INVESTIGATING THE IMPACTS OF GROUND MANAGEMENT ON ARTHROPODS IN ORGANIC CUCURBITA AGROECOSYSTEMS

By

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ABSTRACT

INVESTIGATING THE IMPACTS OF GROUND MANAGEMENT ON ARTHROPODS IN ORGANIC CUCURBITA AGROECOSYSTEMS

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Reduced-disturbance ground management practices such as no-till and strip tillage (a.k.a. conservation tillage) have been demonstrated to provide several agronomic benefits that can enhance crop health. However, the effects of conservation tillage methods on arthropod communities are less understood. In this thesis, I investigated the impacts of soil management practices on pests, natural enemies, and bees with an emphasis on *Eucera pruinosa* (Hymenoptera: Apidae) in organic *Cucurbita* agroecosystems. From 2017-2019, using field experiments and a citizen science survey, I observed that different types of foliar herbivores and natural enemies varied in their response to strip tillage. Aphididae (Hemiptera) comprised the majority of foliar insect pests observed in field experiments and were more abundant in conventional tillage than in strip tillage. "Parasitica" were most frequently observed in strip tillage which may have contributed to lower Aphididae abundance. Several epigeal natural enemy taxa including *Harpalus* spp. (Carabidae) and Araneae were significantly more abundant in strip tillage suggesting that this practice may promote enhanced biological control. In my citizen science study, *Eucera pruinosa* flower visitation was approximately three times greater in reduced tillage and no-till systems than in conventional tillage suggesting that lower intensity ground management can help conserve important wild pollinators. In addition, this study demonstrated the efficacy of citizen science for collecting data across broad geographic areas and engaging the public in addressing ecological issues. Overall, my results suggest that conservation tillage methods have the potential to promote enhanced biological control and pollination services in organic *Cucurbita*.

This thesis is dedicated to my wife and daughters who have inspired, strengthened, and loved me throughout my exciting, yet tumultuous years of higher education. I owe a great deal of my success to their support.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: Habitat structural complexity and managing arthropods in organic *Cucurbita*

Introduction

Varieties of *Cucurbita* (Cucurbitaceae) such as squashes, pumpkins, and zucchini have been domesticated by humans for thousands of years for food and other purposes (1–4), and these crops continue to have major economic importance worldwide (5,6). In 2019, the United States produced 113,200 acres of squash and pumpkins equaling 2.1 billion pounds of fruit valued at over $400 million (7), highlighting the economic importance of these crops. Approximately 13% of the total United States squash and pumpkin acreage in 2019 was grown in Michigan and was valued at over $53 million (7). Organic production has also increased in the United States (8) and in 2016, Michigan produced more than $1.7 million of the approximately $48.3 million worth of organic squash (9).

Insect pests

The most common and important insect pests of both conventional and organic Cucurbitaceae crops are squash bugs (*Anasa tristis*, Hemiptera: Coreidae), striped cucumber beetles (*Acalymma vittatum*, Coleoptera: Chrysomelidae), spotted cucumber beetles (*Diabrotica undecimpunctata howardii*, Coleoptera: Chrysomelidae), squash vine borers (*Melittia cucurbitae*, Lepidoptera: Sesiidae), and to some degree, aphids (several species, Hemiptera), and seedcorn maggots (*Delia platura*, Diptera: Anthomyiidae) (8,10). Although all of these insects can cause deleterious effects to squash production, the first four are of primary concern (10). They damage squash plants and/or fruit via feeding and can transmit diseases that can lead to lower quality grades and reductions in marketable fruit (8,10–12).

Piercing-sucking squash bugs (*A. tristis*) are annual, direct and/or indirect pests, and feed on plant sap through leaves, vines, or fruit which can result in leaf withering, fruit rot, or reduced fruit set (13). Squash bugs have also been shown to vector cucurbit yellow vine disease, a bacterial infection caused by *Serratia marcescens* that can result in stunted growth, plant tissue and fluid discoloration, withering, and death (13,14). More than 50 % yield loss due to squash bug damage has been reported in some cases (15).

Like squash bugs, striped and spotted cucumber beetles are annual, direct and/or indirect pests of squash. However, they are chewing rather than piercing-sucking insects that feed on the leaf tissue, flowers, fruit rind, and roots (larvae only) of *Cucurbita* and many other crops (10). Striped cucumber beetles can also cause bacterial wilt, a disease caused by *Erwinia tracheiphila* (16). Damage and management practices due to striped and spotted cucumber beetles alone costs US growers $100 million per year (8,17).

Squash vine are sporadic, indirect pests that damage squash plants by burrowing and feeding within the vines and are only pests of squash in the larval stage (10,18). Although squash vine borers are often more significant in smaller home gardens, commercial growers have reported yield losses of up to 25 % due to this pest (19).

Organic insect pest management and habitat management for beneficial arthropods

Insecticides often used for conventional *Cucurbita* pest control can pose significant risks to beneficial arthropods and other non-target organisms and can contribute to secondary pest outbreaks (13). Insecticide options for organic *Cucurbita* growers are more limited and can suffer from inconsistent efficacy and high cost highlighting the need for alternative pest management strategies (13,20,21). Cultural and physical pest management practices are commonly implemented to control insect pests such as squash bugs and cucumber beetles while supporting healthy natural enemy populations (8,22–24). Some practices include crop rotation to place distance between overwintering pests and squash fields planted during the subsequent growing season, the use of more herbivory-resistant squash varieties, and the use of transplants rather than direct seeding to avoid exposing plants to herbivory during the initial and most susceptible life stages. Growers may also use trap crops, various types of mulches, intercropping with crops that are less attractive to focal pests, trap cropping, row covers, or suction removal to help alleviate pest pressure on squash plants (20,21).

Habitat management, a type of conservation biological control that typically focuses on providing additional plant resources, can be used to promote healthy, persistent natural enemy populations and improve biological control (25,26). Many agricultural insect pests are thought to thrive in monoculture cropping systems where a single crop provides all the resources that they need. In contrast, habitat management can produce more diverse agroecosystems such as polycultures which may inhibit or deter insect herbivore species through the presence of unattractive plants mixed among their preferred hosts (27,28). Natural enemies can also respond positively to more diverse habitats that provide them with limiting resources that are often absent or lacking in agroecosystems such as shelter, stable alternative food sources, and ideal microclimatic conditions (25–28). Plant residues (mulch), which are typical in reduced tillage systems such as strip tillage and no-till, can also provide similar benefits for biological control (25,29–37) while promoting retention of soil moisture, erosion reduction, lower energy use, and inhibition of weed seed germination (38–41). However, no-till has been shown to reduce yields in some crops and climates and reduced tillage methods such as ST, where only crop rows are tilled, may also lead to lower yields due to slower N mineralization (42–45). However, this issue may be ameliorated by zonally intercropping cover crops such as hairy vetch (*Vicia vilosa* Roth; “vetch”), a N fixing legume, within future crop rows and cereal rye (*Secale cereal* L.; “rye”) between crop rows. This practice may distribute N more efficiently than in conventionally tilled soil with a preceding rye and vetch mixed cover crop and may increase crop N uptake, biomass, and yields while promoting agronomic benefits (42,46,47). However, these effects likely vary depending upon the root systems of different crops (42,48). Previous studies have demonstrated a variety of impacts on *Cucurbita* systems due to ST and cover crop residues (41,49-52), although little is known pertaining to the effects of zonally planted cover crops on *Cucurbita* and associated arthropods. Strip tillage may also enhance pollination services through the provision of undisturbed nesting habitat for fossorial bees such as squash bees (*Eucera* (*Peponapis*) *pruinosa*, Hymenoptera: Apidae) which are economically important, specialist pollinators of *Cucurbita* that nest near their host plants (53–59). However, most previous studies examining the effects of tillage practices on squash bees have only compared no-till and conventionally tilled farms, and results have been mixed (53–56,60). This highlights the need to further elucidate the effects of these and other soil management practices (e.g. strip tillage) on squash bees. Utilizing ecologically based strategies can provide more sustainable pest and pollinator management solutions for *Cucurbita* growers that reduce reliance on chemical insecticides (25). However, previous habitat management research has not always found positive effects on natural enemies, and there are gaps in our knowledge of how these practices affect squash bees.

Thesis objectives

The goal of this study was to determine how beneficial and pest arthropods respond to ground management practices in organic Cucurbita agroecosystems. The first objective examined how a variety of insect pests, natural enemies, and pollinators were affected by different combinations of tillage, segregated cover crops, and cover crop mulches. The second objective used a citizen science survey to examine how flower visitation frequencies of squash bees, honey bees, bumble bees, and other types of bees varied across Michigan *Cucurbita* using different ground management practices. Citizen science provided an opportunity to collect a large amount of data across Michigan while spreading public awareness about the importance native pollinators like squash bees.

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CHAPTER 2: Beneficial and pest arthropod responses to tillage and cover crop residues in organic *Cucurbita* agriculture

Introduction

Reduced tillage methods, such as strip tillage, can be an important tool in farming for retaining soil moisture, preventing erosion, reducing energy use, and inhibiting weed seed germination (1–4). Typically, these methods retain > 30% of plant residues (mulch) from the previous crops on the soil surface between crop rows (5) and have the potential to directly impact crop health. However, reduced tillage practices can also affect both pest and beneficial arthropod communities within crop fields via increased habitat complexity due to plant residues which can provide refuge and/or enhanced microclimatic conditions for arthropods (6–12). This has the potential to benefit or harm crops depending on the types of arthropods that inhabit these undisturbed zones (5,13).

Previous studies have reported increases in certain pest species in reduced tillage cropping systems compared to conventional tillage (5). For example, slugs (Mollusca: Gastropoda) can be serious pests of a wide variety of crops in no-till cropping systems severely damaging crops during early developmental stages (14). Many arthropod crop pests have also been observed in higher abundances in the absence of tillage (15) suggesting that reduced tillage practices may in some cases increase pest pressure. In contrast, reduced tillage practices can also alter community composition, increase diversity, and/or abundance of natural enemies (16,17), and can help ameliorate negative effects of landscape simplification on biological control of crop pests by increasing predator and parasitoid abundance (18). Furthermore, previous research indicates that various forms of intense mechanical disturbance (e.g. moldboard ploughing) directly lead to increased mortality of important arthropod predators such as spiders (19) highlighting the potential benefits of reduced tillage for biocontrol. A recent meta-analysis of findings from 59 studies conducted between 1990 and 2017 that examined tillage impacts on invertebrate pests and predators, found that overall pest and predator abundances were similar in reduced tillage systems compared to systems using conventional tillage (5). However, foliar pests were more abundant and epigeal predators less abundant in conventional tillage compared to reduced tillage systems, suggesting that reduced tillage has the potential to improve biological control (5). In addition to impacting pests and natural enemies, reduced tillage practices can also affect wild bees, however, this has not been investigated for a broad range of taxa (20,21).

This study focused on elucidating the effects of reduced tillage practices and segregated cover crops on pest and beneficial arthropods in organic squash (*Cucurbita pepo*). Several herbivorous insect species including *Anasa tristis* (Hemiptera: Coreidae), *Acalymma vittata* (Coleoptera: Chrysomelidae), *Diabrotica undecimpunctata howardii* (Coleoptera: Chrysomelidae), *Melittia cucurbitae* (Lepidoptera: Sesiidae), and to some degree, Aphididae (Hemiptera), and *Delia platura* (Diptera: Anthomyiidae) can cause significant damage to squash plants and/or fruit and reduce marketable yields (22,23). Reduced tillage has the potential to provide agronomic benefits in squash cropping systems and enhance pest control. Furthermore, reduced tillage practices may improve *C. pepo* pollination by providing undisturbed habitat for fossorial bee species such as *Eucera* (*Peponapis*) *pruinosa* (Hymenoptera: Apidae) which is highly relevant for these pollinator dependent crops. *E. pruinosa* are *C. pepo* specialists that forage from and rest within *C. pepo* flowers as well as dig nests in the soil near squash plants (24–26) suggesting that they likely respond positively to reduced soil disturbance (21,27–29). Furthermore, zonally intercropping cover crops such as hairy vetch (*Vicia vilosa* Roth; “vetch”), a N fixing legume, within future crop rows and cereal rye (*Secale cereal* L.; "rye") between rows may help ameliorate N mineralization deficiencies often associated with reduced tillage practices (30–32).

In this study, foliar arthropods (pests and natural enemies), epigeal natural enemy activity density, and bee flower visitation were compared among *C. pepo* in full tillage and strip tillage treatments with a variety of cover crop (mulch) types to determine if strip tillage and segregated cover crops promote beneficial arthropod populations. Epigeal arthropod abundance was expected to increase in strip tillage treatments overall due to reduced soil disturbance and greater habitat structural complexity. Foliar arthropods and bees other than *E. pruinosa* were not expected to respond strongly to treatments. *E. pruinosa* flower visitation frequency was not expected to vary among full tillage and strip tillage treatments due to the relatively close proximity of experimental plots.

Materials and Methods

Experimental design. The study was conducted at the W.K. Kellogg Biological Station, Hickory Corners, MI (42.4058° N, 85.4023° W) from 2017 to 2019 in separate fields (Area ~ 4236 – 4422 m^2) with fine to course loams (mixed, mesic Typic Hapludalfs). A randomized complete block design with 6 replications was used in all 3 years. Four treatments were examined consisting of several combinations of tillage and cover crop/mulch types: 1) conventional tillage with a previous rye-vetch cover crop mixture grown uniformly across the entire plot and incorporated with tillage; 2) strip tillage with a full-width rye-vetch mixture; 3) strip tillage with a previous rye-vetch zonal cover crop of vetch planted and tilled into future crop rows, rye planted between rows, and rye residue left between crop rows following tillage; and 4) strip tillage with a rye-vetch zonal cover crop and additional rye mulch (~ 3 t/ha) added between crop rows after crop establishment.

Plots were arranged in a randomized complete block design with each treatment replicated 6 times for a total 24 plots per field. Plots were approximately 9.1 by 10.7 m with each containing 6 rows of squash with plants placed 0.6 m apart within rows (Figure S2.1). In 2017, “Honey Bear” acorn squash (*Cucurbita pepo* var. turbinata) was planted. In 2018, 3 varieties of acorn squash (“Honey Bear”, “Taybelle”, “Delicata”) and “Spineless Beauty” zucchini (*Cucurbita pepo*) were planted. In 2019, 2 varieties of winter squash were planted; “Taybelle” (inner 4 rows of each plot) and “Delicata” (outer 2 rows of each plot).

Field operations. Major field operations and corresponding dates are listed in Table S2.1. Cover crops were planted in early September the year before squash planting. In conventional tillage and strip tillage with rye-vetch mixed treatments, cover crops were seeded at a rate of 62.8 kg/ha rye and 22.5 kg/ha vetch. The cover crop seed rate for both rye-vetch zonal and rye-vetch zonal with additional mulch treatment was 78.6 kg/ha rye and 11.1 kg/ha vetch, with two rows of vetch seeded in future squash rows and 6 rows of rye between rows. Cover crops in conventional tillage plots were disk killed in late April prior to being cultivated in late May – early June. Strip-tillage and squash planting occurred in mid – late June. In 2017 and 2018, squash was direct seeded. However, in 2018, due to poor crop emergence (due largely to predation by 13-lined ground-squirrels) , acorn squash and zucchini were transplanted into plots. Due to a shortage of transplants, only 4 rows of squash were grown per plot in 2018 with each row containing a different variety. In 2019, acorn squash was transplanted into all plots. Following squash planting, additional rye mulch (~ 3 t/ha) was spread by hand between crop rows in plots assigned to the additional mulch treatment in each year. Insecticides, fungicides, and herbicides were not used in any of the years. Plots were mechanically cultivated with a combination of between-row rolling cultivators and finger weeders (Kult-Kress) and/or hand weeded as needed throughout each growing season. Zucchini was harvested in late August in 2018, and acorn squash was harvested in late September during each year.

Foliar arthropod surveys. Weekly visual surveys of squash plant foliage were conducted on 10 randomly selected plants from the innermost rows of each plot to determine the effects of the tillage and cover crop treatments on foliar arthropods. Once plants had more than 10 leaves, surveys were conducted on 10 leaves from 10 randomly selected plants per plot. Surveys were conducted between June 28 – August 22, 2017 (9 wk), July 9 – August 24, 2018 (7 wk), and July10 – August 19, 2019 (7 wk). Arthropods observed on foliage were identified in the field to species, genus, family, or order. In 2017, for aphids, we counted the number of colonies per plant, and in 2018 and 2019 the numbers of individual aphids per plant were counted or estimated when numbers were high.

Epigeal natural enemy activity density. To determine the effects of the tillage and cover crop treatments on activity density, 2 pitfall traps were deployed weekly in each plot, one approximately in the center of the innermost crop row (in-row), and one between two of the innermost crop rows (between-row), throughout the growing season during all 3 years (2017: 22 June – 10 Aug; 2018: 17 July – 28 Aug; 2019: 9 July – 20 Aug). Pitfall traps consisted of 946 ml polypropylene cups containing approximately 200 ml of propylene glycol and water (~1:1 v/v). Cups were dug into the soil so that their openings were flush with the soil surface and were covered using galvanized wire poultry netting (2.54 cm mesh size) to prevent larger animals from entering traps while allowing arthropods to fall in. Rectangular pieces of particle board with screws driven through the corners to elevate the covers approximately 4 cm above the ground were placed over pitfall traps to prevent flooding due to rain. Pitfall traps were deployed for approximately 24 hr and then strained in the field. Samples were stored in 75% ethanol and identified to order, family, or genus in the laboratory (33,34) (Albert J. Cook Arthropod Research Collection).

Pollinator surveys. Following the beginning of squash inflorescence, weekly pollinator surveys were conducted to determine the effects of the tillage and cover crop treatments on pollinator visitation. Ten random squash flowers per plot were selected from the innermost rows between July 26 – August 21, 2017 (5 wk), and 12 flowers per plot between August 8 – August 27, 2018 (4 wk) and July 23 – August 20, 2019 (5 wk). Each flower was observed for 1 minute and the numbers of squash bees (Apidae: *Eucera* (*Peponapis*) *pruinosa*), honey bees (Apidae: *Apis mellifera*), bumble bees (Apidae: *Bombus* spp.), and other bees (carpenter bees (Apidae: *Xylocopa* spp.), large bees (> 7 mm), and small bees (< 7 mm)) observed landing in flowers were recorded. Formicidae counts were also recorded when observed in flowers. Surveys were conducted between ~ 9:00 am – 12:00 pm on sunny days with ambient temperatures > 21 °C and little wind.

Statistical analysis. All analyses were performed using R version 3.5.1 (35). Generalized Linear Mixed Models using Laplace approximation and negative binomial distribution were used with the 'glmmTMB' function in the 'glmmTMB' package (36) to determine the effects of treatments on foliar arthropod abundance and activity density. Data from all 3 years (2017 – 2019) were analyzed together with temporal differences accounted for by the inclusion of date and year as random effects in models. Foliar arthropod models contained treatment as a fixed effect with block nested within date and year as random effects. Natural enemy activity density models contained treatment, pitfall trap position (in row or between row), and their interaction as fixed effects with block nested within date and year as random effects. Flower visitation by bees was analyzed as above using Poisson distribution with treatment as a fixed effect and date and year as random effects. The 'Anova' function (Type II Wald Chi-square tests) in the 'car' package (37) was used to determine the significance of fixed effects in models, and the 'emmeans' function in the 'emmeans' package (38) with the 'fdr' p-adjustment method (a.k.a. Benjamini-Hochberg) was used to determine pairwise differences among fixed effect factor levels for significant models. Only arthropod types that comprised at least 5% of total counts of foliar herbivores excluding aphids, foliar natural enemies, and pitfall trap collected natural enemies across all 3 years were analyzed individually. Ants (foliar observations and activity density) were also analyzed separately due to high abundance and the potential for them to tend aphids. Arthropod types that did not meet the 5% threshold are included in models analyzing effects on foliar herbivores overall, foliar herbivores (-aphids), foliar natural enemies overall, and natural enemy activity density overall. As previously noted, aphids were counted by colony in 2017 rather than by counting or estimating the number of individual aphids present on foliage. Therefore, only data from 2018 and 2019 were used to analyze differences in aphid and overall herbivore abundance among treatments.

Results

Foliar arthropods surveys. There were 45,394 arthropods observed on squash foliage from 2017 – 2019 comprised of 42,589 herbivores, 2,231 natural enemies, and 574 Formicidae. Aphididae (2017: n = 3008 aphid colonies; 2018 – 2019: n = 37,513 aphids) was the most abundant herbivore observed followed by *Acalymma vittatum* (Chrysomelidae; n = 1357), Cicadellidae (n = 1291), Thysanoptera (n = 915), *Anasa tristis* nymphs (Coreidae; n = 615), *Anasa tristis* egg masses

(Coreidae; n = 483), *Diabrotica undecimpunctata* (Chrysomelidae; n = 272), *Lygus lineolaris* (Miridae; n = 64), Aleyrodidae (n = 41), and *Anasa tristis* adults (Coreidae; n = 38).

Overall herbivore abundance was influenced by cover crop segregation (strip tillage + rye-vetch mixed vs strip tillage + rye-vetch zonal) and supplemental mulch (strip tillage + rye-vetch zonal vs strip tillage + rye-vetch zonal with added rye mulch). Tillage (conventional tillage + rye-vetch mixed vs strip tillage + rye-vetch mixed) did not have a detectable effect on overall herbivore abundance but did impact several specific insect groups. Mean abundance was 1.3 – 1.9 times greater for Aphididae ($\chi^2 = 20.36$, df = 3, $p < 0.01$) and 1.2 – 1.6 times greater for Thysanoptera ($\chi^2 = 9.14$, df = 3, $p = 0.03$) in full tillage compared to strip tillage treatments (Table 2.1). Conversely, mean abundance was 1.4 – 2.3 times greater for *A. tristis* nymphs ($\chi^2 = 14.87$, df = 3, $p < 0.01$), 1.8 – 2.2 times greater for *A. tristis* egg masses ($\chi^2 = 21.63$, df = 3, $p < 0.01$; Table 2.1), 1.2 – 1.5 times greater for Cicadellidae ($\chi^2 = 24.27$, df = 3, $p < 0.01$), and 1.2 – 1.3 times greater for herbivores excluding Aphididae ($\chi^2 = 29.11$, df = 3, $p < 0.01$) in strip tillage treatments compared to full tillage (Table 2.1). Mean abundance of *A. vittatum* ($\chi^2 = 6.53$, df = 3, $p = 0.09$) and *D. undecimpunctata* ($\chi^2 = 4.29$, df = 3, $p = 0.23$) did not significantly vary across treatments (Table 2.1).

"Parasitica" (parasitic wasps; Apocrita; n = 754) were the most frequently observed natural enemies followed by *Orius* spp. (Anthocoridae; n = 658), Araneae (n = 335), Coccinellidae (n = 129), Dolichopodidae (n = 116), Opiliones (n = 102), Chrysopidae (n = 99), Nabidae (n = 24), *Podisus maculiventris* (Pentatomidae; n = 13), Syrphidae larvae (n = 1). A total of 574 Formicidae were also observed.

Overall, natural enemies were affected by tillage and supplemental mulch, but not by segregation (Table 2.2). Mean abundance was 1.2 – 1.8 times greater for "Parasitica" ($\chi^2 = 36.02$, df = 3, $p < 0.01$) and 1.1 – 1.3 times greater for natural enemies overall ($\chi^2 = 22.28$, df = 3, $p < 0.01$) in strip tillage treatments compared to full tillage (Table 2.2). Mean abundance of Araneae ($\chi^2 = 3.18$, df = 3, p = 0.36), *Orius* spp. ($\chi^2 = 1.03$, df = 3, p = 0.79), and natural enemies overall excluding “Parasitica” ($\chi^2 = 3.42$, df = 3, p = 0.33) did not significantly vary across treatments (Table 2.2). Mean Formicidae abundance was 1.8 – 2.3 times greater in full tillage than in strip tillage treatments ($\chi^2 = 16.70$, df = 3, $p < 0.01$; Table 2.2).

Table 2.1. Data summary and results of statistical analyses for foliar herbivores, 2017-2020. Mean ± SEM foliar herbivores per plant values and statistical significance of models for Aphididae, *A. tristis* egg masses, *A. tristis* nymphs, Cicadellidae, Thysanoptera, *D. undecimpunctata*, *A. vittatum*, herbivores (overall), and herbivores (-aphids) observed on squash foliage. Pairwise differences among treatments for each arthropod type are indicated by different letters following mean ± SEM values (emmeans (fdr p-adjustment); $\alpha = 0.05$).

Treatment	Aphididae[a]	*A. tristis* egg masses	*A. tristis* nymphs	Cicadellidae	Thysanoptera	*D. undecimpunctata*	*A. vittatum*	Herbivores (overall)[b]	Herbivores (-aphids)[c]
FT + RVM	13.87 ± 1.10a	0.05 ± 0.01b	0.07 ± 0.02b	0.18 ± 0.02c	0.21 ± 0.02a	0.04 ± 0.01	0.21 ± 0.02	14.71 ± 1.11abc	0.77 ± 0.04b
ST + RVM	8.83 ± 0.66bc	0.10 ± 0.01a	0.10 ± 0.02a	0.26 ± 0.02ab	0.13 ± 0.02b	0.05 ± 0.01	0.25 ± 0.03	9.89 ± 0.67cd	0.92 ± 0.04a
ST + RVZ	10.99 ± 1.12ab	0.09 ± 0.01a	0.16 ± 0.04a	0.27 ± 0.02a	0.17 ± 0.02ab	0.05 ± 0.01	0.23 ± 0.02	12.10 ± 1.13b	1.01 ± 0.06a
ST + RVZM	7.38 ± 0.52c	0.11 ± 0.01a	0.13 ± 0.04a	0.22 ± 0.02b	0.15 ± 0.02ab	0.05 ± 0.01	0.28 ± 0.03	8.48 ± 0.54d	0.98 ± 0.05a
P value	<0.01	<0.01	<0.01	<0.01	0.03	0.23	0.09	<0.01	<0.01

[a]Aphididae data from 2018 and 2019 only; 2017 data was excluded due to a difference in the data collection method used.
[b]All herbivores observed including Aphididae; data is from 2018 and 2019 only for the same reason mentioned above.
[c]All herbivores observed excluding Aphididae; data is from all 3 years (2017-2019).

Table 2.2. Data summary and results of statistical analyses for foliar natural enemies and ants. Mean ± SEM values and statistical significance of models for Araneae, *Orius*, "Parasitica", natural enemies (overall), natural enemies excluding "Parasitica", and Formicidae observed on squash foliage. Pairwise differences among treatments for each arthropod type are indicated by different letters following mean ± SEM values (emmeans; $\alpha = 0.05$).

Treatment	Araneae	*Orius*	"Parasitica"	Natural Enemies[a]	Natural Enemies (-P)[b]	Formicidae
FT + RVM	0.06 ± 0.01	0.12 ± 0.01	0.10 ± 0.01c	0.35 ± 0.02c	0.25 ± 0.02	0.16 ± 0.02a
ST + RVM	0.06 ± 0.01	0.13 ± 0.01	0.14 ± 0.01b	0.43 ± 0.02ab	0.29 ± 0.02	0.09 ± 0.01b
ST + RVZ	0.05 ± 0.01	0.11 ± 0.01	0.12 ± 0.01b	0.39 ± 0.02bc	0.26 ± 0.02	0.07 ± 0.01b
ST + RVZM	0.07 ± 0.01	0.12 ± 0.01	0.18 ± 0.02a	0.46 ± 0.02a	0.28 ± 0.02	0.09 ± 0.01b
P value	0.36	0.79	<0.01	<0.01	0.33	<0.01

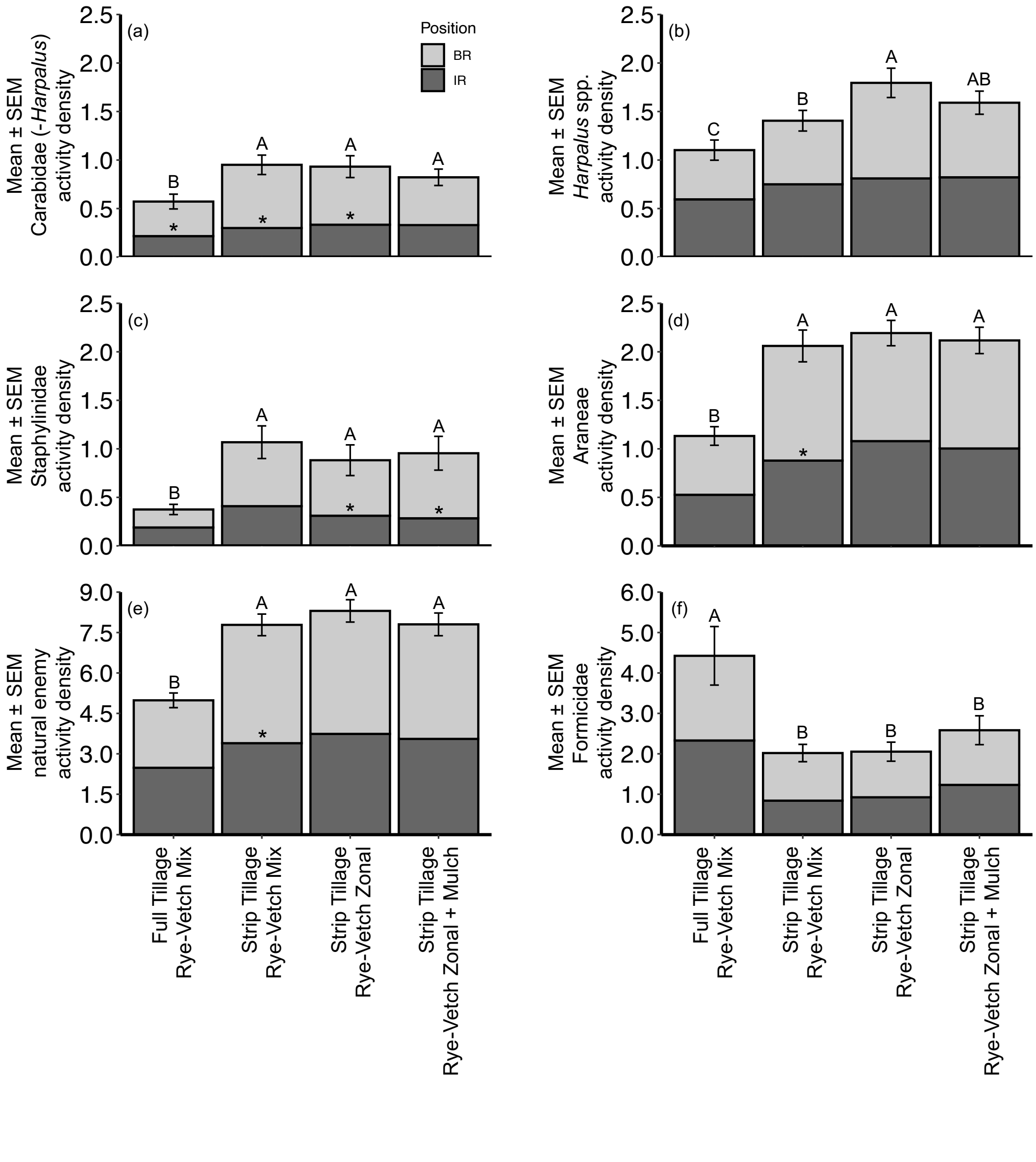
[a]All natural enemies observed including Araneae, *Orius*, and "Parasitica".
[b]All natural enemies excluding "Parasitica".

Epigeal natural enemy activity density. There were 10,548 arthropod natural enemies collected in pitfall traps from 2017 – 2019. Araneae (n = 1981) were the most frequently captured natural enemies followed by *Harpalus* spp. (Carabidae; n = 1556), Opiliones (n = 1052), Staphylinidae (n = 866), Carabidae other than *Harpalus* spp. (n = 865), Wasps (Apocrita; n = 212), Chilopoda (n = 80), Fanniidae larvae (n = 29), and Nabidae (n = 21). A total of 2925 Formicidae were also captured.

Overall, tillage affected epigeal natural enemy activity density but with the exception of *Harpalus* spp. (highest mean activity density in zonal cover crop treatments) cover crop segregation and added mulch did not impact activity density. Mean Araneae activity density was 1.8 – 1.9 times greater in strip tillage treatments than in full tillage ($\chi^2 = 71.64$, df = 3, $p < 0.01$; Figure 2.1d, and 1.0 – 1.3 times greater between crop rows than in rows ($\chi^2 = 8.86$, df = 1, $p < 0.01$; Figure 2.1d). There was no significant interaction between treatment and trap position ($\chi^2 = 4.16$, df = 3, $p = 0.24$). Mean *Harpalus* spp. activity density was 1.3 – 1.6 times greater in strip tillage treatments than in full tillage and zonal cover crop treatments had highest mean activity densities ($\chi^2 = 27.11$, df = 3, $p < 0.01$; Figure 2.1b). Mean *Harpalus* spp. activity density didn't significantly vary by trap position ($\chi^2 = 0.39$, df = 1, $p = 0.53$; Figure 2.1b) and there was no significant interaction between treatment and trap position ($\chi^2 = 3.17$, df = 3, $p = 0.37$). Mean Opiliones activity density did not significantly vary across treatments ($\chi^2 = 1.76$, df = 3, $p = 0.62$). However, mean activity density was slightly greater for Opiliones in crop rows compared to between rows for all treatments except strip tillage with rye-vetch mixed cover crop mulch ($\chi^2 = 5.16$, df = 1, $p = 0.02$) and there was a significant interaction between treatment and trap position ($\chi^2 = 8.32$, df = 3, $p = 0.04$). Mean activity density was 2.4 – 2.8 times greater for Staphylinidae ($\chi^2 = 43.27$, df = 3, $p < 0.01$; Figure 2.1c) in strip tillage treatments than in full tillage, and 1.0 –

2.4 times greater between crop rows than in rows ($\chi^2 = 16.83$, df = 1, $p < 0.01$; Figure 2.1c). There was no significant interaction between treatment and trap position ($\chi^2 = 4.65$, df = 3, $p = 0.20$). Mean Carabidae other than *Harpalus* spp. activity density was 1.4 – 1.7 times greater in strip tillage treatments than in full tillage ($\chi^2 = 15.56$, df = 3, $p < 0.01$; Figure 2.1a) and 1.5 – 2.2 times greater between rows than in rows ($\chi^2 = 31.48$, df = 1, $p < 0.01$; Figure 2.1a). There was no significant interaction between treatment and trap position ($\chi^2 = 2.27$, df = 3, $p = 0.52$). Mean overall natural enemy activity density was 1.6 – 1.7 times greater in strip tillage treatments than in full tillage ($\chi^2 = 92.58$, df = 3, $p < 0.01$; Figure 2.1e) and 1.0 – 1.3 times greater between rows than in rows ($\chi^2 = 11.52$, df = 1, $p < 0.01$; Figure 2.1e). There was no significant interaction between treatment and trap position ($\chi^2 = 2.97$, df = 3, $p = 0.40$). In contrast to natural enemies, mean Formicidae activity density was 1.7 – 2.2 times greater in full tillage than in strip tillage treatments ($\chi^2 = 38.09$, df = 3, $p < 0.01$; Figure 2.1f). Mean Formicidae activity density did not significantly vary by trap position ($\chi^2 = 1.28$, df = 1, $p = 0.26$; Figure 2.1f) and there was no significant interaction between treatment and trap position ($\chi^2 = 2.66$, df = 3, $p = 0.45$).

Figure 2.1. Epigeal natural enemy activity density. Mean ± SEM activity density of (a) Carabidae (-*Harpalus* spp.), (b) *Harpalus* spp., (c) Araneae, (d) natural enemies overall, and (e) Formicidae. Significant pairwise differences among treatments for each arthropod taxa are indicated by different letters (emmeans; $\alpha = 0.05$). Significant differences between trap positions (BR=between row; IR=in row) within treatments are indicated by asterisks.



Pollinator surveys. There a total of 2,117 bees observed visiting *Cucurbita* flowers from 2017 – 2019. *Bombus* spp. (Apidae; n = 1018) were the most frequently observed bees followed by *Apis mellifera* (Apidae; n = 528), *Eucera* (*Peponapis*) *pruinosa* (Apidae; n = 359), and other bees (n = 212). Mean *Bombus* spp. visitation was 1.1 – 1.2 times greater in strip tillage with rye-vetch mixed than in other treatments (χ^2 = 9.19, df = 3, p = 0.03; Table 2.3). Mean *A. mellifera* visitation was 1.1 – 1.4 times greater in strip tillage with rye-vetch zonal and added mulch than in other treatments (χ^2 = 9.92 df = 3, p = 0.02; Table 2.3). Mean *E. pruinosa* visitation was 1.1 – 1.5 times greater in strip tillage with rye-vetch zonal than in other treatments (χ^2 = 8.27, df = 3, p = 0.04; Table 2.3). Mean other bee visitation did not significantly vary across treatments (χ^2 = 2.35, df = 3, p = 0.50; Table 2.3). Overall bee visitation was 1.1 – 1.3 times greater in strip tillage with rye-vetch mixed than in other treatments (χ^2 = 14.93, df = 3, p < 0.01; Table 2.3). Mean abundance of Formicidae in flowers was 3.7 – 12.7 times greater in full tillage than in strip tillage treatments (χ^2 = 245.69, df = 3, p < 0.01; Table 2.3).

Table 2.3. Data summary and results of statistical analyses for flower visitation by bees. Mean ± SEM values and statistical significance of treatment for *E. pruinosa*, *A. mellifera*, *Bombus* spp., other bees, all bees (previous 4 bee types combined), and Formicidae observed per *Cucurbita* flower in 1 min. Pairwise differences among treatments for each bee type are indicated by different letters following mean ± SEM values (emmeans; α = 0.05).

Treatment	*E. pruinosa*	*A. mellifera*	*Bombus* spp.	Other bees[a]	All Bees[b]	Formicidae
FT + RVM	0.08 ± 0.01	0.12 ± 0.01b	0.25 ± 0.02b	0.05 ± 0.01	0.50 ± 0.02c	0.27 ± 0.07a
ST + RVM	0.11 ± 0.01	0.15 ± 0.01ab	0.31 ± 0.02a	0.06 ± 0.01	0.63 ± 0.03a	0.07 ± 0.04b
ST + RVZ	0.12 ± 0.01	0.13 ± 0.01ab	0.28 ± 0.02ab	0.06 ± 0.01	0.58 ± 0.03ab	0.06 ± 0.03b
ST + RVZM	0.08 ± 0.01	0.17 ± 0.02a	0.25 ± 0.02b	0.05 ± 0.01	0.55 ± 0.03bc	0.02 ± 0.01c
P value	0.04	0.02	0.03	0.50	<0.01	<0.01

[a]Other bees: carpenter, large (> 7mm), and small (< 7mm) bee flower visitation data combined.
[b]All bees: *E. pruinosa*, *A. mellifera*, *Bombus* spp., and other bee flower visitation data combined.

Discussion

In this study, foliar arthropod abundance was expected to be similar across tillage treatments due to their weak direct association with management that impacts the soil, compared to epigeal species. Contrary to this expectation, abundances of several foliar insect taxa responded to treatments. Aphididae was by far the most frequently observed foliar insect herbivore and their mean abundance was lower in strip tillage treatments than in full tillage. In addition, there were higher abundances of "Parasitica" observed in strip tillage treatments overall. This may have been due to an indirect impact of our treatments with greater nitrogen uptake by plants in strip tillage treatments compared to conventional tillage. Elevating plant nitrogen can increase size and fecundity of Aphididae and can sometimes result in higher rates of parasitism, emergence, size, longevity, and ratio of female adult "Parasitica" (39,40). However, because strip tillage has often been associated with reduced N mineralization compared to conventional tillage, it is also possible that plant nitrogen was higher in conventional tillage and promoted larger Aphididae populations while providing no benefit to "Parasitica". However, other factors may have mediated differences in both Aphididae and parasitoid wasp abundances. Other foliar natural enemies such as Araneae and *Orius*, did not significantly vary among full tillage and strip tillage treatments. This suggests that the lower abundance of "Parasitica" in full tillage may have contributed to elevated Aphididae abundance. Additionally, many arthropod pests are r-selected and tend to be successful in environments with higher disturbance (5,41). Formicidae was also significantly more abundant in full tillage which may have contributed to increased Aphididae abundance by tending them and actively excluding aphidophagous predators and/or other herbivorous arthropods (42–44).

In contrast to Aphididae, *Anasa tristis* egg masses and nymphs, Cicadellidae, *D. undecimpunctata,* and *A. vittatum* were more abundant in strip tillage treatments than in full tillage (differences not statistically significant for latter 2 species) despite increased abundance of overall natural enemies in strip tillage. As previously noted, "Parasitica" appear to account for the overall increase in natural enemies in strip tilled plots. Parasitoids such as *Eumicrosoma* spp. (Hymenoptera: Platygastridae) and *Trichopoda pennipes* (Diptera: Tachinidae) can be effective at controlling herbivore pests such as *A. tristis* (45). However, considering the increased abundance of *A. tristis* in strip tillage treatments despite increased parasitoid abundance, it may be that species relevant for *A. tristis* control were not among the more common parasitoids present. Additionally, Araneae, *Orius*, and natural enemies overall excluding "Parasitica" did not vary in abundance among treatments. This suggests that factors other than natural enemies such as increased structural habitat complexity (5), host plant quality (46), weed densities (47–49), refuge and microclimatic conditions (46,49,50), or some other emergent property of strip tillage treatments may have led to differences in abundance of foliar herbivores.

As predicted, activity density was greater in strip tillage treatments than in full tillage for all epigeal natural enemies except Opiliones. This is consistent with several previous studies indicating that reduced tillage methods like strip tillage can significantly increase beneficial arthropods (5,6,8,11,12,17–19), although see counterexamples (7,51,52). *Harpalus* spp. such as *Harpalus pensylvanicus* (Carabidae), important omnivores in many agricultural systems, were the most frequently captured beetle taxa and were more abundant in strip tillage than full tillage. Previous research has also demonstrated a positive relationship between *H. pensylvanicus* activity density and cover crops, as well as negative impacts of disturbance and the absence of cover crops (53). Similarly, mean activity density of Carabidae other than *Harpalus* spp., and Staphylinidae were also significantly higher in strip tillage than full tillage. This suggests that strip tillage can support larger populations of beneficial epigeal beetles (11) that can be important predators of pest arthropods and weed seeds (54). Furthermore, mean Araneae activity density was significantly higher in strip tillage than full tillage providing additional evidence that strip tillage can increase activity density of many generalist predators (12) which has the potential to improve biological control.

Although natural enemy activity density overall was significantly higher in strip tillage than full tillage, this was not accompanied by decreased abundance of foliar herbivores with the exception of Aphididae. It is possible that strip tillage created conditions that were beneficial for both foliar herbivores and epigeal natural enemies, and that epigeal natural enemy abundance was relatively inconsequential for foliar herbivores due to a spatial separation. This highlights the importance of establishing populations of natural enemies that control the specific types of pests that are present, rather than overall natural enemy diversity (6,55). However, several important natural enemies of major *Cucurbita* pests were captured significantly more in strip tillage treatments overall. Therefore, it may also be that herbivores such as *A. tristis* were more abundant in strip tillage due to beneficial conditions despite being predated upon/parasitized by natural enemies. In contrast to epigeal natural enemies, Formicidae activity density was significantly greater in full tillage than in strip tillage treatments. This observation coincides with greater foliar Formicidae abundance and further suggests that Formicidae may have played a role in decreased abundance of other foliar arthropods in full tillage via removal/exclusion (42–44).

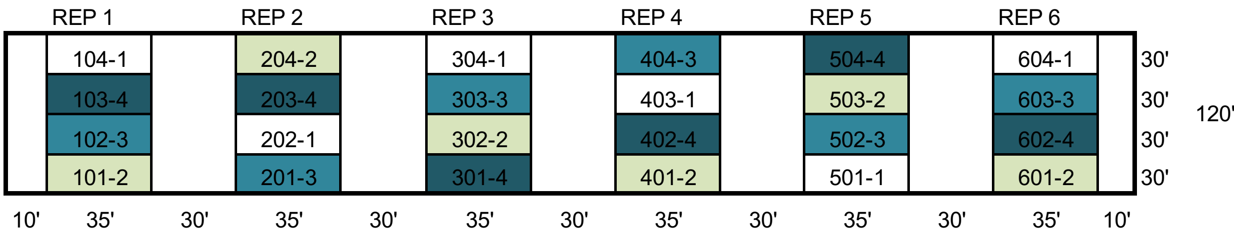
Bee flower visitation was not expected to vary among treatments. Non-fossorial bees (e.g. *A. mellifera*), do not interact with the soil surface, therefore, tillage and/or ground cover were expected to be inconsequential. Flower visitation by fossorial species, *E. pruinosa* and *Bombus* spp., was not expected to vary due to the close proximity of experimental plots which would allow them to visit flowers even in plots they may not have preferred to nest in, and *Bombus* spp. likely nest and forage on floral alternatives outside the field (56). Nonetheless, flower visitation by *A. mellifera*, *Bombus* spp. and bees overall varied among treatments. It is not likely that treatments directly caused these differences, but they may have indirectly impacted bee visitation through increased floral abundance and/or floral resources. However, tillage practices in squash systems are likely to disturb nests of fossorial bee species, particularly *E. pruinosa* which nests within squash fields, impacting overall abundance and flower visitation frequency over time (21,27,28).

Similar to foliar and activity density observations, Formicidae abundance in flowers was significantly higher in full tillage than in strip tillage treatments, particularly when supplemental mulch was added. Interestingly, these findings contrast with those of several previous studies in which Formicidae abundance was higher in reduced tillage or no-till compared to full tillage (57–59). This suggests that Formicidae may have been attracted to plants in full tillage that already supported high Aphididae abundance due to increased plant nitrogen (no data collected), reduced natural enemy pressure, or a combination of factors that promoted larger populations of Aphididae compared to strip tillage treatments. Formicidae may have subsequently influenced Aphididae population dynamics in full tillage via tending behaviors.

In summary, reduced tillage was found to support higher abundances of some foliar herbivores. However, Aphididae which accounted for the majority of foliar herbivores was significantly more abundant in full tillage overall, and cover crop segregation and/or additional rye mulch between crop rows impacted abundance. Other factors such as squash plant nutrition, level of habitat disturbance, decreased natural enemy abundance, and tending by Formicidae may have also contributed to these patterns. “Parasitica” were the only foliar natural enemy found to vary in abundance among treatments and they were more frequently observed in strip tillage overall and highest abundance in strip tillage + rye-vetch zonal and added rye mulch. This may have been due to increased prey abundance and/or resources provided by plant residues between crop rows. Epigeal natural enemies were also more abundant in strip tillage treatments overall and cover crop segregation was mostly inconsequential suggesting that reduced tillage practices can improve biological control despite cover crop planting methods. In this study, strip tillage did not significantly increase flower visitation by *E. pruinosa*, an agriculturally important specialist pollinator of squash. However, it is likely that these bees respond positively to lower disturbance tillage practices such as strip tillage. Overall, the results of this study and previous research indicate that reduced tillage can increase natural enemy and potentially pollinator abundance, leading to improved biological control and pollination compared to full tillage systems.

APPENDIX

Figure S2.1. Experimental field layout and dimensions. Each year (2017-2019) plots were arranged in a randomized complete block design with each treatment replicated 6 times for a total of 24 plots per field.



Treatment	Tillage	Cover Crop
1	Chisel	Rye-vetch mixed
2	Strip	Rye-vetch mixed
3	Strip	Rye-vetch zonal
4	Strip	Rye-vetch zonal + mulch

Table S2.1. Dates of major field operations in 2017, 2018, and 2019.

Field Operation	Date			
	2016	2017	2018	2019
Cover crop killed in full tillage		25 April	26 April	23 April
Full tillage treatment cultivated		17 May	4 June	16 May
Strip tillage		13 June	18 June	26 June
Squash planted		14 June	18 June	27 June
Rye mulch spread in additional mulch treatment		15 June	20 June	27 June
Cultivation		22,29 June; 17 July	7 Aug	3,12,19 July
Summer squash harvested		NA	17,20,22 Aug	NA
Winter squash harvested		6 Sep	27 Sep	24 Sep; 5 Oct
Rye and vetch planted	6 Sep	5 Sep	21 Sep	NA

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LITERATURE CITED

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CHAPTER 3: Citizen science improves our understanding of the impact of soil management on wild pollinator abundance in agroecosystems

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Abstract

Native bees provide essential pollination services in both natural and managed ecosystems. However, declines in native bee species highlight the need for increased understanding of land management methods that can promote healthy, persistent populations and diverse communities. This can be challenging and costly using traditional scientific methods, but citizen science can overcome many limitations. In this study, we examined the distribution and abundance of an agriculturally important wild bee species, the squash bee (*Eucera* (*Peponapis*) *pruinosa*, Hymenoptera: Apidae). They are ground nesting, specialist bees that depend on cultivated varieties of *Cucurbita* (squash, pumpkins, gourds). The intimate relationship between squash bees and their host plants suggests that they are likely sensitive to farm management practices, particularly those that disturb the soil. In this study, citizen scientists across Michigan used a survey to submit field management and bee observation data. Survey results indicated that squash bees occupy a wide

geographic range and are more abundant in farms with reduced soil disturbance. Citizen science provided an inexpensive and effective method for examining impacts of farm management practices on squash bees and could be a valuable tool for monitoring and conserving other native pollinators.

Introduction

Pollinators are important in both natural and managed ecosystems to maintain plant genetic diversity, contribute to ecosystem stability, and sustain crop production (1). Of the most commonly produced crops globally, 35% rely on or benefit from animal pollination which is provided mostly by insects such as western European honey bees (*Apis mellifera*, Hymenoptera: Apidae) and a wide variety of wild, native bees (1,2). Honey bees are the most prolific pollinators of pollinator dependent crops (3), however annual losses of managed honey bees can currently reach as high as 50% due to a suite of factors such as exposure to pesticides, reduced forage availability, parasites, and diseases (4,5). As a result, researchers are investigating the role of wild bees as crop pollinators, which are declining due to human disturbances such as habitat loss/fragmentation (6), landscape simplification (7), and increased pesticide use (8). Management practices that increase abundance and species richness of native bees can ameliorate crop pollination deficiencies (9), especially for crops that are more effectively pollinated by native bees (10–15).

Studying changes in insect populations is often challenging (16,17), and in order to collect baseline abundance and distribution data, insect monitoring has in some cases turned to citizen science as an effective method for gathering large datasets across broad geographic areas with low costs compared to traditional methods (18,19). Although citizen science can suffer from limitations such as data accuracy and participant retention, these issues can be negated with proper planning and participant training as demonstrated by many successful citizen science projects. For example, citizen science can be an effective method for monitoring native bees (20–22). Citizen scientist observations can describe bee species dynamics as well as specimens collected by professional researchers (23), provide data on specific aspects of bee biology, including the nesting habits of solitary bee species (24), and the impacts of flowers and surrounding natural land cover on plant-bee interactions (25). Participants in pollinator citizen science projects often volunteer because of a desire to learn about bees and to contribute to science (26). This provides opportunities for large-scale, cost-effective studies that simultaneously allow scientists to educate the public about ecological issues such as the loss of biodiversity. Actively engaging with the public through hands-on experiences provides more impactful education that can enhance learning and inspire continued action (27).

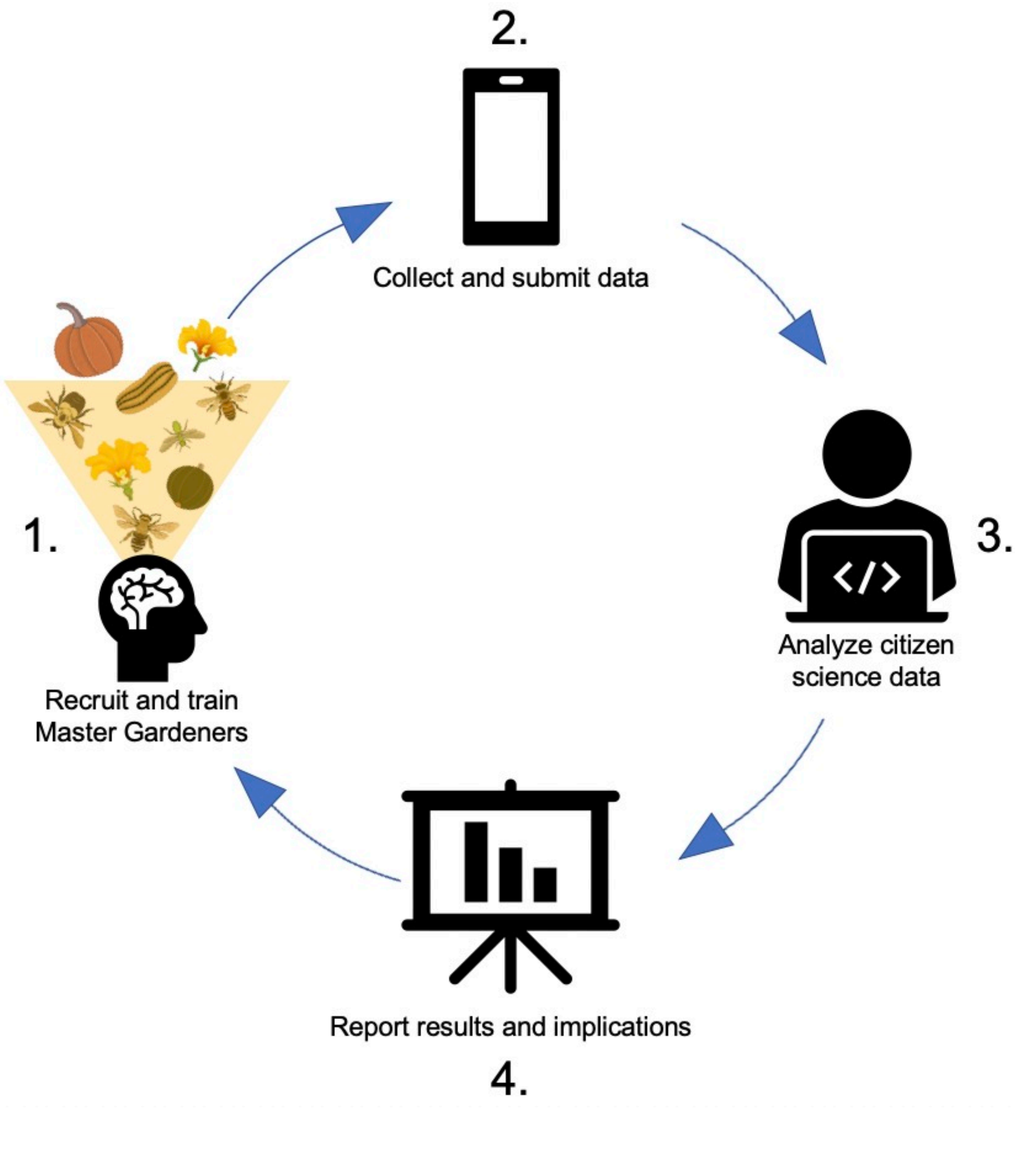
Our study focused on squash bees (*Eucera* (*Peponapis*) *pruinosa*, Hymenoptera: Apidae), an important specialist pollinator of *Cucurbita* (e.g. pumpkins, squash, gourds). This plant genus is dependent on pollination and is an ideal system to promote native bees because of their mutualism with squash bees. These specialist bees forage for nectar and pollen on *Cucurbita* flowers, rest within closed flowers, and excavate nests in the soil in and around *Cucurbita* plants (28–30). The intimate relationship between squash bees and their host plants indicates that squash bees are potentially sensitive to farm management practices, especially those that manipulate the soil. However, studies examining the relationship between squash bees and farm management practices have produced differing results. For example, tillage can destroy squash bee nests, reduce the number of surviving offspring, alter sex ratios and emergence timing (31), and reduce squash bee flower visitation (32). Conversely, another study observed similar adult squash bee abundance in tilled and untilled pumpkin fields, and squash bees preferred to nest in irrigated soil near host plants regardless of whether or not the soil was tilled (33). However, more recent findings suggest that squash bees prefer to nest in tilled soil (34). Mulching is another ground management practice commonly used in *Cucurbita* production that may deter or inhibit squash bee nesting. Although, previous attempts to compare squash bee nesting frequency in bare soil and soil covered by different types of mulch were inconclusive due to low sample size (35).

Although multiple studies have examined the impacts of farm management on squash bees, the scope of investigation has often been limited to one management practice at a time, sample sizes have been relatively low, and results have often been mixed (31–34). Here, we used a citizen science survey to determine how squash bee abundance varies according to multiple farm management practices including tillage type, depth, and mulch, and ascertain the distribution of squash bees in Michigan. Citizen science allowed us to increase sampling while providing opportunities to spread awareness among the public about the importance of squash bees which may pollinate about two-thirds of squash grown commercially in the United States (36). Previous research indicates that citizen science projects are more successful if the participants have prior interest in the subject matter (26,37,38) thus we recruited Michigan State University (MSU) Extension Master Gardeners because of their interest in agriculture, their level of scientific knowledge, and their commitment to educate others in their communities.

Materials and Methods

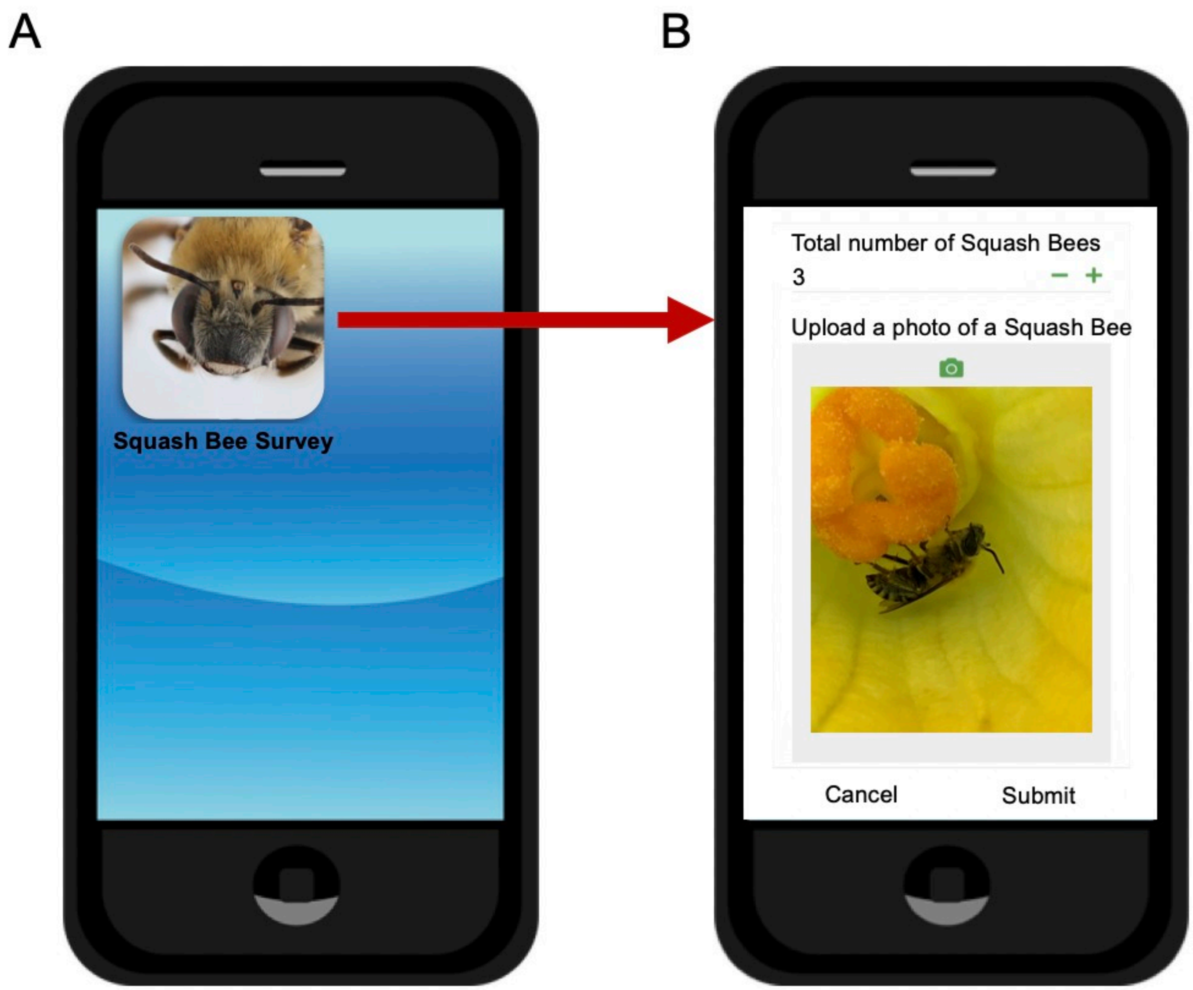
Citizen scientist recruiting and training. Master Gardeners were contacted by the program coordinator via email and recruited to participate in our squash bee survey. To train Master Gardeners we invited them to webinar presentations held in June 2017 and 2018 where the methods, project goals, and preliminary results were discussed. Educational workshops (~3h) were held for participants at several locations throughout Michigan in July 2017 (Novi, MI: July 20; Holland, MI: July 21), 2018 (Mason, MI: July 16; Novi, MI: July 20; Grand Rapids, MI: July 25; Lincoln, MI: July 27), and 2019 (Novi, MI: July 18; Grand Rapids, MI: July 25). Each workshop included a classroom presentation during which participants were taught about the biology of cucurbit flowers, squash bees, bee identification, the importance of native pollinators, and the methods for collecting data and submitting surveys. Master Gardeners were provided with supplemental educational materials including a factsheet with information pertaining to the pollination system of cucurbits and their relationship with squash bees, and a brief bee identification guide. Presentations were followed by an outdoor session in a squash garden or farm where participants practiced identifying squash bees at flowers and filling out the squash bee survey (Figure 3.1).

Figure 3.1. Conceptual diagram illustrating the process of training, data collection, data analysis, and reporting for this citizen science project. Michigan State University Extension Master Gardeners were taught about the pollination system of cucurbits, the importance of squash bees, and how to identify bees visiting squash flowers (1). Master Gardeners collected data on squash bees which they submitted using the Squash Bee Survey smartphone application (2). Surveys could also be submitted through a web browser or via paper copies. Data was analyzed and verified with photos submitted by participants (3). Results were shared with Master Gardeners via webinars, presentations, and a factsheet (4).



Squash bee survey and observation protocol. The primary method used for data collection and survey submission was the Squash Bee Survey smartphone application (Figure 3.2) developed in the MSU Vegetable Entomology Lab using Google forms (39) and AppSheet (40). Surveys were also made available for participants in printable PDF and web browser versions accessible through the MSU Vegetable Entomology website (https://vegetable.ent.msu.edu). In each survey, Master Gardeners provided the last four digits of their phone number as unique, confidential identifiers, and were asked several questions pertaining to the location and management of the farm where they conducted surveys (Figure S3.1). Tillage type was one of the primary factors of interest in our study and participants could select no tillage, reduced tillage, or full tillage. No tillage is characterized by a lack of soil disturbance between harvesting and planting crops resulting in the presence of crop stubble or residues. Reduced tillage (a.k.a. conservation tillage) is defined by lower tillage intensity resulting in the retention of some crop residues on the soil surface. Both of these methods help to prevent soil erosion, increase water retention, and conserve energy resources. Full tillage (a.k.a. conventional tillage) uses cultivation (e.g. ploughing, harrowing) as the primary means of weed control and seedbed preparation resulting in a loose soil surface and lack of plant residues on the soil surface (41). Tillage depth (0 cm, 3-14 cm, 15-25 cm), and mulching practices (none, plant material, plastic) were also of interest, and participants selected all categories that represented the practices used in a particular crop field. Surveys submitted electronically via the smartphone application or web browser option were automatically entered into a Google Sheets spreadsheet with a timestamp and stored in Google Drive via AppSheet (40). Printed surveys received by mail were entered into the spreadsheet manually upon receipt. There was no limit to the number of surveys each participant could submit.

Figure 3.2. **Squash bee survey smartphone application installed on a smartphone (A). Screenshot from the squash bee survey (B).** The smartphone application was the primary platform for Master Gardeners to submit information about the bees observed in cucurbit flowers and management practices used on farms.



Master Gardeners were asked to conduct bee surveys on cucurbit flowers in the morning (~07:00 – 12:00) while squash bees were active, on sunny days with no more than light winds and air temperatures of at least 21 ºC. For each survey, five separate cucurbit flowers were observed for 1 min each for a total of 5 min of observations, and the numbers of squash bees, honey bees, bumble bees, and other bees (any other type of bee) observed visiting the flowers were summed and recorded. At the end of the survey, participants were asked to take a picture of a bee that they identified as a squash bee to be submitted with their electronic data. These photos were used to assess participants' squash bee identification accuracy; photo verifications were not performed for surveys that were submitted by mail. IRB Number is x17-688e; i054192; this survey was deemed exempt and was not subjected to review by an institutional review board or ethics committee.

Statistical analysis. Squash bee observations were mapped by county and compared to previous county records of this species (42). The number of surveys and the number of different people participating were calculated for each year. The proportions of squash bees, honey bees, bumble bees, and other bees were calculated to identify the most common type of bee observed during surveys. Generalized Linear Mixed Models using Laplace approximation and negative binomial distribution were used with the 'glmmadmb' function in the 'glmmADMB' package (43) to determine the effects of various management practices on the number of squash bees observed during surveys. Each model contained a single fixed effect (tillage type, tillage depth, mulch, irrigation, insecticides, farm area, farm area devoted to cucurbits; S1 Fig) with date nested within county as random effects. Models were individually compared to a null model using the 'anova' function in the 'stats' package (44). The 'AICctab' function in the 'bbmle' package (45) was used to compare models based on AIC (Table 1). The 'emmeans' function in the 'emmeans' package

(46) with the 'fdr' p-adjustment method was used to determine pairwise differences between factor categories for models that differed significantly from the null model.

Kruskal-Wallis tests were performed using the 'kruskal.test' function in the 'stats' package (44) to determine the effects of the previously mentioned management practices on honey bees, bumble bees, and other bees ($\alpha = 0.05$). This analytical method was used for these bee categories due to non-convergence of the generalized linear mixed model method used for squash bee analyses. Surveys submitted with incorrectly identified squash bee photos, factor categories with less than 5 responses, and numeric outliers (bee counts greater than the third quartile plus 1.5 times the interquartile range for each respective bee category) were excluded from analyses. Squash bees are known to forage solely on species in the genus *Cucurbita* (29,47) consequently, surveys observing only cucumber or melon flowers (*Cucumis*) were also excluded from analyses. When Kruskal-Wallis tests were significant, post hoc analyses were conducted using the 'dunn.test' function in the 'dunn.test' (48) package with the 'holm' p-adjustment method to control family-wise error rates, to determine differences in bee abundances among groups for factors with more than 2 groups ($\alpha < 0.05$). All analyses were performed using R version 3.5.1 (44).

Results

Number of surveys received per county and types of bees observed. Of the 291 surveys received, 276 (2017: 56 electronic surveys; 2018: 70 electronic and 7 print surveys; 2019: 101 electronic and 42 print surveys) were used for analysis from 21 Michigan counties and 1 Indiana county. Eleven out of 21 Michigan counties reported observing squash bees (Figure 3.3A). Of the 11 Michigan counties that reported squash bees, only four overlapped with historical reports (42), and the remaining seven provide new county records. A total of 59 people participated in this study

(Figure 3.3B), 87% of whom submitted observations from organic farms, with some participating in multiple years (2017: 19 different participants; 2018: 27 different participants; 2019: 23 different participants). Out of all surveys, 48% included photos, 90% of which were correctly identified as squash bees. Squash bees accounted for 51% of bees reported over the combined 3 years (Figure 3.4) and were the most common type of bee reported in each year (2017: 67%, 2018: 33%, 2019: 54%).

Figure 3.3. Squash bees reported in Michigan counties. Counties from which squash bee surveys were received, the number of surveys submitted from each county, and previous county records of squash bees (A). The number of different participants per county (B). A total of 5 surveys were received from 2 different participants in Floyd Co. Indiana, both of which reported squash bees (Floyd Co. Indiana not displayed on map).

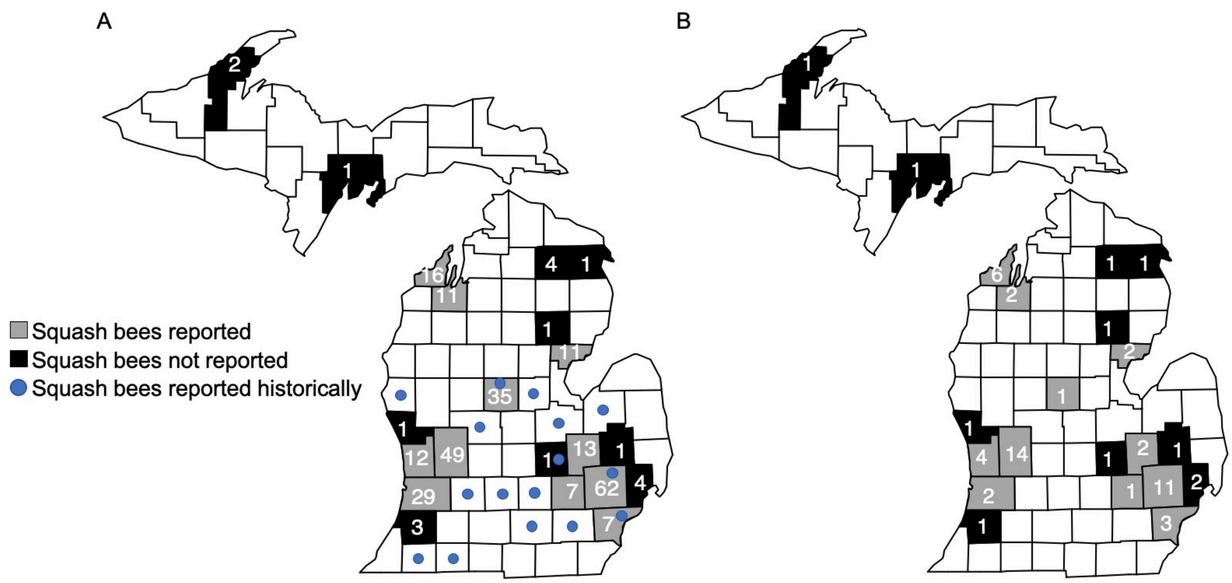
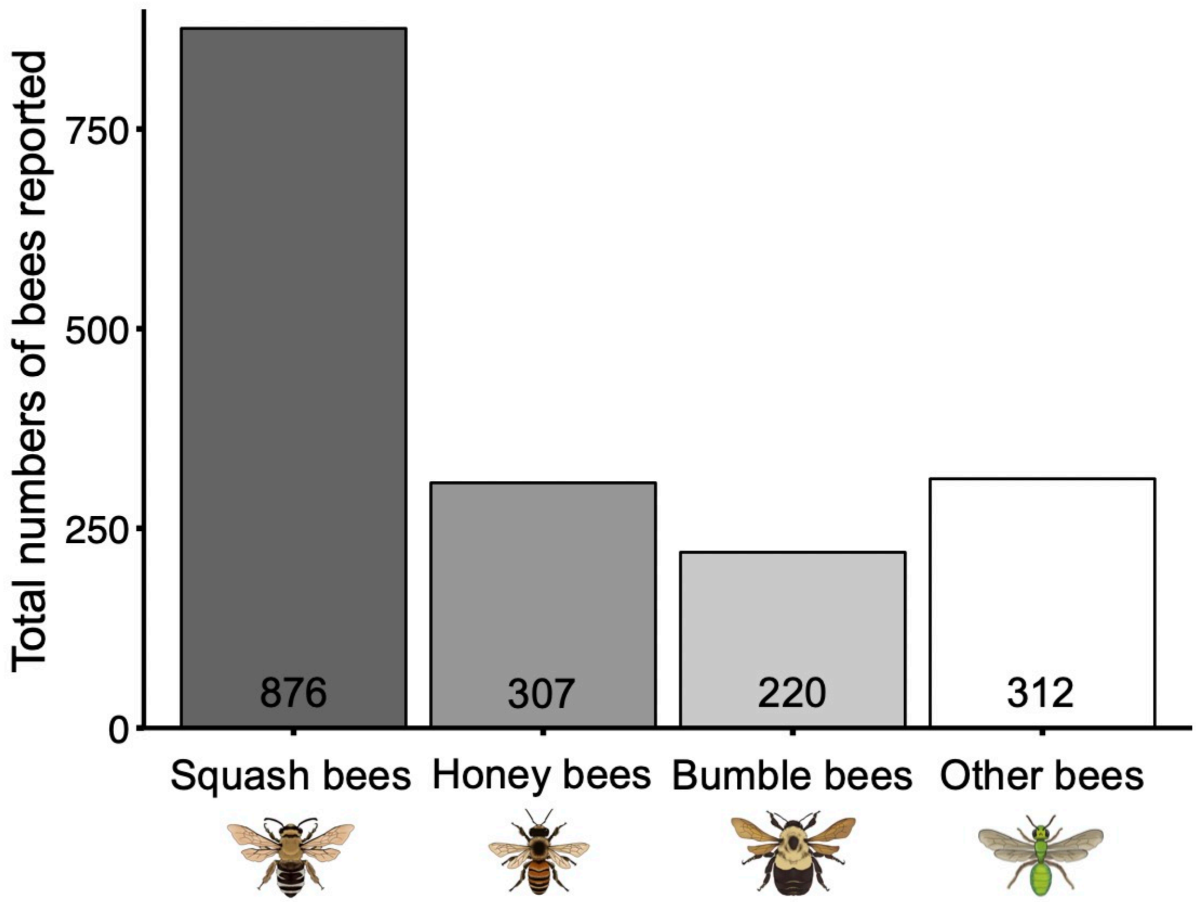
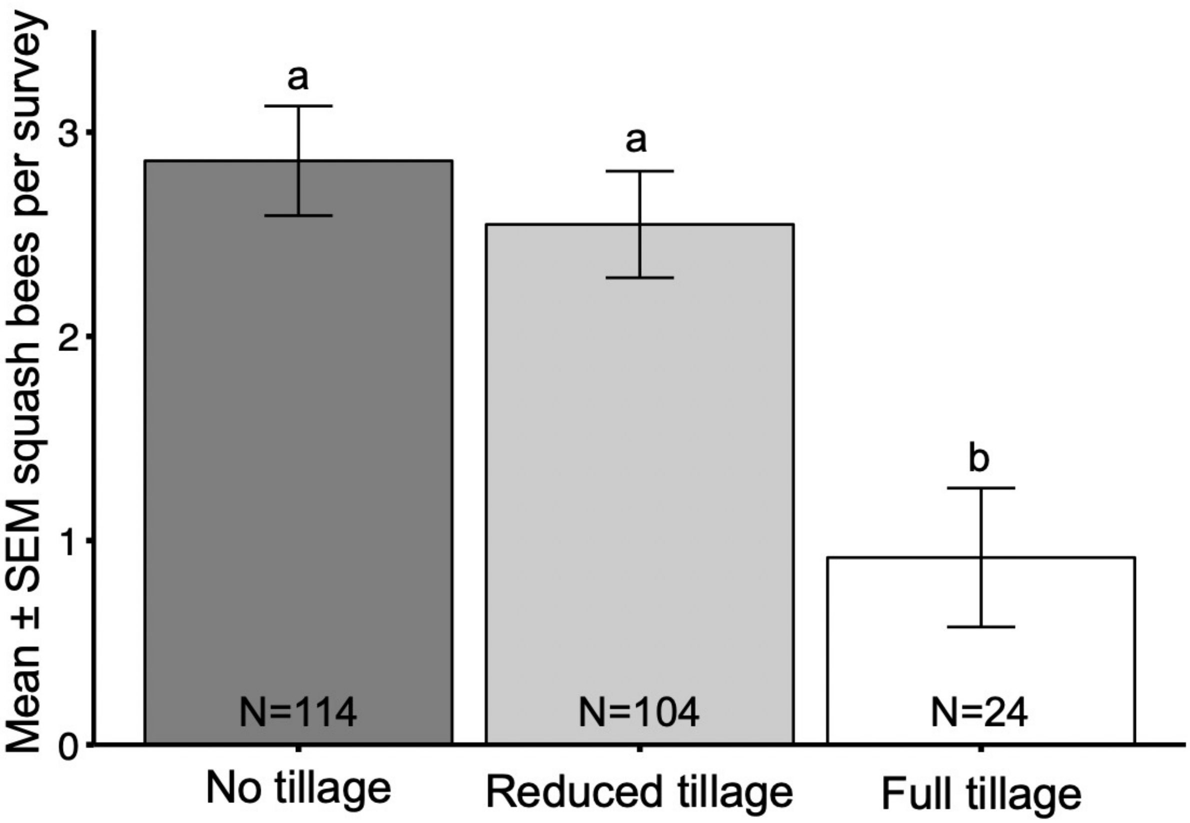


Figure 3.4. Types of bees reported by citizen scientists. Total numbers of squash bees, honey bees, bumble bees, and other bees observed by citizen scientists visiting cucurbit flowers over the summers of 2017, 2018, and 2019 combined.



Effects of management practices on squash bees. The number of squash bee visits reported per survey varied according to tillage type ($\chi^2 = 11.18$, df = 2, $p < 0.01$; Figure 3.5). The mean number of squash bees reported in farms using no tillage (mean = 2.86 ± 0.27 (SE)) was more than 3 times greater than the mean number in full tillage (mean = 0.92 ± 0.34 (SE); p = 0.02), but only slightly greater than in reduced tillage (mean = 2.55 ± 0.26 (SE); p = 0.78). The mean number of squash bees reported in reduced tillage farms was about 3 times greater than in those using full tillage (p = 0.02).

Figure 3.5. Squash bees by tillage type. Mean ± SEM number of squash bees reported in a squash bee survey conducted by citizen scientists in farms using different types of tillage, combined for 3 years (2017, 2018, 2019).



Tillage depth did not affect squash bee visitation ($\chi^2 = 2.91$, df = 2, p = 0.23; Figure 3.6). Likewise, squash bee visitation did not significantly vary by mulch type ($\chi^2 = 5.98$, df = 3, p = 0.11) (Figure 3.7). However, the mean number of squash bees reported in the 'Plastic' (mean = 4.50 ± 1.67 (SE)) and 'Plastic + Plant Material' (mean = 3.86 ± 1.18 (SE)) groups were more than 1.5 times greater than in both the 'None' (mean = 2.46 ± 0.30 (SE)) and 'Plant Material' (mean = 2.36 ± 0.20 (SE)) groups.

Figure 3.6. Squash bees by tillage depth. Mean ± SEM number of squash bees reported in a squash bee survey conducted by citizen scientists in farms using different tillage depth (cm), combined for 3 years (2017, 2018, 2019).

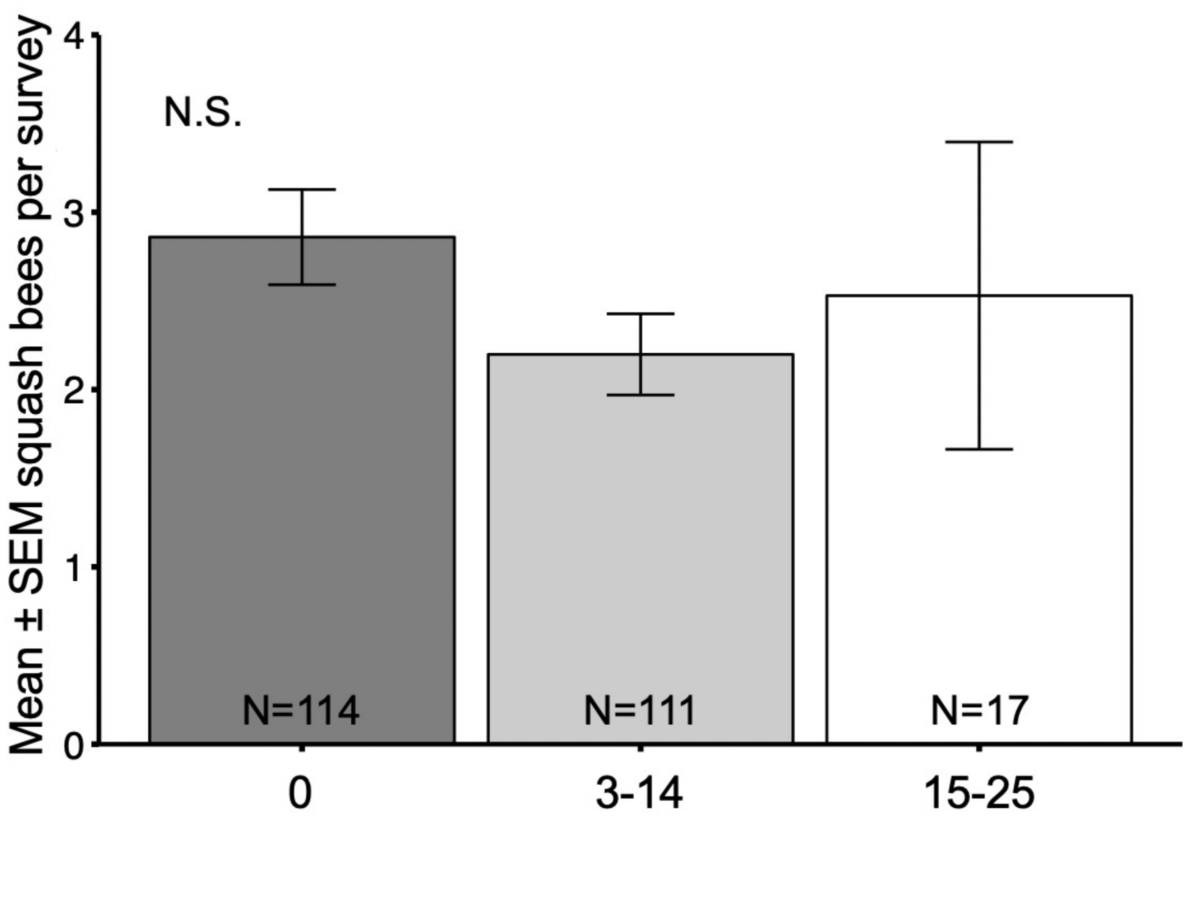
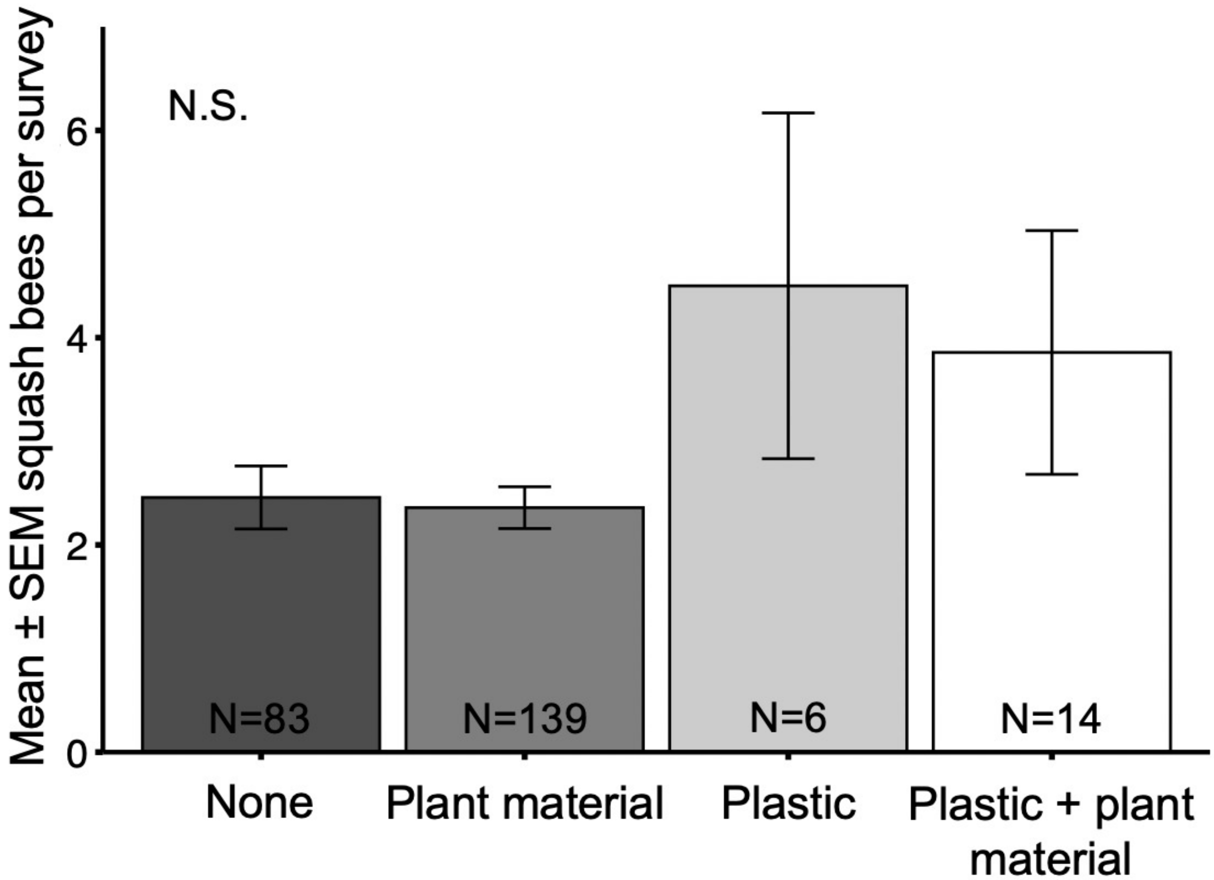


Figure 3.7. Squash bees by mulch type. Mean ± SEM number of squash bees reported in a squash bee survey conducted by citizen scientists in farms using different mulching practices, combined for 3 years (2017, 2018, 2019).



Squash bee visitation was not affected by insecticides ($\chi^2 = 2.53$, df = 2, p = 0.28), irrigation ($\chi^2 = 3.97$, df = 2, p = 0.14), the type of vine crop observed ($\chi^2 = 5.68$, df = 3, p = 0.13), or amount of area devoted to cucurbit growth ($\chi^2 = 2.10$, df = 1, p = 0.15). Based on AIC model comparison, tillage type (ΔAICc = 0.0, df = 6, weight = 0.858) explains the patterns in squash bee visitation better than other analyzed factors (Table 3.1).

Table 3.1. AIC comparisons of GLMMs testing the effects of different cucurbit management practices in a citizen science survey, 2017-2019. Different fixed effects were compared using the difference in AIC (ΔAICc) between the model of the lowest AIC and all other models. AIC weight indicates the probability that a model best describes the data. The model with the lowest ΔAICc and the highest AIC weight is assumed to better fit the data than other models.

Fixed Effect	ΔAICc	df	Weight
Tillage Type	0.0	6	0.858
Cucurbit Area	7.0	5	0.026
Null	7.0	4	0.026
Irrigation	7.2	6	0.023
Mulch	7.3	7	0.022
Vine crop observed	7.6	7	0.019
Tillage depth	8.3	6	0.014
Insecticides	8.7	6	0.011

Effects of management practices on honey bees, bumble bees, and other bees. No relationship was observed between honey bee visitation and tillage type (χ^2 = 1.40, df = 2, p = 0.50), tillage depth (χ^2 = 1.05, df = 2, p = 0.59), insecticides (χ^2 = 1.13, df = 2, p = 0.57), irrigation (χ^2 = 0.30, df = 2, p = 0. 86), or the type of vine crop observed (χ^2 = 6.50, df = 3, p = 0.09; Table S3.1). However, mulch affected honey bee visitation (χ^2 = 12.02, df = 3, p = 0.01). The mean number of honey bees reported in farms using plant material mulch (mean = 0.44 ± 0.06 (SE)) was more than 3 times greater than when mulch (mean = 0.13 ± 0.04 (SE)) was not present ($p < 0.01$). No other significant differences were observed among mulch types, however, the mean number of honey bees observed when plant material and plastic (mean = 0.33 ± 0.14 (SE)) or plastic alone (mean = 0.40 ± 0.40 (SE)) were present was approximately 2.5-3 times greater than when mulch was not present (Table S3.1). Average honey bee visitation was also 2 times greater when cucurbit area was greater than 0.4 hectares (mean = 0.64 ± 0.24 (SE)) compared to less than 0.4 hectares (mean = 0.31 ± 0.04 (SE); χ^2 = 2.92, df = 1, p = 0.09; Table S3.1). No relationship was observed between bumble bee visitation and tillage type (χ^2 = 2.96, df = 2, p = 0.23), tillage depth (χ^2 = 5.05, df = 2, p = 0.08), mulch (χ^2 = 1.56, df = 3, p = 0.67), insecticides (χ^2 = 3.62, df = 2, p = 0.16), or irrigation (χ^2 = 4.13, df = 2, p = 0.13). Interestingly, bumble bee visitation was 3.5 times greater when cucurbit area was less than 0.4 hectares (mean = 0.54 ± 0.05 (SE)) compared to greater than 0.4 hectares (mean = 0.15 ± 0.10 (SE); χ^2 = 3.60, df = 1, p = 0.06). Bumble bee visitation also varied by the type of vine crop observed (χ^2 = 20.56, df = 3, $p < 0.01$). The mean number of bumble bees observed visiting winter squash varieties (*C. pepo,* mean = 0.80 ± 0.09 (SE)) was more than two times greater than when mixed varieties (combination of *C. pepo* and *Cucumis*; mean = 0.35 ± 0.12 (SE); $p < 0.01$) or summer squash varieties alone (*C. pepo,* mean = 0.36 ± 0.06 (SE); p <

0.01) were observed. No other significant differences were found among vine crop types; however, the mean number of bumble bee visits in surveys where summer and winter squash flowers were observed together (mean = 0.70 ± 0.18 (SE)) was approximately 2 times greater than when mixed or summer squash flowers alone were observed. Other bee visitation did not vary by mulch (χ^2 = 4.26, df = 3, p = 0.23), insecticides (χ^2 = 4.77, df = 2, p = 0.09), irrigation (χ^2 = 0.58, df = 2, p = 0.75), or the type of vine crop observed (χ^2 = 6.07, df = 3, p = 0.11; Table S3.1). Although, visitation by other bees varied according to tillage type (χ^2 = 11.04, df = 2, p < 0.01) with the mean number of other bees observed in farms using no tillage (mean = 1.33 ± 0.14 (SE)) and full tillage (mean = 1.36 ± 0.32 (SE)) being approximately 2 times greater than in farms using reduced tillage (mean = 0.68 ± 0.09 (SE); p < 0.01; p = 0.16). Tillage depth also affected other bee visitation (χ^2 = 8.66, df = 2, p = 0.01) with the mean number of other bee visits in farms using no tillage (mean = 1.33 ± 0.14 (SE)) being around 1.5 times greater than in farms with 3-15 cm tillage depth (mean = 0.78 ± 0.10 (SE); p = 0.01) or 15-25 cm (mean = 0.96 ± 0.24 (SE); p = 0.71; Table S3.1).

Discussion

This study was the first to successfully use citizen science to gather a large dataset to examine an agriculturally significant native bee's distribution and to determine how their flower visitation frequency varies according to crop management. Some of the successes of our project are due to identifying an appropriate target audience for involvement with the project, a thorough volunteer education process, simplicity of the survey protocol, and ease of data submission. These allowed us to not only sustain the project for 3 years, a longer duration than many other pollinator citizen science projects (23,49,50), but increase data collection in each project year. Pollinator citizen science projects with more complex, time consuming experiments tend to have problems retaining or increasing participant numbers (51), which was one of the reasons for keeping our protocols relatively simple. For example, we simplified the data collection methods by asking participants to count bees at flowers instead of using bee nest count data which would have been a more direct measure of the impact of soil management practices, but this would have required more time and effort from citizen scientists. Furthermore, nesting data would be prone to error and difficult for us to verify via photos. Since squash bees spend the majority of their time in *Cucurbita* flowers and tend to nest close to their host plants (33), flower visitation frequency is likely directly related to overall squash bee abundance. In addition to adjusting sampling methods to the ability level of participants, incorporating technological advances were also important for success as has been demonstrated in previous citizen science studies (52). We used smartphones as our primary method for data collection and photo submission as many of our citizen scientists were familiar with this technology. To keep the survey relatively short, we omitted some questions that would have provided us with valuable data, for example, recording soil-type, time of day, weather conditions, flower sex, and flower abundance would have allowed us to answer additional questions. Despite these limitations, we find that citizen scientists are eager to be involved with these types of data collection efforts and that they can contribute valuable information to science.

Our survey focused mainly on soil management methods because of the need to better understand their intimate interactions with ground-nesting bees. As soil conservation methods, such as strip-tillage, gain more acceptance in agriculture (53–55), their impacts on beneficial arthropods need to be evaluated. The amount of area and depth of soil disturbance as well as mulching practices were our primary interests, since these are likely to destroy nests or interfere with nesting behavior. Survey results suggest that on average, flowers in non-tilled farms received approximately three times more squash bee visits than when full tillage was used. This is concurrent with previous study results that also found increased squash bee flower visitation (32) and offspring emergence (31) when soil was not tilled. Additionally, surveys of flowers on reduced tillage farms reported only slightly fewer average squash bee visits than no till surveys which indicates that both of these practices can contribute to squash bee population conservation at similar levels.

It is possible that tillage is correlated with other types of management practices that are responsible for changes in squash bee abundance, such as crop rotation or insecticide use. Considering that squash bees nest close to their host plants (33) and that *Cucurbita* crops are typically rotated, the number of squash bees visiting flowers is likely influenced by the management of previous year's fields, and the distance between these and current plantings. This highlights the relevance of ground management not only within individual fields but at the farm level. However, previous research demonstrating significant impacts on squash bee abundance due to soil management combined with a lack of observed impacts on generalist pollinators like honey bees and bumble bees suggests that although other forms of farm management may have some impact on squash bees, soil tillage is likely to impose strong effects (32).

Strip-tillage is often accompanied by the presence of cover crop residues (mulch) between strips of tilled soil which can help maintain soil moisture, reduce soil erosion, and inhibit weed seed germination (56). We did not observe a significant decrease in average squash bee visitation where mulch was present, but rather a numerical increase. Although this increase was not statistically significant, overall, mulch did not appear to inhibit or deter squash bees from visiting flowers. This is an important finding since we expected that mulch may deter females from digging nests, which are typically observed in bare soil (28). Conversely, our results suggest that squash bees may successfully build nests despite the presence of mulch which is similar to previous observations where squash bees nested in vegetated soil (57).

Surprisingly, tillage depth had no observed effect on squash bee flower visitation although we expected that shallower tillage may lead to increased squash bee visitation due to conservation of nests. It is possible that although deeper tillage destroys more squash bee nests it is not directly related to bee numbers counted at flowers if bees nest in the field perimeters where they are protected from soil disturbance. In addition, it may have been difficult for some citizen scientists to accurately approximate tillage depth, resulting in mis-categorizations. However, considering squash bee abundance was significantly lower in fully tilled fields, the amount of tilled area may have a greater impact than the depth of tillage. Our results suggest that in crops where tillage is necessary, reduced tillage can provide similar levels of native soil nesting bee conservation compared to no tillage.

Citizen science was also an effective means of examining the current geographic range of the squash bee because of the relatively broad participation in our study. Citizen scientists reported 7 new county records for this species, and while geographic range expansion may be responsible for such patterns, we hypothesize that a more likely explanation is a lack of historical reports and/or an increase in the number of small organic farms in Michigan which more often practice farm management methods that can promote native bees (58–60).

In our survey, squash bees were observed visiting flowers about 3-4 times more often than honey bees, bumble bees, or other bees, comprising more than 50% of the total number of bees reported (Fig 4). We did not expect that honey bees would respond to mulching or tillage because they are not ground nesting bees and therefore do not directly interact with the soil. We observed a positive effect of plant mulches on honey bee abundance which may be due to an indirect impact of soil management practices on these bees through affecting plant health or flower abundance. Bumble bees did not respond to ground management practices, and although they are ground nesting bees, they can cover long distances during foraging and are likely nesting outside of squash fields (32,61–63). The overall lack of response by honey and bumble bees to most soil management practices could also be because they are dietary generalists feeding on other available sources of pollen and nectar (64). Additionally, honey bees in particular visit squash flowers primarily for nectar as indicated by their preference for pistillate squash flowers (65). Therefore, squash flower abundance, quality, and/or field attributes dictated by soil management may be less consequential for these bees.

In summary, implementing management practices such as reduced tillage can help conserve native bees by providing suitable nesting habitat and allow farmers to take advantage of natural pollination services. Declines in both native and managed bees highlight the need to increase these types of conservation efforts (66–68) and non-traditional scientific methods like citizen science can provide new solutions. Despite its limitations, citizen science has proven to be an effective tool and it should be utilized when possible due to its ability to yield large amounts of quality data and provide citizens with an impetus for action towards issues like native pollinator conservation.

APPENDIX

Figure S3.1. Squash bee survey filled out by citizen scientists, 2017 – 2019. Surveys were submitted via a smartphone application, web browser, or mail.

Page 1

Please contact us if you have any question about the survey
Logan Appenfeller at appenfel@msu.edu or Zsofia Szendrei at szendrei@msu.edu

By filling out this form you are participating in a citizen science research project focusing on squash bees. It was initiated by Michigan State University's Vegetable Entomology Lab. With your help, our goal is to learn about the abundance and distribution of squash bees that specialize on cucurbits and are native to Michigan.
This project is funded by the USDA Organic Program.
You indicate your voluntary agreement to participate by completing and returning this survey. Your participation in this survey is voluntary, and thank you for participating!
Questions marked with an Asterisk are required for survey submission, others are optional.

Name (optional): ____

Contact (email preferred, optional): ____

*What are the last 4 digits of your phone number? ___________________

*What vine crops are grown in the garden? (Check all that apply)

- o Summer Squash (Yellow, Zucchini, Pattypan, Crookneck, etc.)
- o Winter Squash (Butternut, Acorn, Buttercup, Delicata, Hubbard, Kabocha, Pumpkin, Spaghetti, etc.)
- o Cucumber (Salad, Pickling, Slicing, etc.)
- o Melon (Cantaloupe, Honeydew, Muskmelon, Watermelon, etc.)

*Do you consider yourself an organic grower? o Yes o No

*What type of tillage do you use?

- o Full Tillage (100% soil cultivation)
- o Reduced Tillage (partial soil cultivation)
- o No Tillage (no cultivation)

*What is your tillage depth (inches)?

o 0 o 1-5 o 6-10 o 11-20

*How many types of vegetable crops do you grow? __________________________________

*What is the total area of your garden/farm?

o < 1 acre o 1 - 5 acre o > 1 acre

*How much area do you grow cucurbits on?

o < 0.5 acre o 0.5 - 1 acre o > 1 acre

*How do you irrigate your vine crops? (Check all that apply)

o Trickle/Drip o Overhead/Sprinkler/By hand o None

*What insecticides do you use on your vine crops? (Check all that apply)

- o Approved for organic use, Biopesticides
- o Restricted use or conventional synthetic pesticides
- o None

*What type of mulch do you use with your cucurbit plants? (Check all that apply)

o Plastic o Plant Material o None

RETURNING THE SURVEY: Please EMAIL this survey to Zsofia Szendrei at szendrei@msu.edu
or MAIL to 1129 Farm Lane, Room 348, East Lansing MI 48824

Figure S3.1 (cont'd)

Page 2

Please contact us if you have any question about the survey
Logan Appenfeller at appenfel@msu.edu or Zsofia Szendrei at szendrei@msu.edu

Bee Observation Protocol

Please observe 5 flowers on your vine crop during peak bloom.
Observe each flower for 1 minute, for a total of 5 minutes.
Add up the number of bees observed during the 5 minute observation and record in the categories below. Please avoid counting the same bee twice.
Bee observations should only be done in the morning of sunny days with no more than light winds, air temperature should be 70°F or above.
You can submit as many observations as you would like. For each observation, please use a new observation form.
Please categorize bees into one of 4 groups: **squash, honey, bumble, 'other'**
To see pictures of bees and get help with identification go to https://goo.gl/Yb0VFS
To watch a video about squash bees vs. honey bees go to https://youtu.be/a2UcgRx9ugE

*Date: ______________________________

*What is the nearest town/city to your current location? (Town/City, State)

____

*What vine crop are you observing?

- Summer Squash (Yellow, Zucchini, Pattypan, Crookneck, etc.)
- Winter Squash (Butternut, Acorn, Buttercup, Delicata, Hubbard, Kabocha, Pumpkin, Spaghetti, etc.)
- Cucumber (Salad, Pickling, Slicing, etc.)
- Melon (Cantaloupe, Honeydew, Muskmelon, Watermelon, etc.)

	# of Squash Bee	# of Honey Bee	# of Bumble Bee	# of Other Bees
Flower # 1				
Flower # 2			> 5 acre	
Flower # 3				
Flower # 4				
Flower # 5				

RETURNING THE SURVEY: Please EMAIL this survey to Zsofia Szendrei at szendrei@msu.edu
or MAIL to 1129 Farm Lane, Room 348, East Lansing MI 48824

Table S3.1. Data summary and results of statistical analyses for honey bees, bumble bees, and other bees. Number of observations by cucurbit management method and mean ± SEM, Kruskal-Wallis test results for honey bees, bumble bees, and other bees in a citizen science survey conducted in 2017-2019. Significant pairwise differences among factor levels were determined using Dunn's test and are indicated by different letters following mean ± SEM values ($\alpha < 0.05$).

			Tillage type					
		None	**Reduced**	**Full**		χ^2	**df**	***P* value**
Honey bees	N	109	97	19				
	mean ± SEM	0.29 ± 0.06	0.33 ± 0.06	0.47 ± 0.18		1.40	2	0.50
Bumble bees	N	103	101	24				
	mean ± SEM	0.43 ± 0.06	0.58 ± 0.07	0.67 ± 0.17		2.96	2	0.23
Other bees	N	110	111	25				
	mean ± SEM	1.33 ± 0.14a	0.68 ± 0.09b	1.36 ± 0.32ab		11.04	2	<0.01
			Tillage depth (cm)					
		0	**3-14**	**15-25**		χ^2	**df**	***P* value**
Honey bees	N	109	96	20				
	mean ± SEM	0.29 ± 0.06	0.35 ± 0.06	0.35 ± 0.13		1.05	2	0.59
Bumble bees	N	103	102	23				
	mean ± SEM	0.43 ± 0.06	0.65 ± 0.08	0.39 ± 0.14		5.05	2	0.08
Other bees	N	110	112	24				
	mean ± SEM	1.33 ± 0.14a	0.78 ± 0.10b	0.96 ± 0.24ab		8.66	2	0.01
				Mulch				
		None	**Plant**	**Plastic**	**Plastic + Plant**	χ^2	**df**	***P* value**
Honey bees	N	78	124	5	18			
	mean ± SEM	0.13 ± 0.04a	0.44 ± 0.06b	0.40 ± 0.40ab	0.33 ± 0.14ab	12.02	3	<0.01
Bumble bees	N	79	119	6	24			
	mean ± SEM	0.56 ± 0.08	0.53 ± 0.07	0.33 ± 0.33	0.42 ± 0.13	1.56	3	0.67
Other bees	N	81	135	6	24			
	mean ± SEM	0.85 ± 0.12	1.19 ± 0.12	1.83 ± 0.91	0.63 ± 0.16	4.26	3	0.23
			Irrigation					
		Overhead	**Drip**	**Overhead + Drip**		χ^2	**df**	***P* value**
Honey bees	N	178	37	10				
	mean ± SEM	0.31 ± 0.05	0.37 ± 0.11	0.30 ± 0.15		0.30	2	0.86
Bumble bees	N	176	39	13				
	mean ± SEM	0.57 ± 0.06	0.41 ± 0.11	0.23 ± 0.12		4.13	2	0.13
Other bees	N	190	43	13				
	mean ± SEM	1.01 ± 0.09	1.21 ± 0.23	1.00 ± 0.23		0.58	2	0.75
			Insecticides					
		None	**Organic**	**Conventional**		χ^2	**df**	***P* value**
Honey bees	N	184	31	10				
	mean ± SEM	0.31 ± 0.04	0.42 ± 0.12	0.30 ± 0.21		1.13	2	0.57
Bumble bees	N	181	34	13				
	mean ± SEM	0.56 ± 0.06	0.29 ± 0.09	0.62 ± 0.21		3.62	2	0.16
Other bees	N	196	35	15				
	mean ± SEM	1.14 ± 0.10	0.63 ± 0.15	0.67 ± 0.23		4.77	2	0.09
		Cucurbit area (hectare)						
		< 0.4	**> 0.4**			χ^2	**df**	***P* value**
Honey bees	N	214	11					
	mean ± SEM	0.31 ± 0.04	0.64 ± 0.24			2.92	1	0.09
Bumble bees	N	215	13					
	mean ± SEM	0.54 ± 0.05	0.15 ± 0.10			3.60	1	0.06
Other bees	N	232	14					

Table S3.1 (cont'd)

						χ^2	df	*P* value
	mean ± SEM	1.06 ± 0.09	0.79 ± 0.19			0.01	1	0.93
		Vine crop observed						
		Mixed	**Summer**	**Summer + Winter**	**Winter**	**χ^2**	**df**	***P* value**
Honey bees	N	35	107	18	65			
	mean ± SEM	0.29 ± 0.11	0.26 ± 0.06	0.50 ± 0.17	0.40 ± 0.08	6.50	3	0.09
Bumble bees	N	34	105	20	69			
	mean ± SEM	0.35 ± 0.12a	0.36 ± 0.06a	0.70 ± 0.18ab	0.80 ± 0.09b	20.56	3	<0.01
Other bees	N	39	113	20	74			
	mean ± SEM	0.95 ± 0.21	1.21 ± 0.13	1.10 ± 0.30	0.81 ± 0.14	6.07	3	0.11

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LITERATURE CITED

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CHAPTER 4: Conclusions and future directions

In this thesis, I have investigated the effects of ground management practices (tillage and cover crop mulches) on pests and beneficial arthropods in organic *Cucurbita* agroecosystems. In recent years, many studies have examined how pests and natural enemies respond to conservation tillage methods, however results have been mixed across cropping systems (1,2). Furthermore, few studies have determined how ground management affects native bees, which are important pollinators of many crops (3,4). The research presented in this thesis has helped to elucidate these questions and provides implications for *Cucurbita* growers who can utilize conservation tillage practices to improve biological control, pollination, and receive agronomic benefits.

In Chapter 2, my research focused on determining if foliar arthropod abundances, epigeal natural enemy activity density, and bee flower visitation frequency varied among treatments consisting of different combinations of tillage cover crop mulches. Previous studies examining how foliar arthropods and epigeal natural enemies respond to these factors have yielded mixed results with some finding that conservation tillage regimes (e.g. strip tillage, no tillage) reduced pest abundances directly or indirectly through improved biological control (5–9). Others observed inconsistent effects on natural enemies (2,10,11) or sometimes increased pest pressure compared to conventional tillage systems (12,13). This research demonstrated that strip tillage has the potential to significantly increase activity density of several epigeal natural enemy taxa suggesting that strip tillage may promote biological control of weeds and epigeal pests. This is likely due to increased habitat complexity in strip tillage systems which can offer valuable resources for natural enemies such as refuge, ideal microclimatic conditions, and alternative food sources (5,10,11). Some foliar arthropods also responded to strip tillage, and although some foliar pests were slightly more abundant in strip tillage, Aphididae, the dominant pest observed, and Formicidae were significantly more abundant on plants in full tillage. This suggests that more disturbed habitats may favor population growth of certain pests like Aphididae. Formicidae, which in previous studies has been more abundant in conservation tillage systems (14,15), may be attracted to plants with large Aphididae populations and may exacerbate Aphididae pressure via tending behavior. Bee visitation frequency also varied among treatments, for *A. mellifera* and *Bombus* spp. However, this was likely indirectly due to treatment effects, such as flower abundance or quality considering these bee taxa are not likely to interact with the soil surface in *Cucurbita* fields. *Eucera pruinosa* flower visitation did not vary significantly among treatments which was possibly due to their sole reliance on *Cucurbita* flowers, whereas *A. mellifera* and *Bombus* spp. may utilize other floral resources within or outside experimental plots (e.g. weeds, other crops). I expected that mulch residues in strip tillage plots may negatively impact *E. pruinosa* flower visitation by obstructing the soil surface near their host plants. However, similarly to the results of my citizen science project, I did not observe this which suggests that mulch may not deter or inhibit *E. pruinosa* from visiting flowers. This absence of apparent treatment effects also suggests that tillage type was inconsequential for *E. pruinosa* flower visitation in this experiment. Although, this is contrary to the results of my citizen science project which suggested that farms with reduced soil disturbance supported larger *E. pruinosa* populations as indicated by increased flower visitation in no-till and reduced tillage farms compared with conventional tillage. This is likely because different fields were used during each year of this experiment which eliminated the possibility for post-harvest tillage to impact *E. pruinosa* larvae overwintering in the soil. In contrast, in my citizen science project bee observations were conducted in farms where *Cucurbita* had previously been grown and post-harvest tillage practices had time to impact overwintering *E. pruinosa* larvae. The results of this experiment suggest that *E. pruinosa* flower visitation frequency is not directly impacted by tillage and/or mulch, but over time this rate can be modified depending on the number of *E. pruinosa* larvae that survive post-harvest ground management practices.

In Chapter 3, my research focused primarily on determining the effects of ground management practices on *E. pruinosa* due to their importance as *Cucurbita* pollinators. Previous studies examining the effects of tillage on *E. pruinosa* have yielded mixed results, and most have only compared no-till farms to those using conventional tillage (4,16–18). The effects of mulch on *E. pruinosa* were even less understood prior to my research (19). Furthermore, I collected data for multiple other management practices such as tillage depth, mulch, area devoted to cucurbits, type of cucurbits grown, insecticides, and irrigation that were not considered in many previous *E. pruinosa* studies. MSU Extension Master Gardeners were recruited/trained to participate in a citizen science survey where they submitted data primarily using a smartphone application. In contrast to some other pollinator citizen science projects (20–22), our participant numbers increased over the years which provided a large dataset from multiple *Cucurbita* farms in Michigan. I found *E. pruinosa* to be the most common bee visiting flowers in *Cucurbita* farms, and tillage type was the most important factor affecting *E. pruinosa* abundance. *E. pruinosa* flower visitation frequency was nearly 3 times greater in no-till farms and farms using reduced tillage than farms using full tillage, indicating that reduced soil disturbance may promote larger populations of these important specialist bees. Tillage depth did not significantly impact *E. pruinosa* flower visitation suggesting that the amount of tilled area may be more consequential for population size. Likewise, mulch did not significantly impact *E. pruinosa* visitation. However, visitation was numerically highest in fields using plastic mulch or a combination of plastic and plant material mulch. Therefore, mulch may not deter or inhibit *E. pruinosa* nesting. No other management practices were observed to impact *E. pruinosa* abundance. Ground management practices did not appear to impact visitation by generalist bees such as *A. mellifera* and *Bombus* spp. which was expected due to their lack of interaction with the soil in *Cucurbita* fields. My results indicate that reduced tillage methods can provide suitable, undisturbed nesting habitat for fossorial bees such as *E. pruinosa* compared to conventional tillage. Reduced tillage could be a useful contributor to native bee conservation and improve wild bee provided pollination services for growers.

Overall, my thesis helped elucidate the effects of ground management practices on pests and beneficial arthropods in organic *Cucurbita* management, and contains the first citizen science study to successfully examine an agriculturally important wild bee's distribution and the impacts of farm management on their abundance. According to my research, reduced tillage practices such as strip tillage can significantly increase activity density of several epigeal natural enemy taxa which can improve biological control. Strip tillage can also lead to reduced abundance of some foliar herbivores such as Aphididae, and Formicidae may contribute to Aphididae population growth. My results suggest that this habitat management method has the potential to reduce reliance on chemical insecticides. Furthermore, my thesis demonstrated that reduced tillage practices to contribute native bee conservation and enhance pollination services in *Cucurbita*, and that citizen science is an effective tool for native bee conservation studies. Taken together, the results suggest that decreasing soil disturbance in farms can have positive impacts on beneficial arthropods that provide biological control and pollination services. Future research should examine the effects of practices such as strip tillage on *Cucurbita* arthropod communities while also examining plant health and vigor (e.g. nitrogen uptake, size, floral abundance, yield) to gain a better understanding of the mechanisms driving patterns in arthropod communities.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2020-01

Author: Logan R. Appenfeller

Title of thesis: Investigating the impacts of ground management on arthropods in organic Cucurbita agroecosystems

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Apidae	*Eucera pruinosa*	adult	20	pinned
Carabidae	*Harpalus* sp.	adult	20	pinned

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