Nesting habitat enhancement for wild bees within soybean fields increases crop production

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Abstract – Bee diversity is declining in agricultural landscapes, altering pollination services that may support or enhance crop yields. Pollination services provided by wild bees are often limited to field margins adjacent to natural habitats, but methods to increase pollinators and their ecosystem services within field interiors remain elusive. Evidence is growing that wild bees enhance yields of flowering crops, including soybean (*Glycine max* (L.) Merrill). We tested responses of bee communities and soybean yield following pollinator enclosure and nesting manipulation experiments within production areas of the United States. We found that pollinator enclosures resulted in lower seed yield of soybean plants, and providing nesting substrate resulted in a yield increase as well as increases in ground-nesting bees. We provide evidence for soybean-associated bees and suggest these species may benefit from supplemental nesting substrate within production areas and increase soybean yields.

Agroecosystems / Enclosure / Food security / Pollination / Bee communities / Nesting habitat provision

1. INTRODUCTION

Habitat loss is a driving force of declining bee diversity and pollination services to crops and wild plants (Klein et al. 2007; Potts et al. 2010; Winfree et al. 2007a). Agricultural intensification displaces habitat for native bees and disrupts mutualisms through the reduction of plant diversity (Burke et al. 2013; Garibaldi et al. 2011; Kremen et al. 2002). Therefore, pollinators rely on semi-natural habitat bordering production areas for food and nesting resources in agricultural landscapes. These refuges support local pollinator communities and enhance ecosystem services to

adjacent crop fields (Diekötter et al. 2013; Tschamtker et al. 2005; Winfree et al. 2007b). However, unless bees and other pollinators can inhabit production areas, growers cannot take full advantage of the free crop pollination services provided by native bees (Christmann and Aw-Hassan 2012; Sardiñas et al. 2016). Intensive management practices, loss of floral diversity, and restrictions of nesting substrates act as barriers that prevent pollinators access to crop field interiors. Resident bee communities of agricultural landscapes and their pollination services could be enhanced with greater knowledge of their limiting resources (Burke et al. 2017).

Agricultural intensification in North America is associated with large monocultures of self-pollinating crops including the legume, soybean (*Glycine max*). In general, legumes are bee pollinated, and even self-pollinating legumes have outcrossing potential (Suso et al. 2016; Smýkal et al. 2018). Reduced floral size and reward

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(nectar and pollen) associated with domestication often result in decreased insect attraction and pollination (Meyer and Purugganan 2013). Nonetheless, honeybees can increase soybean production, but variation in yield differences between cage and field experiments suggested a cultivar-dependent pollination role for wild bees (Ahrent and Caviness 1994; Erickson 1975; Erickson et al. 1978; Free 1993). Pollination enhancement of soybeans by wild bees was further demonstrated in cage experiments (Roumet and Magnier 1993) and recently inferred in crop fields (Milfont et al. 2013; Monasterolo et al. 2015; Zelaya et al. 2018). Dennis (2018) found increases in soybean seed set but not overall yield. Although wild bees may enhance soybean pollination and yield, bee communities within agricultural landscapes are dependent on resources of nearby semi-natural habitats (Winfree et al. 2007a). Without food and nesting resources within the production area, it is unlikely that soybeans and other cultivated crops can benefit from wild bee services (Burkle et al. 2017).

Improving habitat quality along field margins may increase bee abundance and diversity, thereby increasing spillover of pollination services to production areas (Garibaldi et al. 2011; Kennedy et al. 2013; Tscharntke et al. 2005; Winfree et al. 2007b). In large fields, however, pollination by bees declines exponentially with distance from semi-natural habitats (Garibaldi et al. 2011; Ricketts et al. 2008). This is partly due to foraging limitations, with larger bee species capable of foraging farther distances than smaller bees (Greenleaf et al. 2007). Typical foraging distances are context dependent on the spatial variation of the nest location in relation to host floral resources (Everaars et al. 2018). Foraging distances for native and smaller bee species are largely unknown. For example, a review by Greenleaf et al. (2007) categorized typical foraging distances as 50% of maximum foraging distances due to insufficient studies available for the review. However, mark-recapture studies of native solitary bees suggest typical foraging ranges can be much less (<1%) than these predicted values (Sardiñas et al. 2016), and empirical studies suggest that small and medium-sized bees primarily forage less than 100 m from nests (Cutler et al. 2015; Steffan-Dewenter and Tscharntke 1999).

This contradiction likely reflects bees balancing energy expenditures to acquire pollen (Everaars et al. 2018), and the disproportionately greater foraging distances of large and often managed bee species (Greenleaf et al. 2007). Bees are more likely to inhabit provisioned nests that are close to a used resource (Gathmann and Tscharntke 2002) and will nest within flowering crop fields that are intensively managed (Julier and Roulston 2009; Krug et al. 2010; Sardiñas et al. 2016). Therefore, nesting sites could be established in exposed soil following crop planting (Cane 1991), but the lack of floral resources limits nest establishment by early-season species. Late-season solitary and multivoltine bees that search for nesting sites before or during early soybean flowering are limited by dense crop cover and little exposed soil nesting substrate. Thus, pollination services in large, intensively managed fields are likely restricted for most wild bees (Tscharntke et al. 2005).

Collectively, the effects of soybean floral domestication, limited bee foraging ranges, and intensive management lead to the assumption that soybean production areas are inhospitable habitats for bees (Garibaldi et al. 2011; Kennedy et al. 2013; Kremen et al. 2002; Tscharntke et al. 2005). However, there is growing evidence for a community of ground-nesting social and solitary bees that is tolerant of production areas (Gill and O'Neal 2015; Wheelock et al. 2016; Dennis 2018; Raudenbush et al. 2018). This includes species that found colonies early in the season, as well as solitary bees with emergence times that coincide with soybean flowering. Although it is unknown if bee assemblages depend more on soybean crops or field margins, yields in other crop systems are higher when bees nest within fields (Cane 2008; Julier and Roulston 2009; Sardiñas et al. 2016).

Here, we tested two hypotheses on the roles of wild bees in soybean pollination and yield. First, we hypothesized that bee pollination enhances soybean yield over wind or self-pollination. Second, we tested whether greater nesting substrate availability within intensively managed production areas increases bee diversity, bee abundance, and soybean yield. We sampled the bee community and quantified bee effects on soybean yield using pollinator enclosures over

soybean flowers in fields with and without nesting habitat enhancements for ground and cavity-nesting species. We predicted that preventing pollinators access to soybean flowers would result in a lower yield compared to plants with flowers available to pollinators. We also predicted that soybeans in fields with supplemental nesting substrate would have increased soybean yield due to increased abundance of bee pollinators and decreased foraging distance between nests and crop plants.

2. MATERIALS AND METHODS

Study sites The study was conducted in summer 2014 at the Ecology Research Center (ERC) of Miami University near Oxford, Ohio, USA (39.5325°N, -84.7232°W; Online Resource 1). The ERC consists of temperate and secondary growth deciduous forest, soybean and corn agricultural fields, old field, and lawn. We established four sites of experimental soybean fields in a paired plot design ($n=8$ plots) to account for variation in surrounding environmental factors. Each site consisted of a control and enhancement plot, with each plot 60×70 m (0.4 ha) in size. Indeterminate glyphosate-resistant Steyer® variety 835BCU77 soybeans without a seed coating were planted in 76-cm (30-in.) rows in all plots. We implemented flowerless buffers of 10–15 m around each plot with weekly tillage or mowing to reduce the effects of nearby pollen and nesting sources on the nesting enhancement treatments of the plot pairs. Short buffers were used to ensure that all species in the adjacent area of both plots could access the center of either plot in a site. To reduce bee visitation between plot pairs, the plots within a site were positioned diagonally (Online Resource 1). The distance between sites varied from 0.18 to 0.80 km.

Nesting habitat enhancements Nesting treatments were applied within crop fields before (13th–17th July) soybean flowering to target late-season and multivoltine bee species. Although social species (early-season founders) may nest in soil, the lack of floral resources in crop fields during spring and early summer would prevent these bees from using early-season nest treatments. Centered

within each plot, a 1×10 -m rectangular strip of ground was cleared by hand-pulling soybean plants to provide habitat for ground-nesting bees. Nest treatment areas in control plots, abbreviated N-, were covered with 0.125 mm^2 white, nylon netting to exclude ground-nesting insects with minimal alteration to soil properties (i.e. sunlight, soil temperature and moisture), while nest treatment areas of experimental plots, abbreviated N+, were not covered.

Bee community sampling Bees were sampled weekly from 31st July – 4th September using 0.59 L (20 oz) fluorescently painted pan traps (white, yellow, and blue; Droege 2008). Four raised platforms were placed in each plot halfway between nest treatment corners and the nearest plot corner (along the diagonals). Three pan traps (one of each color) containing soapy water were placed on each platform for 24 h. Platform height was adjusted weekly to vegetation height (Geroff et al. 2014). Bees from all four platforms were pooled for analyses.

Pollinator exclosures To evaluate the effects of pollinators on soybean production, pollinator access to soybean plants was manipulated in a paired design within each plot (Dennis 2018, Kalisz and Vogler 2003, Milfont et al. 2013). On 22nd July, when the first flower was observed at sites, 1-m tall mesh cages constructed with 0.125 mm^2 white, nylon netting were placed over 1-m segments of planting rows of soybean plants (~10 adjacent plants), hereafter, C+. Another 1-m row of plants directly adjacent to C+ plants were open to pollinators and served as the control plants, indicated as C-. We removed all insects and eggs on C+ and C- plants immediately prior to caging soybeans and visually inspected cages for pests and structural integrity weekly. Plots within each site had identical configurations of mesh cages, with one to the east and one to the west of the plot center. Assigned randomly, two sites contained mesh cages at distances of 6 and 18 m from each plot center, while the other two sites had cages 12 and 24 m from plot centers. Mesh cages were removed from 2nd – 8th September once soybeans were finished flowering. Average plant height was recorded when cages were removed

to evaluate potential effects of herbivory (C- plants) and shading (C+ plants) on plant growth.

Soybean harvest To quantify the effects of nest enhancements on soybean pollination, a transect of 10 soybean plants was harvested 10 m and 20 m east and west of nest treatments in all plots for a total of four harvested transects per plot. Within each transect, one soybean plant was sampled every 10 plants (~1 m) along the row to represent plants across the entire length of the nest treatment (10 m). C+ and C- plants were harvested following senescence to evaluate effects of pollinators on seed set. To quantify fruit and seed set, the number of pods and seeds within each pod, respectively, were recorded for all sampled plants. Aborted pods were not counted. To quantify seed mass, seeds of each plant were pooled and oven dried at 100°C for 24 h to achieve constant mass (0% moisture).

Bee community analyses Species richness, abundance, diversity, and ground-nesting bees were analyzed using linear and generalized linear mixed effects models in R version 3.3.1 (R Core Team 2016) with the *lme4* package (Bates et al. 2015). We tested hypotheses that nest treatments affected response variables by performing likelihood ratio tests of models with and without the nest treatment variable. All models had random site intercepts to account for differences in site-specific factors, such as variation in the surrounding environmental factors (e.g. vegetation type, other available floral resources, soil differences). Response variables included abundance, rarified species richness, Shannon's Index, and ground-nesting species richness and abundance of the bee community. Species richness and abundance responses were modeled using Poisson error distributions.

Soybean yield analysis The effects of pollinators on soybean yield were determined by modeling yield differences between C+ and C- plants using maximum likelihood estimation. All model selection was performed using the *AICcmodavg* package (Mazerolle 2016). Soybean yield responses for C+ models included seed set, fruit (pod) set, seed mass, and quantity of pods with one, two,

three, and four seeds. All models ≤ 2 AICc units of the best model (lowest AICc) were evaluated and the best model was used in analyses (Online Resource 2). We used the response $\ln(y_i) - \ln(x_i)$, where y_i is the average per plant yield response among 1 m of C- plants of cluster i , where $i = 1:16$ (two clusters per plot), and x_i is the average yield response of the adjacent 1 m of C+ plants of cluster i . Models included a random intercept of site or plot to account for spatial variation that was unrelated to pollinator exclusion. Results are reported as mean intercepts with 95% CIs and interpreted as a significant effect size if CIs do not include zero.

To determine if distance from nest enhancements affected soybean yield, likelihood ratio tests were performed on linear mixed effects models with and without a nest treatment variable. The average production per plant within a transect (1 transect = 10 plants pooled), was log-transformed to meet the assumptions of normality. Site was included as a random effect and distance from plot centers (10 and 20 m) was a continuous fixed effect in all models.

Plant-pollinator analysis To determine which bee species were most associated with soybean yield, we analyzed seed set responses to nest treatment, distance from plot center, and the abundance of individual bee species. Seed set was the average number of seeds per plant harvested from all four transects within a plot ($n = 40$ plants). We allowed intercepts to randomly vary with site. The response variable, seed set, and the predictor, individual bee species abundance, were log-transformed. Since the most common species are likely responsible for crop pollination (Kleijn et al. 2015), only bee species that were present in every plot were included in the analyses. Model selection was conducted as described above. Chi-squared test statistics of likelihood ratio tests are reported for the final model predictors.

3. RESULTS

We sampled 1742 individual bees within flowering soybean fields and used 1724 individuals of 53 species for analyses (Online Resource 3).

Due to uncertainty in identification, seven *Lasioglossum* (*Dialictus*) males and 11 individuals with missing body parts were omitted from analyses. The effect of nest treatments differed depending on the bee group (Table 1), but did not affect rarified species richness ($\chi^2(1) = 0.13$, $p = 0.72$) or Shannon's Index ($\chi^2(1) = 0.06$, $p = 0.80$), but tended to increase overall abundance ($\chi^2(1) = 3.35$, $p = 0.07$) of the bee community. Ground-nesting bee abundance in N+ plots (mean $\pm$ SE: 43.6 $\pm$ 6.6) was greater than N- plots (mean $\pm$ SE: 38.7 $\pm$ 6.4; $\chi^2(1) = 4.93$, $p < 0.05$). Three bee species, *Lasioglossum callidum*, *Lasioglossum hitchensi*, and *Agapostemon virescens*, were more abundant in N+ plots. Species richness of ground-nesting bees was not different between N- and N+ plots ($\chi^2(1) = 0.51$, $p = 0.47$). We found fewer honey bees, *Apis mellifera*, in N+ compared to N- plots.

Yield differences between C+ and C- plants demonstrated that pollinators increased seeds by 23.1% (effect size = 0.160 ± 0.154) and pods by 24.4% (0.171 ± 0.154), although there was a trend of decreasing seed mass in C- plants (-0.053 ± 0.078) (Fig. 1). Increases in C- seed and pod set were primarily due to increases in pods with two (0.430 ± 0.170) seeds, although pods with one (0.657 ± 0.791), three (0.131 ± 0.136) and four (0.634 ± 0.732) seeds tended to increase when exposed to pollinators (see Online Resource 4 for untransformed values). The average ($\pm$ SE) heights of C+ (73 ± 3 cm) and C- (72 ± 2 cm) plants were similar and did not warrant further consideration for statistical analysis.

Figure 1. Intercepts of soybean production models with and without pollinators. Responses (seed set, pod set, and mass per seed) are evaluated as $\ln(\text{pollinators}) - \ln(\text{no pollinators})$, such that positive values indicate increased production when pollinators are allowed flower access. Each hollow circle represents averaged response of one cluster of soybean plants. Solid squares indicate averages, and vertical lines represent limits of 95% CI.

N+ had increased soybean seed and pod production, but differences were not statistically significant. N+ increased seeds per plant on average by 31.0% at 10 m and 28.7% at 20 m from nest treatments ($\chi^2(1) = 3.47$, $p = 0.06$) and increased pod set ($\chi^2(1) = 3.16$, $p = 0.08$) (Fig. 2). Seed mass also tended to increase in N+ plots ($\chi^2(1) = 2.57$, $p = 0.11$). Changes in counts of pods with one, two, three, or four seeds were not uniform (Fig. 3). There was no effect of N+ on number of pods with one ($\chi^2(1) = 0.20$, $p = 0.65$), two ($\chi^2(1) = 1.29$, $p = 0.26$), or four seeds

Table 1. Response means and standard errors of model with and without supplemental nesting habitat. Chi-squared statistics and associated p values are derived from likelihood ratio tests between models.

Response	Control $\pm$ SE	Nesting Enhancement $\pm$ SE	Chi-squared Statistic	p value
Ground bee abundance	38.7 $\pm$ 6.4	43.6 $\pm$ 6.6	0.44	0.51
Ground bee richness	9.3 $\pm$ 1.0	10.1 $\pm$ 0.9	0.64	0.42
Overall richness	12.1 $\pm$ 1.1	12.5 $\pm$ 1.1	0.09	0.77
<i>L. callidum</i> abundance	0.9 $\pm$ 0.3	2.6 $\pm$ 0.7	14.89	<0.0005
<i>L. hitchensi</i> abundance	15.6 $\pm$ 3.7	19.9 $\pm$ 4.4	8.86	<0.005
<i>A. virescens</i> abundance	1.5 $\pm$ 0.4	2.9 $\pm$ 0.8	7.93	<0.005
<i>A. mellifera</i> abundance	2.6 $\pm$ 0.8	1.4 $\pm$ 0.5	5.97	<0.05

Figure 2. Distribution of seed (*upper*) and pod (*lower*) set per plant with (gray) and without (black) nesting substrate enhancements 10 m and 20 m from plot centers. Hollow circles represent average of one transect (10 plants). Vertical lines indicate 95% CIs and solid squares show means.

($\chi^2(1) = 2.30, p = 0.13$) seeds. Instead, seed and pod set increases were due to higher quantities of pods with three seeds ($\chi^2(1) = 4.03, p < 0.05$).

Figure 3. Pod set (y-axis) with one, two, three, and four seeds (x-axis) with (gray) and without (black) nesting substrate enhancements. Vertical lines indicate 95% CIs and solid squares show means. * $P < 0.05$.

Statistical models of individual bee species abundances and average soybean seed set resulted in four competing models (Table 2). Nest treatment was the strongest predictor and was included in all models. The abundance of *L. callidum* was a positive predictor of seed set in all models. *Apis mellifera* was present in two models and *Lasioglossum imitatum* in one model, showing weak associations with increased seed set. *Lasioglossum versatum* and *Augochlora pura* were negatively associated with seed set.

4. DISCUSSION

Effects of soybean flower access and nesting enhancements We demonstrated positive relationships between pollinator access and soybean fruit and seed production. Soybean plants exposed to pollinators while flowering yielded a 23% higher seed set and 24% higher fruit set compared to those excluded from pollinators, regardless of nest treatments. This is consistent with previous findings that wild pollinators play a role in cultivated soybean pollination (Milfont et al. 2013; Monasterolo et al. 2015). Milfont et al. (2013) found increases in pods with three seeds with supplemental pollinators, while we observed increases in pods with two seeds when soybean flowers were exposed to ambient pollinators. Though this difference could be cultivar-dependent, it suggests pollination limitation of soybeans by native bees outside of production areas. Although we did not control herbivory or shade, there was no observed difference in heights or damage of caged and open plants (Dennis 2018).

An association of some wild bee species with soybean suggests that use of soybean flowers enhances their distribution and abundance within agricultural landscapes (Kleijn et al. 2015). We did not find a relationship between overall bee diversity and nesting enhancement, but the trend of increased bee abundance resulted from ground-nesting species. Of the 53 bee species sampled from the soybean fields, only 10 species were found to occur in every plot, which together comprised 81% of the total bee abundance (Table 3). Of these species, three increased in abundance with nesting enhancements: *L. callidum*, *L. hitchensi*, and *A. virescens*. All are ground-

Table II. Competing models for natural log responses of average seed set within plots ($n=40$ plants per response value). Predictor coefficients with standard errors and Chi-squared (χ^2) statistics with associated p -

values from likelihood ratio tests are provided for each variable within a model. Shading indicates one competing model. χ^2 statistic without asterisks indicates a p value > 0.05 .

Species	Coef	$\chi^2(1)$	Coef	$\chi^2(1)$	Coef	$\chi^2(1)$	Coef	$\chi^2(1)$
Intercept	4.604 ± 0.110	NA	4.585 ± 0.113	NA	4.587 ± 0.114	NA	4.593 ± 0.107	NA
Nest Enhancement	0.240 ± 0.025	41.76****	0.254 ± 0.025	43.00****	0.249 ± 0.024	43.83****	0.248 ± 0.025	42.65****
<i>Lasioglossum callidum</i>	0.068 ± 0.022	8.49 ***	0.062 ± 0.021	7.76**	0.072 ± 0.021	10.00***	0.065 ± 0.022	7.94 ***
<i>Augochlora pura</i>	-0.054 ± 0.018	8.04 ***	-0.062 ± 0.018	10.17***	-0.050 ± 0.019	6.37 *	-0.059 ± 0.018	9.17 ***
<i>Apis mellifera</i>	NA		0.034 ± 0.022	2.33	0.046 ± 0.022	4.07 *	NA	
<i>Lasioglossum versatum</i>	NA		NA		-0.028 ± 0.017	2.53	NA	
<i>Lasioglossum imitatum</i>	NA		NA		NA		0.024 ± 0.021	1.19
ΔAIC_c	0.00		0.87		1.79		2.00	

**** $P < 0.0001$; *** $P < 0.005$; ** $P < 0.01$; * $P < 0.05$

nesting bees and comprised 42% of all sampled bees. *Lasioglossum callidum* and *L. hitchensi* are social and generalist species of the same subgenus of small-bodied sweat bees, *Dialictus*, while *A. virescens* is a late-season and communal nesting generalist. Interestingly, 85% of the individuals of *A. virescens* were male, which may forage on soybean nectar but do not actively collect pollen. Since the mechanism of soybean pollination enhancement by bees and other insects is largely unknown, it is unclear if male bees benefit yield, though males are efficient pollinators in other systems (Cane et al. 2011). We also

observed a colony of *Halictus ligatus* nesting within a habitat enhancement. The typical foraging range of this small social and generalist species is unknown, but Ginsberg (1985) found that *H. ligatus* visited flowers in a nearest-neighbor pattern and did not fly over available floral resources. Therefore, the bare ground enhancement likely provided suitable nesting sites within this flowering crop field. Increased abundance of dominant ground-nesting bee species suggests that nesting substrate may be a limiting factor for these bees within crop production areas.

The nesting enhancements also resulted in a 30% increase in seed and pod set 10 and 20 m from plot centers. Milfont et al. (2013) observed increased yield due to bees was primarily due to higher production of pods with three seeds. Considering provisioned nesting substrate also increased the presence of ground-nesting bees, our findings from both the pollinator-exclosure and nesting enhancement suggest that soybean pollination is limited by the availability of bee pollinators. Nesting enhancement was a strong predictor of increased seed set. The magnitude of the increase in seed and pod set due to nest enhancements was greater than the abundance response of ground-nesting bees, implying that other bee species were involved in soybean pollination but sampled less effectively in pan traps.

Table III. Rank ordered abundances of species ($n=10$) present at all sites.

Species	Total Abundance
<i>Lasioglossum hitchensi</i>	602
<i>Melissodes bimaculata</i>	200
<i>Augochlora pura</i>	167
<i>Lasioglossum versatum</i>	129
<i>Agapostemon virescens</i>	74
<i>Apis mellifera</i>	68
<i>Lasioglossum callidum</i>	58
<i>Lasioglossum imitatum</i>	51
<i>Halictus confusus</i>	30
<i>Halictus ligatus</i>	25

We detected several positive associations between individual bee species and increased soybean yield, including *L. callidum*, which increased with nesting enhancements. *Lasioglossum imitatum* and *A. mellifera* were also significant predictors of increased soybean yield, suggesting that insect pollination of soybeans is due to the complementary effects of a diverse assemblage of pollinators. The positive associations between small sweat bees and crop yield may be a consequence of bee traits and flower morphology. Soybean flowers exhibit a closed morphology that requires maneuverability of keel petals to access floral rewards. Since *Dialictus* are only a few millimeters in size, they may be small enough to navigate soybean flowers to obtain pollen and nectar stores. However, not all soybean-associated bees are small. For instance, *A. mellifera* is a medium-sized bee, and is a known pollinator of soybeans. Since honeybees were not a part of the experimental design, hives were located 200–800 m from experimental plot edges and often with other flowering crops in closer proximity. This is likely the source of the weak relationship between honeybees and soybean production. Alternatively, it could be due to the short handling time *A. mellifera* spent servicing soybean flowers (Online Resource 5). In general, the weak effect sizes of individual species suggest that many bee species are needed to enhance soybean pollination and yield, perhaps because a given bee species may visit a single flower infrequently but visits by multiple bee species increase the likelihood of pollen transfer among flowers. Nonetheless, effective pollinators of this system are likely to include the more dominant species found in every plot (Kleijn et al. 2015) with the exception of *A. pura*, which showed a negative relationship with soybean production and was likely a reflection of the shade from nearby forests. Such variation in species-specific bee responses supports the hypothesis that a subset of the overall ground-nesting bee assemblage is responsible for yield enhancement of soybeans.

A soybean-associated bee community. Identifying native bee species associated with soybean production areas of Midwestern North America is a recent interest (Gill and O’Neal 2015, Wheelock

et al. 2016, Dennis 2018, Raudenbush et al. 2018), even though attention to wild bees within soybean crops dates back decades (Rust et al. 1980). The heterogeneous habitat types (forest, grassland, crops) surrounding our study sites and small plot size (0.40 ha) likely contributed to the high bee diversity (Online Resource 6) sampled within crops and among sites (Dauber et al. 2003). Less than 10 individuals were sampled from 37 species, suggesting most of these species are not commonly found in soybean crop fields and are unlikely to play a role in soybean pollination (Kleijn et al. 2015). Bee surveys in Ohio (Raudenbush et al. 2018), Indiana (Dennis 2018), and Iowa (Gill and O’Neal 2015) soybean fields found diverse assemblages similar to our study (Online Resource 3). Specifically, *Lasioglossum (Dialictus)* sp. (identified here as *L. hitchensi*), *A. virescens*, *Melissodes bimaculata*, and other *Lasioglossum (Dialictus)* species were found in high abundances in these states, supporting the notion of a soybean-associated bee community.

The associations among ground-nesting bees, soil-substrate enhancements, and increased soybean yield emphasizes the potential importance of *Dialictus* bees. Due to the difficulties differentiating species of *Dialictus*, they are often lumped as a single taxonomic unit in pollination studies (Gibbs 2011). However, four species in our study belong to *Dialictus*, and three of those either benefited from nesting substrate enhancements, increased soybean yield, or both. Important traits, such as sociality and foraging behaviors, vary widely among these species and highlight the need to understand their roles in soybean and other crops. We also observed *Xylocopa virginica*, *Bombus impatiens*, and *M. bimaculata* visiting soybean flowers, all of which are large-bodied bees and known to be under sampled with pan traps (Droege 2008; Geroff et al. 2014). *Xylocopa virginica* (large carpenter bee) is a communal generalist that nests in wood cavities. The small flower morphology of soybean may be attractive to this short-tongued species when within typical foraging range. In contrast, *M. bimaculata* is a solitary, ground-nesting generalist with a seasonal activity that

largely overlaps with soybean flowering. Despite its large size, *M. bimaculata* was the second most abundant species and we found multiple individuals in soybeans with full pollen loads on tibial scopa, which may be of soybean origin (Gill and O'Neal 2015; Rust et al. 1980). As with most *Bombus* spp., *B. impatiens* are large-bodied generalists that colonize old rodent burrows underground (Michener 2000). Bumblebees are known to forage on another mass-flowering crop that does not require insect-pollination (i.e. canola; Riedinger et al. 2015; Westphal et al. 2003) and were under sampled in Iowa soybean fields using pan traps as well (Wheelock et al. 2016). Additionally, we observed *B. impatiens* in crop fields visiting soybean flowers. *Bombus impatiens* is one of the few bumblebees not declining in the fragmented agricultural landscapes of the Midwestern United States (Grixti et al. 2009), and its success may be partly due to its use of crop floral resources. Although a managed species, *A. mellifera* is a strong flyer with long-range foraging abilities and was likely under sampled as well. *Bombus impatiens* (Herrmann et al. 2017), *A. mellifera*, and possibly others regularly forage at far distances from their nest (Greenleaf et al. 2007). Therefore, it is possible that some individuals of these species visited more than one pair of experimental plots in our study.

Implications of ground-nesting substrate enhancements for soybean production. Increased soybean yields spanned at least 20 m from the nesting enhancement, with only slight decreases in effect size with distance from nesting enhancements, suggesting that pollinators of soybean will use nesting substrate within the production area. Closed canopies of intensively managed fields limit bee pollination of soybean plants within foraging ranges, and likely restricts small pollinators like *Dialictus* to areas near the field margins (Greenleaf et al. 2007). Exposing soil within soybean production areas provided bees nesting substrate, which increases their pollination services at farther distances within the crop field.

Providing exposed soil substrate for pollinators removes crop plants. Therefore, yield increases due to bee pollination should be weighed against loss of crop area. Based on the 1-m row of plants we sampled at 32 locations within our 0.4-ha (1-acre) plots, we estimate there were 9.0 plants m^{-1} . Following Lee and Herbek (2005), this translates to an average of 47,950 plants within a plot of our system. Their average seed weight of 1134 seeds per kilogram (2500 seeds per pound) estimates a yield of 1.1 tons per ha (32 bushels per acre) without pollinator influence. The estimated yield in our system was 1.3 tons per ha (39 bushels per acre). The application of nesting enhancement strips resulted in a loss of 0.001 ha (<0.0025 acres) per 0.4 ha harvested. If the observed 29.9% increase in yield due to nesting enhancements occurs throughout the plot, we expect a yield of 1.7 tons per ha (>50 bushels per acre). This suggests supplemental insect pollination of soybean in a conventional system could be economically beneficial, and that the area needed for bee pollination services is small relative to the potential increase in yield.

Our findings support previous reports that several wild bee species may be associated with soybean crops (Gill and O'Neal 2015; Wheelock et al. 2016; Rust et al. 1980; Dennis 2018; Raudenbush et al. 2018), and provide further evidence of insect pollination enhancement of soybean crops. These findings indicate wild bee species play a significant role in soybean pollination and yield enhancement, especially if nesting substrate is available. We provided evidence for this at a plot scale and during the soybean bloom, but studies in larger production areas over many years are needed to determine whether this approach will support native bee populations and enhance pollination of soybeans and other crops within intensively managed agricultural landscapes. Crop rotation, tillage practices, and other crop systems should be incorporated into future studies to ensure that attracting bees into the crop field does not instead serve as an ecological trap in space or time.

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AUTHOR CONTRIBUTIONS

All authors have conceived the study design, MCM executed the field work, and all authors contributed to the analysis and writing.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest The authors declare that they have no potential conflict of interest in relation to the study in this paper.

L'amélioration de l'habitat de nidification des abeilles sauvages dans les champs de soja augmente la production agricole.

agroécosystèmes / zone d'exclusion / sécurité alimentaire / pollinisation / communautés d'abeilles / habitat de nidification.

Verbessern der Nesthabitate für Wildbienen in Sojafeldern erhöht die Nutzpflanzenproduktion.

Agrar-Ökosysteme / Ausschlussfläche / Lebensmittelsicherheit / Bestäubung / Bienengemeinschaften / Nesthabitatbereitstellung.

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