0.66	0.04*	-2.46	0.91	0.007**
	% Woody Vegetation within 800 m	-0.16	0.49	0.74	-0.08	0.45	0.86	NA	NA	NA
	Margin Floral Richness	0.26	0.35	0.46	0.05	0.32	0.88	0.51	0.30	0.09.
Leafcutter	Treatment: PT1[1]	-0.68	0.97	0.48	0.14	0.85	0.87	-0.15	1.68	0.93
	Treatment: PT Public[1]	-0.94	0.75	0.21	-0.81	0.70	0.24	-0.66	1.49	0.66
	Treatment: Treated Private[1]	-0.50	0.83	0.55	0.13	0.70	0.86	-1.37	1.10	0.21
	Treatment: Treated Public[1]	-1.86	1.12	0.10	-1.53	1.08	0.16	-1.40	2.94	0.64
	% Agriculture within 800 m	0.29	0.27	0.29	0.11	0.22	0.62	0.17	0.38	0.66
	Margin Floral Richness	-0.57	0.34	0.09.	-0.58	0.34	0.09.	-0.10	0.80	0.90

Example Model: Response ~ Treatment + Floral_Rich + % Ag + (1|Site/Field_ID)

Significance Codes: '***' <= 0.001 '**' = 0.01 '*' = 0.05 '.' = 0.1

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT Public – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

Table 2.5: Results of mixed-effects models evaluating treatment and site-specific habitat variables (margin floral richness and % woody vegetation) on resin and leafcutter bee nesting effort and success in 2018 and 2019 for study fields located on public land. Models had random intercept terms for Field.ID nested within Site and a random intercept for Year.

		Response								
		Nest Cells			**Cavities Used**			**% Emergence**		
Nest Type	**Fixed Effects Variables**	β	± SE	*P*	β	± SE	*P*	β	± SE	*P*
Resin	Treatment: PT Public[1]	1.10	0.40	0.006*	1.22	0.38	0.001**	-0.39	0.42	0.36
	Treatment: Treated Public[1]	-0.23	0.45	0.62	0.06	0.45	0.89	-0.87	0.49	0.07.
	% Woody Vegetation within 800 m	1.81	0.90	0.04*	2.03	0.85	0.02*	NA	NA	NA
	Margin Floral Richness	-0.14	0.38	0.71	-0.45	0.37	0.22	0.23	0.20	0.25
Leafcutter	Treatment: PT Public[1]	-0.16	0.37	0.67	-0.22	0.37	0.54	-0.14	0.46	0.76
	Treatment: Treated Public[1]	-0.61	0.41	0.14	-0.39	0.40	0.32	-0.79	0.51	0.12
	Margin Floral Richness	0.05	0.15	0.73	-0.05	0.15	0.74	-0.13	0.19	0.51

Example Model: Response ~ Treatment + Floral_Rich + %Woody + (1|Site/Field_ID) + (1|Year)

Significance Codes: ‘***’ <= 0.001 ‘**’ = 0.01 ‘*’ = 0.05 ‘.’ = 0.1

[1] **Treatment Categories:** PT Public - Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

FIGURES: CHAPTER 2

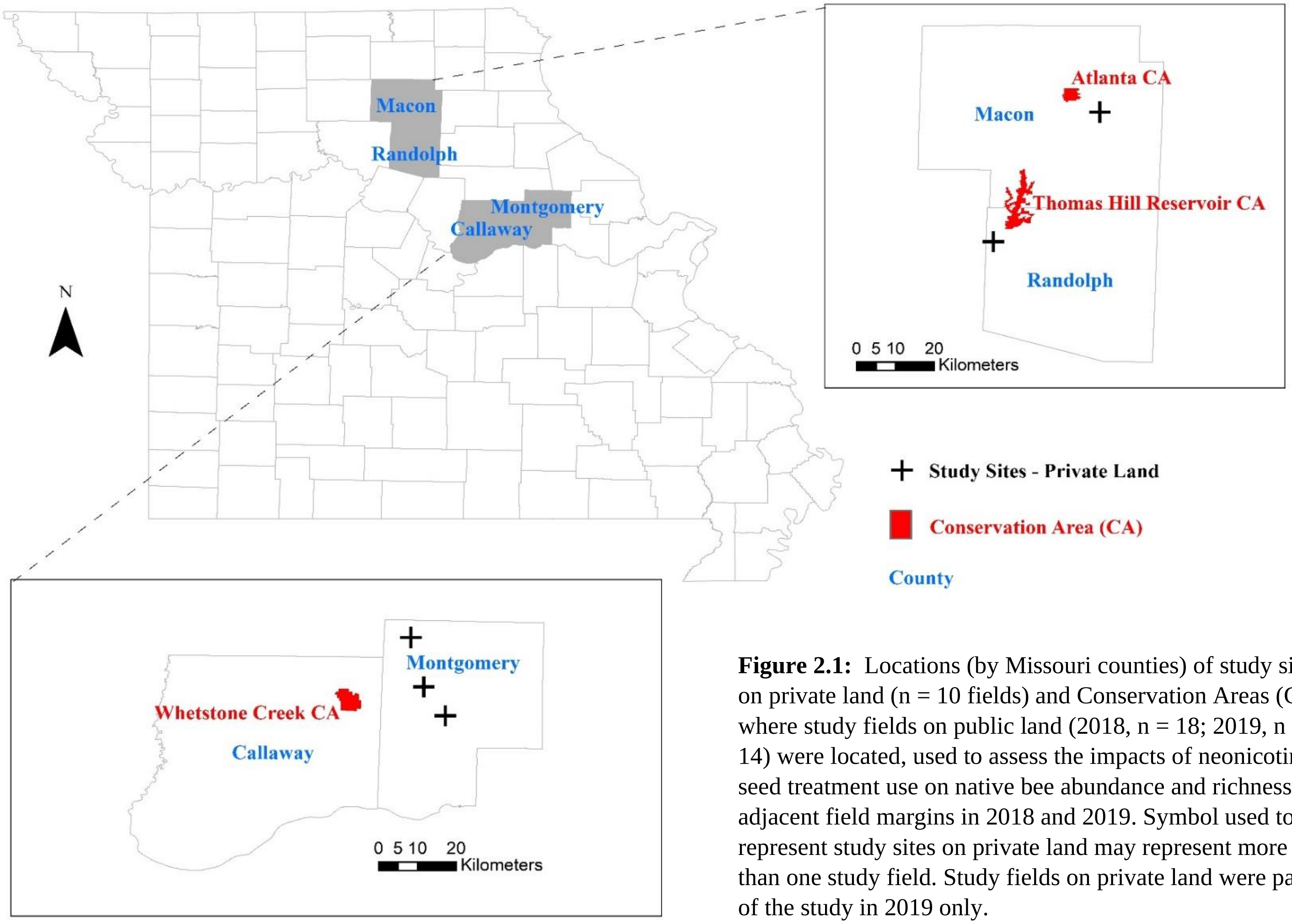

Figure 2.1: Locations (by Missouri counties) of study sites on private land (n = 10 fields) and Conservation Areas (CA), where study fields on public land (2018, n = 18; 2019, n = 14) were located, used to assess the impacts of neonicotinoid seed treatment use on native bee abundance and richness in adjacent field margins in 2018 and 2019. Symbol used to represent study sites on private land may represent more than one study field. Study fields on private land were part of the study in 2019 only.

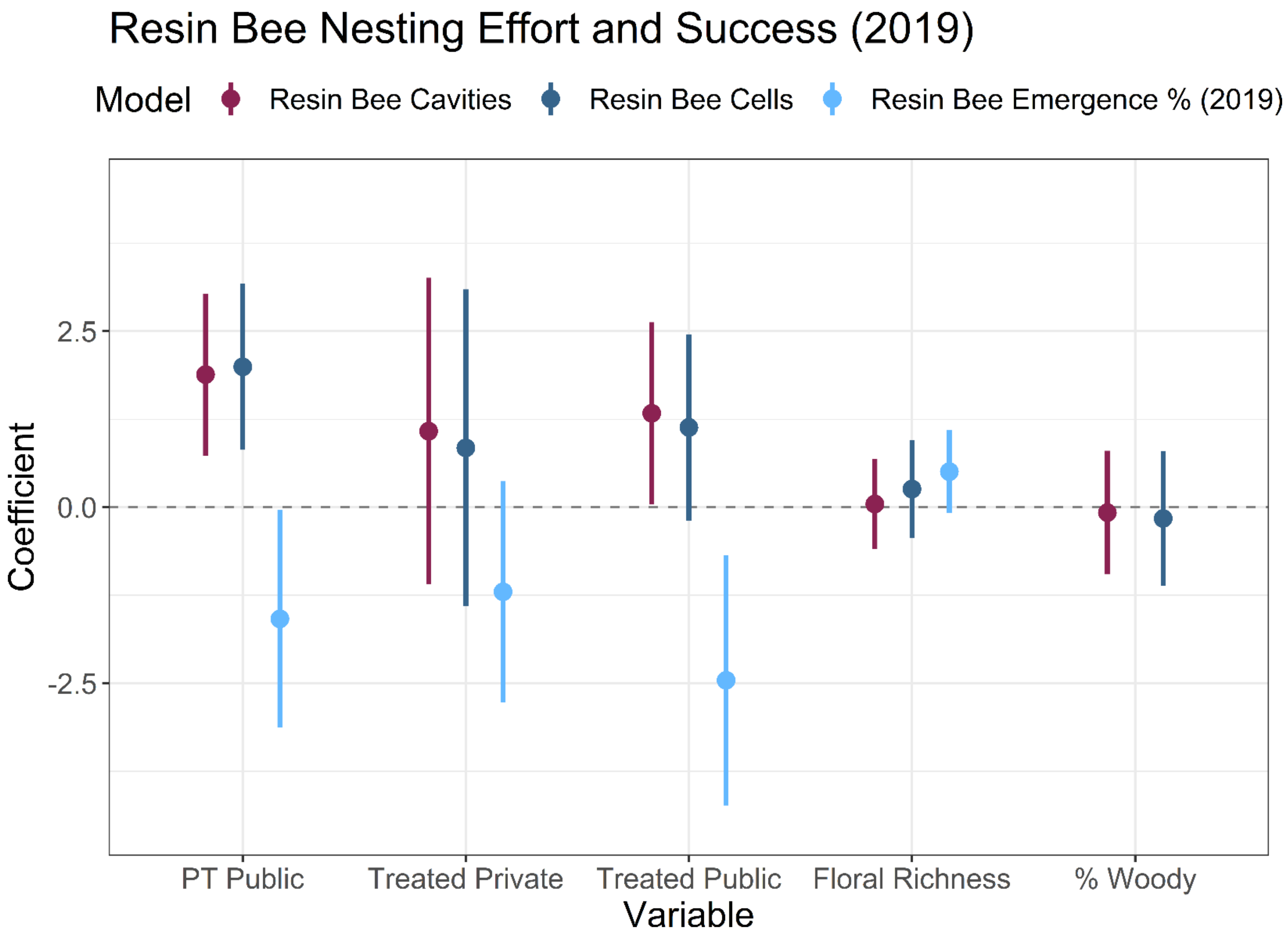

Figure 2.2: Coefficients ± 1.96 * standard error from models evaluating resin bee nesting effort and success in field margins in 2019. Model variables include treatment, as well as the field-specific habitat variables margin floral richness and % woody vegetation within 800 m of study field (nesting effort models only).

Treatment Categories: PT Public – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds in 2019 (and years prior) and located on private land. Treated Public – Fields planted with neonicotinoid-treated seeds in 2019 (and years prior) and located on public land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in 2019 or any year prior and were located on public land. PT1 fields had no resin bee nests and were not included in resin bee models.

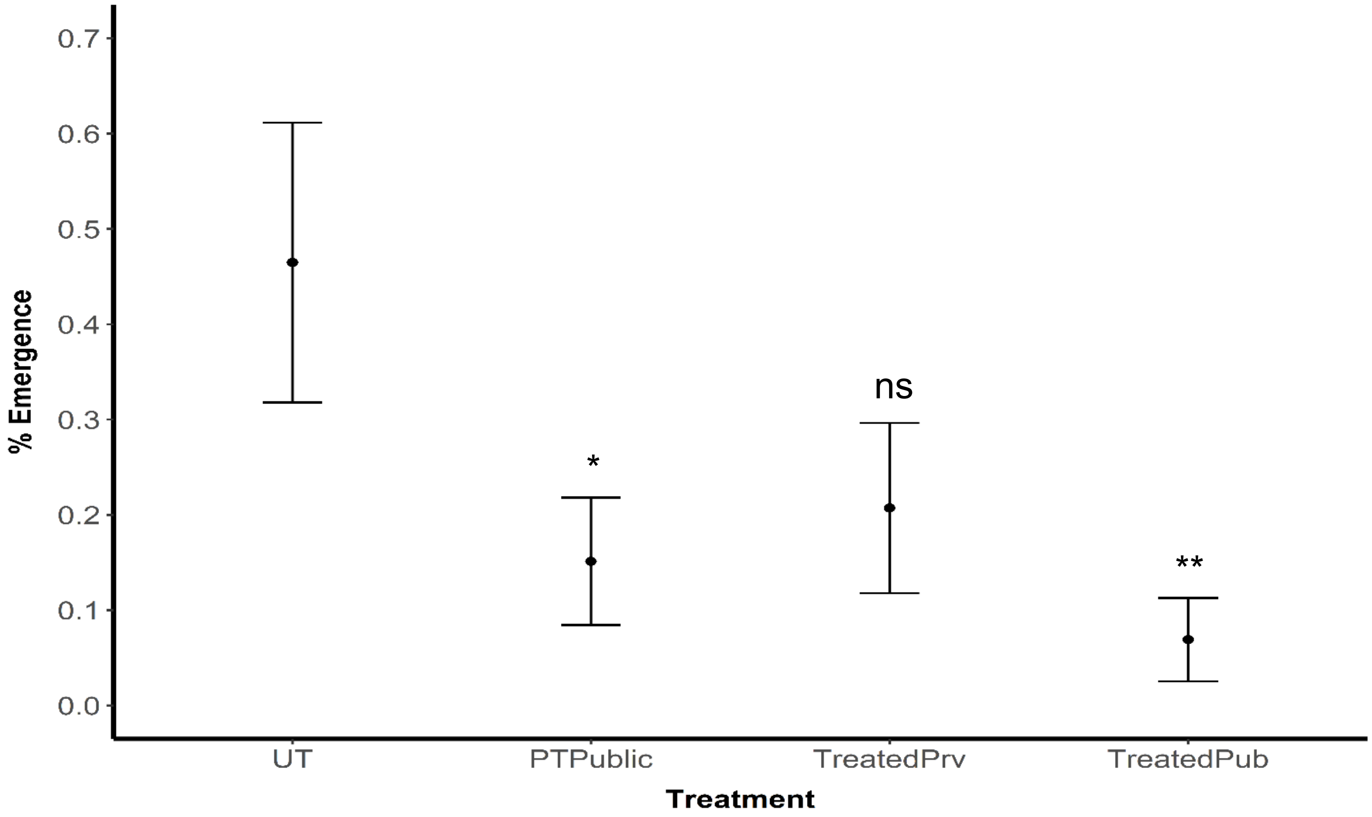

Figure 2.3: Point plot of mean % emergence of resin bees (collected from field margins in 2019) by field treatment. Significance codes indicate differences in relation to untreated (UT) fields. Error bars represent the standard error. Treatment categories: UT – fields not planted with neonicotinoid-treated seed in the study year or any year prior and located on public land; PTPublic – fields last planted with neonicotinoid-treated seed in 2016 and located on public land; TreatedPrv – fields planted with neonicotinoid-treated seeds during the study period (and at least one year prior) and located on private land; TreatedPub – fields planted with neonicotinoid-treated seeds during the study period (and at least three years prior) and located on public land.

Significance Codes: '**' = 0.01 '*' = 0.05 'ns' = not significant

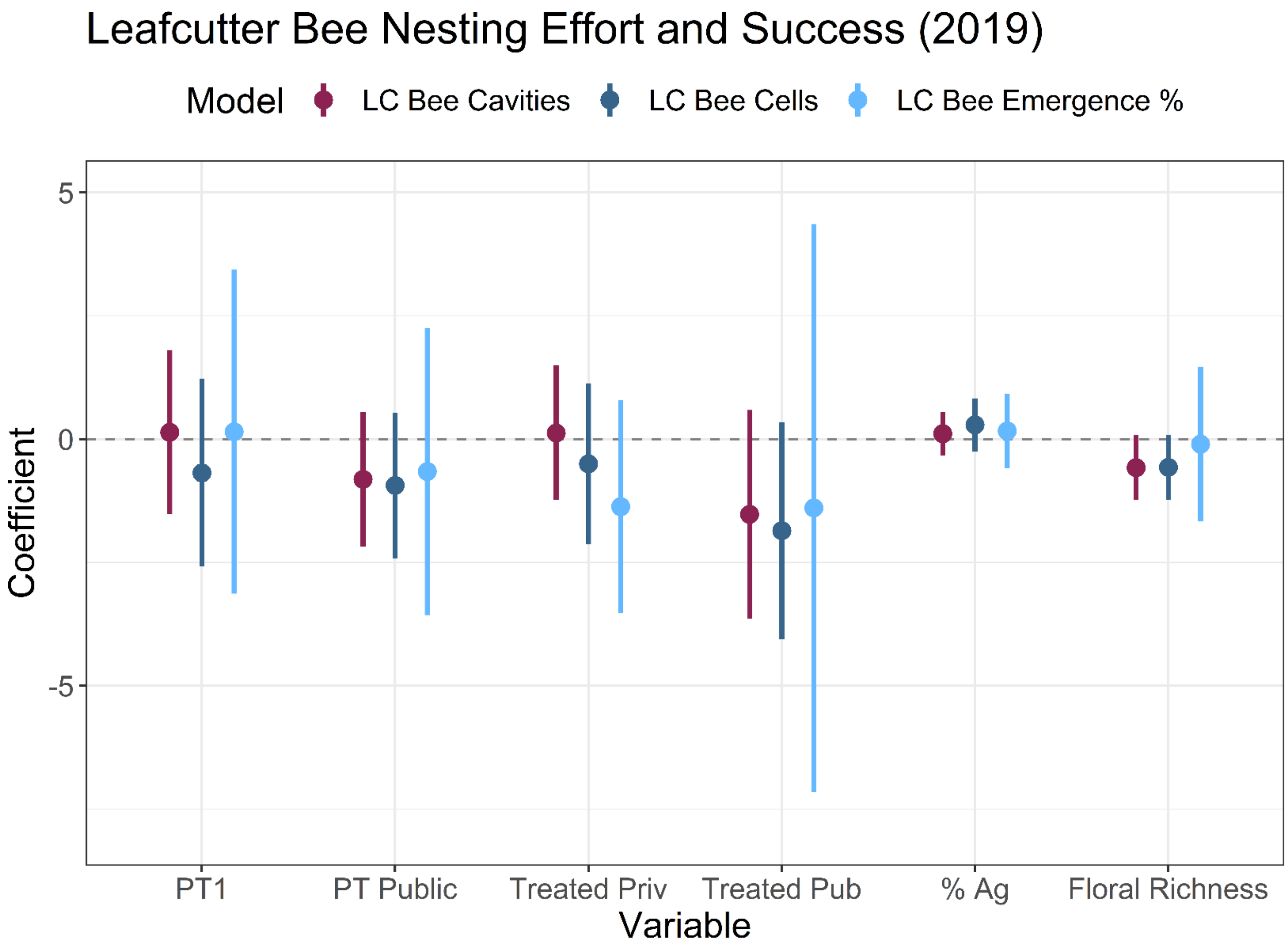

Figure 2.4: Coefficients ± 1.96 * standard error from models evaluating leafcutter bee nesting effort and success in field margins in 2019. Model variables include treatment, as well as the field-specific habitat variables % cultivated agriculture within 800 m of study field and margin floral richness. Treatment Categories: PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT Public – Fields last planted with neonicotinoid-

treated seed in 2016 and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds in 2019 (and years prior) and located on private land. Treated Public – Fields planted with neonicotinoid-treated seeds in 2019 (and years prior) and located on public land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in 2019 or any year prior and were located on public land.

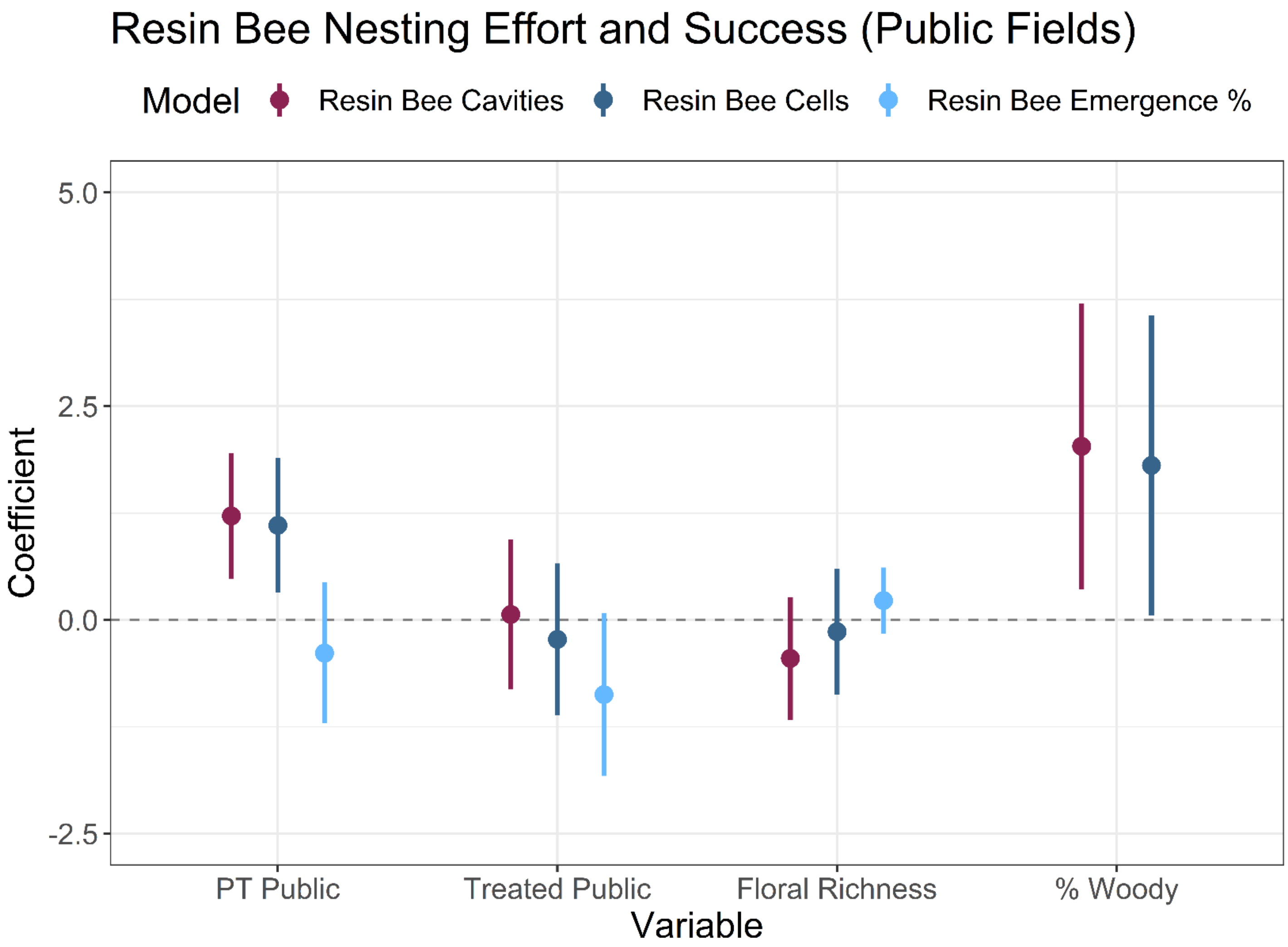

Figure 2.5: Coefficients ± 1.96 * standard error from models evaluating resin bee nesting effort and success in public field margins (2018-2019). Model variables include treatment, as well as the field-specific habitat variables margin floral richness and % woody vegetation within 800 m of study field (nesting

effort models only). Treatment Categories: PT Public – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during both study years (2018 and 2019) and located on public land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in the study years or any year prior and were located on public land.

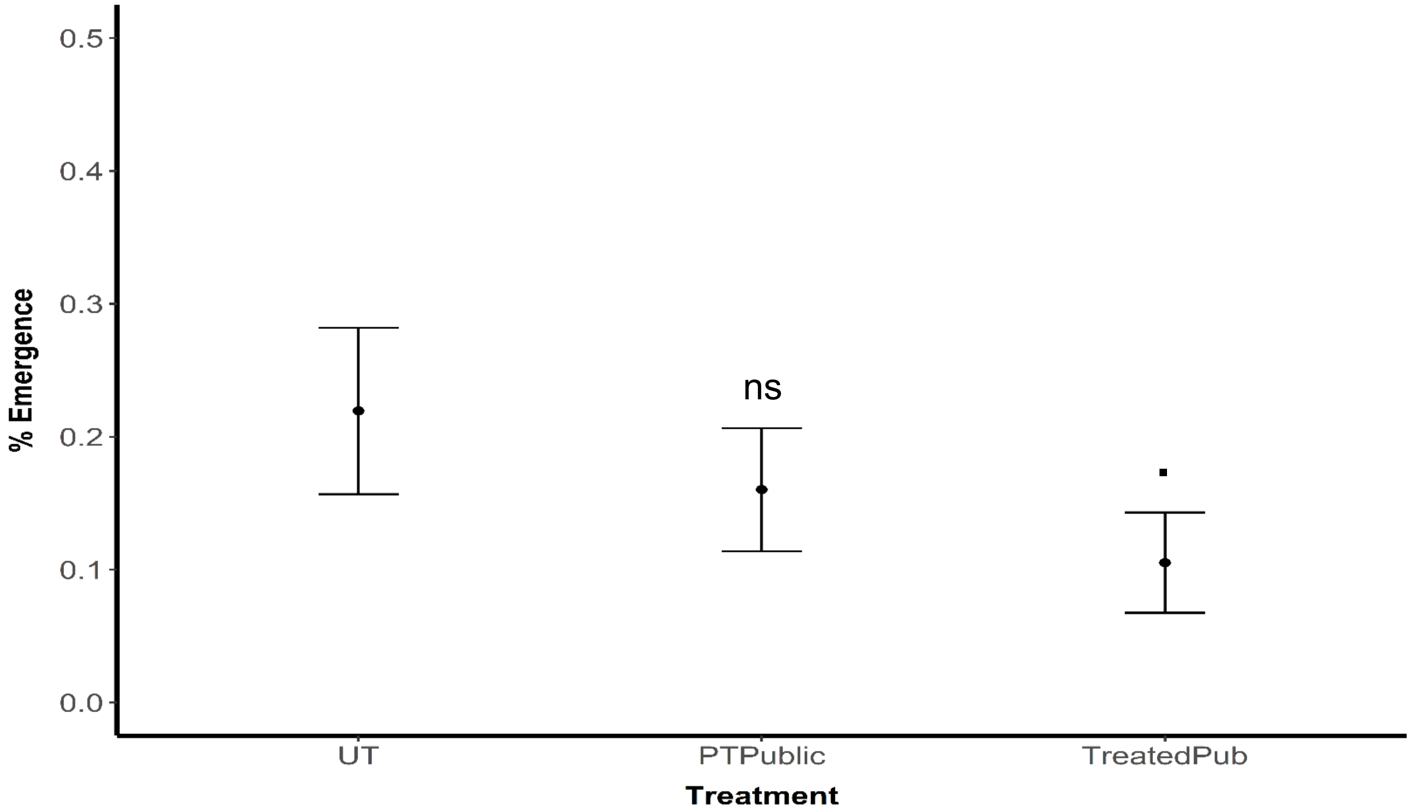

Figure 2.6: Point plot of % emergence of resin bees (collected from public field margins in 2018 and 2019) by field treatment. Significance codes indicate differences in relation to untreated (UT) fields. Error bars represent the standard error. Treatment categories: UT – fields not planted with neonicotinoid-treated seed in the study year or any year prior and located on public land; PTPublic – fields last planted with neonicotinoid-treated seed in 2016 and located on public land; TreatedPub – fields planted with neonicotinoid-treated seeds during the study period (and at least three years prior) and located on public land.

Significance Codes: '.' = 0.1 'ns' = not significant

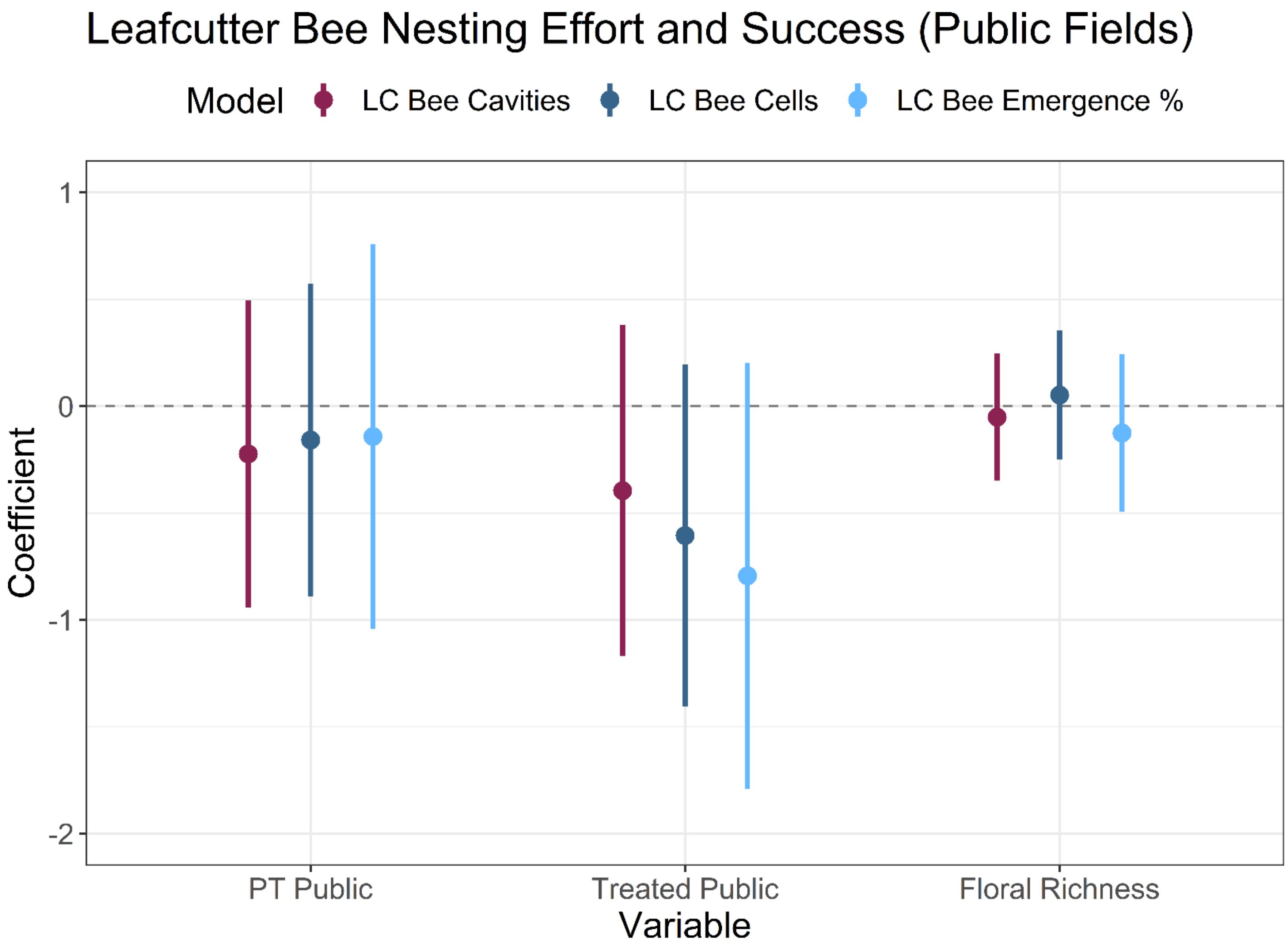

Figure 2.7: Coefficients ± 1.96 * standard error from models evaluating leafcutter bee nesting effort and success in public field margins. Model variables include treatment and the field-specific habitat variable margin floral richness. Treatment Categories: PT Public – Fields last planted with neonicotinoid-treated seed in

2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds in 2019 (and years prior) and located on public land. Reference treatment category is UT (untreated) fields, which were not planted with neonicotinoid-treated seed in 2019 or any year prior and were located on public land.

APPENDICES

Appendix A: List of study fields, including their ownership status, treatment category, field area (ha), margin area (ha), and GPS coordinates. Coordinates for privately-owned fields were removed to protect the privacy of landowners.

Field ID	Site[1]	Ownership	Treatment[2]	Field Area (ha)	Margin Area (ha)	GPS Coordinates
ATP1	ATP	Private	PT1	2.79	1.85	NA
ATP2	ATP	Private	PT1	2.17	5.02	NA
ATP3	ATP	Private	PT1	6.01	1.87	NA
ATP4	ATP	Private	PT1	14.11	5.09	NA
ATT1	AT CA	Public	Treated Public	0.66	2.55	39°53'19.9"N 92°30'21.1"W
ATT2	AT CA	Public	Treated Public	2.13	0.79	39°53'22.6"N 92°29'36.1"W
ATT3	AT CA	Public	PT3	0.83	3.03	39°53'11.3"N 92°29'14.6"W
ATT4	AT CA	Public	PT3	1.02	1.03	39°53'14.8"N 92°29'05.2"W
ATUT1	AT CA	Public	Untreated	1.87	2.40	39°53'07.2"N 92°30'22.8"W
ATUT2	AT CA	Public	Untreated	0.64	1.32	39°52'24.3"N 92°31'43.6"W
THRP1	THRP	Private	Treated Private	3.13	0.88	NA
THRP2	THRP	Private	Treated Private	1.82	0.89	NA
THRT1	THR CA	Public	Treated Public	2.04	0.62	39°36'19.1"N 92°36'47.7"W
THRT2	THR CA	Public	Treated Public	1.66	1.82	39°36'05.4"N 92°36'46.8"W
THRT3	THR CA	Public	PT3	2.99	5.94	39°36'04.7"N 92°36'10.8"W
THRT4	THR CA	Public	PT3	1.40	3.12	39°35'48.3"N 92°36'32.1"W
THRUT1	THR CA	Public	Untreated	1.33	0.47	39°35'57.6"N 92°37'13.3"W
THRUT2	THR CA	Public	Untreated	2.05	2.97	39°36'08.3"N 92°37'22.8"W
WCP1	WCP	Private	Treated Private	2.91	2.56	NA
WCP2	WCP	Private	Treated Private	6.56	3.50	NA
WCP3	WCP	Private	Treated Private	2.31	2.58	NA
WCP4	WCP	Private	Treated Private	2.11	3.24	NA
WCP5	WCP	Private	Treated Private	7.75	2.77	NA
WCT1	WC CA	Public	Treated Public	1.11	4.30	38°56'19.4"N 91°41'45.7"W
WCT2	WC CA	Public	Treated Public	1.65	0.82	38°57'16.4"N 91°42'14.0"W
WCT3	WC CA	Public	PT3	1.45	5.05	38°58'37.9"N 91°42'57.9"W
WCT4	WC CA	Public	PT3	2.54	2.98	38°56'32.8"N 91°42'30.4"W
WCUT1	WC CA	Public	Untreated	1.32	1.21	38°58'04.1"N 91°42'41.3"W
WCUT2	WC CA	Public	Untreated	0.66	0.59	38°58'23.9"N 91°43'50.6"W

[1] **Site Description:** AT CA – Public fields located on Atlanta Conservation Area; ATP – Privately-owned fields located 2 – 20 km from Atlanta Conservation Area; THR CA – Public fields located on Thomas Hill Reservoir Conservation Area; THRP – Privately-owned fields located 2 – 20 km from Thomas Hill Reservoir Conservation Area; WC CA – Public fields located on Whetstone Creek Conservation Area; WCP – Privately-owned fields located 2 – 20 km from Whetstone Creek Conservation Area.

[2] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land; Untreated – Fields which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

Appendix B: Mean, minimum, and maximum field area (ha), margin area (ha), and percentage of land in row crop agriculture within an 800m radius of study fields for each treatment category.

		Field Area (ha)			**Margin Area (ha)**			**Agriculture 800m (%)**		
Treatment	Ownership	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
PT1 (n = 4)	Private	6.27	2.17	14.11	3.46	1.85	5.09	39	28	45
Treated Private (n = 7)	Private	3.80	1.82	7.75	2.35	0.88	3.5	35	11	67
PT3 (n = 6)	Public	1.71	0.83	2.99	3.52	1.03	5.94	7	4	11
Treated Public (n = 6)	Public	1.54	0.66	2.13	1.82	0.62	4.3	2	1	5
Untreated (n = 6)	Public	1.31	0.64	2.05	1.49	0.47	2.97	3	1	6

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land; Untreated – Fields which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.

Appendix C: Field margin habitat characteristics vegetation height (cm), bare soil (%), number of blooming plants, total floral richness, and grass (%) for each treatment category. We collected all data using a 50 cm x 50 cm quadrat in 2019. Bare soil (%) and grass (%) represent visual estimates of the percentage of the quadrat occupied by bare soil and grass, respectively. We averaged vegetation height and number of blooming plants for the twelve quadrat samples taken at each field during each sampling period. Total floral richness represents the summed total of unique flowering plant species from the twelve quadrat samples taken at each field, as opposed to an average.

		Vegetation Height (cm)			**Bare Soil (%)**			**# of Blooming Plants**			**Total Floral Richness**			**Grass (%)**		
Treatment[1]	Ownership	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
PT1 (n = 4)	Private	85	73	110	8	0	22	2	0	7	10	6	15	36	8	57
Treated Private (n = 7)	Private	89	47	117	8	0	26	2	0	6	8	3	17	43	14	71
PT3 (n = 6)	Public	99	63	166	4	0	13	1	0	5	9	3	20	48	10	82
Treated Public (n = 6)	Public	94	49	126	7	0	22	2	0	7	10	5	22	26	3	66
Untreated (n = 6)	Public	94	55	148	5	0	13	2	0	7	9	3	19	49	11	74

[1] **Treatment Categories:** PT1 – Fields last planted with neonicotinoid-treated seed in 2018 and located on private land; PT3 – Fields last planted with neonicotinoid-treated seed in 2016 and located on public land; Treated Public – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on public land; Treated Private – Fields planted with neonicotinoid-treated seeds during the study period (and years prior) and located on private land; Untreated – Fields which were not planted with neonicotinoid-treated seed in the study year or any year prior and were located on public land.