Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes region

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Abstract

Understanding the interactions between plants and pollinators within a system can provide information about pollination requirements and the degree to which species contribute to floral reproductive success. Past research has focused largely on interactions within monocultured agricultural systems and only somewhat on wild pollination networks. This study focuses on the culturally significant Three Sisters Garden, which has been grown and tended by Anishinaabe and Haudenosaunee peoples for generations. Here, the plant-pollinator network of the traditional Three Sisters Garden with the inclusion of some additional culturally significant plants was mapped. Important visitors in this system included the common eastern bumble bee, *Bombus impatiens* Cresson (Hymenoptera: Apidae), and the hoary squash bee, ~~*Eucera*~~ *Xenoglossa pruinosa* (Say) (Hymenoptera: Apidae), as determined by their abundances and pollinator service index (PSI) values. Understanding the ~~important~~ key pollinators in the Three Sisters Garden links biological diversity to cultural diversity through the pollination of culturally significant plants. Further, this information could be of use in supporting Indigenous food sovereignty by providing ~~information~~ knowledge about which wild pollinators could be supported to increase fruit and seed set within the Three Sisters Garden. Our findings can also lead to more effective conservation of important wild pollinator species.

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Introduction

Pollination is a mutualistic interaction between two levels of the food web—plants and their pollinators (Jordano, 1987; Carvalheiro et al. 2008; Ings et al. 2009; Willis Chan and Raine, 2023). Network theory has been used in the evaluation of mutualistic interactions, and the interactions are cumulatively referred to as a plant-pollinator network (Jordano, 1987; Ings et al. 2009; Kaiser-Bunbury and Blüthgen, 2015; Jolls et al. 2019). While plant-pollinator network characteristics (such as asymmetry and nestedness) (Bascompte et al. 2003; Bascompte et al. 2006; Montoya et al. 2006) make them theoretically robust, there is potential for anthropogenically driven environmental disturbance to eventually collapse plant-pollinator networks (Kearns et al. 1998; Potts et al. 2010; Brosi and Briggs, 2013; Tucker and Rehan, 2016; Tscharntke 2021).

Plant-pollinator networks are negatively impacted by various factors including habitat fragmentation and land use changes (Spiesman and Inouye, 2013), conventional agricultural practices (e.g., pesticides), non-native species introductions (Kearns and Inouye, 1998), and increasingly climate change (Memmott et al. 2007). Documenting these mutualisms and modeling how they respond to change are integral to the conservation and restoration of ecological networks (Memmott, 2009; Kaiser-Bunbury and Blüthgen, 2015; Tucker and Rehan, 2016). Studying plant-pollinator networks helps to fill in baseline information about the ecological role of wild bees and to understand the stability and/or resiliency of the network to environmental change (Tucker and Rehan, 2016). The loss of even a single species can have significant effects on reproductive success of the plants within a system (Brosi and Briggs, 2013). Declines of pollinators within a network can contribute to negative feedback of less floral reproduction, which then in turn contributes to fewer resources for pollinators (Tscharntke, 2021). Wild pollinators ~~are~~ provide~~ing~~ significant levels of pollination services to crops (Garibaldi et al. 2011), yet the details (e.g., the level of pollinator abundance or diversity required to provide adequate pollination) of these relationships remain relatively unknown (Kovacs-Hostyanszki 2017; Danforth et al. 2019). Pollination deficits are a threat to global food security (Tscharntke 2021).

There have been efforts recently to increase food production without increasing the level of environmental harm from agriculture (Pretty 2014; Tscharntke 2021; Ramirez and Wright 2023). Intercropping is a practice that may ~~have the ability to~~ increase yield and promote sustainable land and resource use (Tscharntke 2021; Ramirez and Wright, 2023). Intercropping has also been suggested as a method of reducing agricultural causes of pollinator decline (Kovacs-Hostyanszki 2017; Tscharntke 2021). The Three Sisters method of cultivation is a polyculture practice (intercropping) involving the growth of multiple crops simultaneously (Eames-Sheavly, 1993; Kuepper et al. 2016).

Archaeobotanical remnants in the forests and prairies of Canada show evidence of corn domestication as early as 500 A.D. (Boyd and Surette, 2010) and common bean and squash cultivation in the Woodland period (1000 B.C.–1000 A.D.) (Boyd et al. 2014). The Three Sisters was grown for 500 years pre-contact by the Seneca people in western New York and was referred to as “Diohe'ko”, which translates to “these sustain us” (Lewandowski, 1987). The Haudenosaunee people (people of the long house) of ~~e~~the Eastern United States and Canada have traditionally planted the Three Sisters Garden (Eames-Sheavly, 1993). Broadly, it has been reported that the Three Sisters was grown by all tribes who practiced agriculture in northeastern North America (Lewandowski, 1987).

This study aims to better understand the pollinator community and plant-pollinator network in a Three Sisters Garden (TSG). The Three Sisters Garden is composed of corn (*Zea mays* L. (Poales: Poaceae)), common bean (*Phaseolus vulgaris* L. (Fabales: Fabaceae)), and squash (*Cucurbita* L. sp. (Cucurbitales: Cucurbitaceae)) (Boyd et al. 2014).

Cucurbita plants (pumpkins, squash, gourds) are monoicous and rely on insect pollination; each plant has both pistillate (female) and staminate (male) flowers (Stapleton et al. 2000; Whitaker and Davis, 2012; Brochu et al. 2021, Willis Chan and Raine, 2021). Flowers open at dawn and close by noon each day, and pollination must occur within this window (Nepi and Pacini, 1993, Willis Chan and Raine, 2021). ~~*Eucera*~~*Xenoglossa pruinosa* is an oligolectic bee species, foraging only on the flowers of *Cucurbita* crops (Hurd and Linsley, 1964; Willis Chan 2020; Brochu et al. 2021). The hoary squash bee’s natural geographic range has increased over the past 1000 years following the spread of squash planting for agricultural purposes (Brochu et al. 2021). Domesticated squash has been receiving pollination by wild pollinators prior to the introduction of the western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) (Lopez-Uribe et al. 2016). *Phaseolus vulgaris* (L. Fabaceae) is self-compatible, i.e., able to self-pollinate as the flower opens (Ibarra-Perez et al. 1999, de Souza Paulino et al. 2023). It is also noted, however, that the reproductive success of the plant (seed yield) can be increased by visits by larger bees (e.g., carpenter bees, bumble bees, etc.) (Ibarra-Perez et al. 1999). *Phaseolus coccineus* has been found to set few pods without the presence of insect visitors (Darwin, 1876, Free, 1966, Free and Racey 1968, Kendall and Smith, 1976). *Zea mays* is wind pollinated and therefore does not rely on insects for pollination~~,~~; however, insects may visit the flowers (Johnson and Hayes, 1932).

In some cases, sunflowers would be grown along one side of the Three Sisters Garden (Kuepper et al. 2016); it has been reported that this was done to attract pollinators to the garden (Native Seeds Search, 2020; Rodale Institute, 2020). Other plants, including Hopi tobacco (*Nicotiana rustica* L. (Solanales: Solanaceae)), purple coneflower (*Echinacea purpurea* (L.) Moench (Asterales: Asteraceae)), common milkweed (*Asclepias syriaca* L. (Gentianales: Apocynaceae)), wild bergamot (*Monarda fistulosa* L. (Lamiales: Lamiaceae)), Oswego tea/bee balm (*Monarda didyma* L.), and American vervain (*Verbena hastata* L. (Lamiales: Verbenaceae)), are also planted in some food and medicine gardens (Our Sustenance, 2020; PAN and TRCA, 2020).

The Three Sisters Garden (TSG) is a growing method with long biological and cultural roots, and medicine plants are important to many Indigenous cultures (Densmore, 1928; Lewington, 1990; Padulosi et al. 2004; Genuisz, 2015; PAN and TRCA, 2020). A better understanding of the pollinator community and plant-pollinator network in the Three Sisters

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Garden will provide information about the wildlife that provides ecological services to this kind of garden and thus the pollinators that are connected to Indigenous food and medicine sovereignty. Intercropping also offers a sustainable agricultural practice that may be useful, specifically in urban agriculture (Ramirez and Wright, 2023).

The objective of this study is to map the plant-pollinator network in the culturally significant Three Sisters garden and determine if and how the pollinator community in the garden differs from the local wild pollinator community based on pan-trap sampling in adjacent, natural sites and in the context of other regional studies. These baseline conditions will be useful for predicting how the Three Sisters Garden system may be impacted by environmental change into the future.

~~Human societies across the globe have developed in concert with the local landscape, including particular plant and animal species (Lewington, 1990). In the geopolitical nation now called Canada, various Indigenous nations have long-standing connected relationships with the land (Turner, 1973; Hellson, 1974; Arnanson, 1981; Marles et al. 2000; Uprety et al. 2012; Turner et al. 2013; Brokenleg and Tornes, 2013; Genuisz, 2015). For the Ojibway people situated near the Great Lakes, plants are a part of living *Mino-bimaadizi*, or the good life (Densmore, 1928). Plants are regarded as sacred by the Ojibway due to their connection to nourishment for and healing of the body (Densmore, 1928).~~

~~Many Indigenous communities are experiencing a dramatic loss of their cultural practices, including traditional stories, land use and language (Brokenleg and Tornes, 2013; Reyes-García et al. 2013). These losses have been found to be intertwined with biological losses on the landscape, e.g., if a plant is no longer present, the word for it is eroded out of language and social memory (Davidson-Hunt and Berkes, 2003). Whereas it is through these means that cultural diversity and biological diversity can be lost, biocultural diversity can be conserved in tandem with biodiversity through food (Hill et al. 2019). At the intersection of environmental protection and Indigenous reconciliation is food sovereignty, where the rights of people to define and control their own food production systems are supported and valued (Armstrong, 2020; Settee and Shukla, 2020). Achieving food security and food sovereignty will require Indigenous peoples to have access to, and the right to grow, their culturally appropriate foods (Armstrong, 2020; Settee and Shukla, 2020). The UN Sustainable Development Goal #2 is to end hunger, achieve food security and improve nutrition, and promote sustainable agriculture (UN, 2023). Target 2.3 refers to increasing agricultural productivity of small-scale farmers and Indigenous peoples (UN, 2023). Target 2.4 refers to increasing agricultural systems that maintain ecosystems, mitigate climate change, and increase soil health (UN, 2023). These targets highlight the importance of studying the relationship between culturally significant plant systems and pollinators to better understand how to manage these systems.~~

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Domesticated squash has been receiving pollination by wild pollinators prior to the introduction of the western honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) (Lopez-Uribe et al. 2016). The Three Sisters method of cultivation is a polyculture practice (intercropping) involving the growth of multiple crops simultaneously (Eames-Sheavly, 1993; Kuepper et al. 2016). In some cases, sunflowers would be grown along one side of the Three Sisters Garden (Kuepper et al. 2016); while not explicitly stated in the scientific literature, it has been reported elsewhere that this was done to attract pollinators to the garden (Native Seeds Search, 2020; Rodale Institute, 2020). Each plant brings a valuable service to the garden: the corn provides long stalks for the beans to climb, the beans fix nitrogen in the soil, and the squash shades the soil to supress weeds and retain moisture (Eames-Sheavly, 1993; Kuepper et al. 2016). This ecological agriculture method also discourages pests with the diverse cropping method (Eames-Sheavly, 1993). It has also been reported that growing the Three Sisters Garden has higher yields than growing the same crops in rows on the same land (Creasy, 1988; Mt. Pleasant, 2001; Elliot, 2004; Trotman Martinez, 2007). The "Three Sisters" when eaten together also provide a balanced meal nutritionally (Lewandowski, 1987; Mt. Pleasant, 2001; Trotman Martinez, 2007).

Other culturally important plants, including Hopi tobacco (*Nicotiana rustica* L. (Solanales: Solanaceae)), purple coneflower (*Echinacea purpurea* (L.) Moench (Asterales: Asteraceae)), common milkweed (*Asclepias syriaca* L. (Gentianales: Apocynaceae)), baby-saver plant/wild bergamot (*Monarda fistulosa* L. (Lamiales: Lamiaceae)), Oswego tea/bee balm (*Monarda didyma* L.), and American vervain (*Verbena hastata* L. (Lamiales: Verbenaceae)), are also planted in some Indigenous food and medicine gardens, particularly of Haudenosaunee and Anishinaabe peoples (Our Sustenance, 2020; PAN and TRCA, 2020). Traditionally, some of these plants would have been gathered growing in the wild (Densmore, 1928; Genuisz, 2015); now, they are also grown in representative gardens showcasing and supporting culturally significant plants and diversified growing methods (PAN and TRCA, 2020). Hopi tobacco is a culturally significant plant sacred to Anishinaabe people and other North American Indigenous peoples and is used in daily life to give thanks and to carry prayers to the Creator and is also used at many ceremonies and events (Struthers & Hodge, 2004; Brokenleg & Tornes, 2013; Sadik, 2014; Genuisz, 2015). Oswego tea has medicinal and stimulant properties but is also used as a drinking tea, while baby-saver plant is used more commonly for its medicinal purposes, such as for infants with colic, by the Anishinaabe (Genuisz, 2015). The Ojibway people used common

~~milkweed for medicinal purposes (women's health), as food, and in charms, and American vervain for medicine and treating ailments (e.g., nosebleeds) (Densmore, 1928). The Three Sisters Garden is a growing method with long biological and cultural roots, and medicine plants are important to many Indigenous cultures (Densmore, 1928; Lewington, 1990; Padulosi et al. 2004; Genuisz, 2015; PAN and TRCA, 2020). A better understanding of the pollinator community and plant-pollinator network in the Three Sisters Garden will provide information about the wildlife that provides ecological services to this kind of garden and thus the pollinators that are connected to Indigenous food and medicine sovereignty.~~

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Materials & Methods

Study Sites and Land Acknowledgment

This research was undertaken on the traditional territories of multiple First Nations. The ~~sites located in Tkaranto, including the~~ campus of York University, ~~are~~ is located on the traditional territory of the Anishinabek Nation, the Haudenosaunee Confederacy, the Huron-Wendat, and the Métis. The current treaty holders in this location are the Mississaugas of the New Credit First Nation, and the land is subject to the Dish with One Spoon Wampum Belt Covenant. The eastern Ontario sites are located on unceded Algonquin territory and are subject to Treaty 27 and Treaty 27 1/4. We acknowledge the generations of caretaking of the land by the many Indigenous peoples who have and still do call these places home. Plot A is located in Arnprior, Ontario, Plot B is located in Pakenham, Ontario, and Plot C is located in Lanark, Ontario (Table 1).

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Planting

Seeds for this study were sourced from Urban Harvest (www.urbanharvest.ca) and included scarlet runner (*Phaseolus coccineus* L.) and true red cranberry beans (*Phaseolus vulgaris*), delicata (*Cucurbita pepo* L. 'Delicata') and pattypan squash (*Cucurbita pepo* 'Patty Pan'), bloody butcher corn (*Zea mays* 'Bloody Butcher'), purple coneflower (*Echinacea purpurea*), bee balm (*Monarda didyma*), and sunflowers (*Helianthus annuus* L. 'Autumn Beauty' (Asterales: Asteraceae)). Pots with purple coneflower, Oswego tea, and American vervain (*Verbena hastata* L.) were added to the garden sites since growing these plants from seeds is a multi-year process. Sunflowers and medicine plants were added to replicate the addition of these plants to the traditional TSG system. Seeds were started indoors in April (2019 and 2020) using Organic ProMix and Jiffy pots (4"). The soil was kept moist until germination, and light was provided using a TOLYS 1000W LED light. At each site, the earth was tilled with a shovel, and soil amendments were added: two 85L bags of Organic ProMix and 4 bags of composted sheep manure per plot.

Planting methods followed those described by Six Nations Our Sustenance (2020), which include mounds of soil placed with the centre of each mound 5 ft from the centre of the next mound for a total of 18 mounds. Each mound was 18” across and 12” high. Four corn plants were planted in a 6” square in alternating mounds. Beans were planted 3” from each corn plant, creating a square in each corn/bean mound. Squash plants were planted in the remaining mounds, with 2 plants spaced 4” apart (alternating mounds of ‘Patty Pan’ and ‘Delicata’ squash). ~~In 2019, Plots A and B in eastern ON were used, and each contained a 15 ft x 15 ft Three Sisters Garden, as well as smaller pots of the plants located at two sites in Toronto, and one site in Toronto at a community garden. In 2020,~~ Plots A, B, and C in eastern ON were used, and each contained a 30 ft x 30 ft garden plot of the Three Sisters. Plot A also had purple coneflower, Oswego tea, milkweed, and American vervain growing in the garden. Plots B and C also had purple coneflower, bee balm, and sunflowers growing.

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Insect Interaction Observations, Collection & Curation

~~In 2019, two days per week, 20-minute sweep netting sessions were conducted at each of the five garden sites (Toronto – three sites, eastern Ontario – two sites).~~ Four days per week sweep-net sampling for the plant-pollinator network was conducted, rotating between morning and afternoon sessions, resulting in a total of four sampling sessions per garden per week at three sites in eastern Ontario (Plots A, B, and C). Morning sessions were between 8 AM and noon, and afternoon sessions between 1 PM and 5 PM (as in Tucker and Rehan, 2016). Previous work sampling the plant-pollinator network in a garden setting has used similar methods (Gotlieb et al. 2011). The garden plot was sampled walking down each of the rows and up the next row for 20 minutes. Each pollinator on a flower or inflorescence was collected using a sweep net and placed in an insect vial and the plant species was noted on the vial. It was not determined whether pollen was deposited, rather a visit to the flower was used to signal a visit. Vials were placed in a small lunch cooler containing icepacks until the end of the 20-minute session. At the end of each session, the cooler was emptied, with each specimen that could be identified on the wing identified and released (*Apis mellifera*, *Bombus* spp.). A voucher of each species was collected to represent each species collected in the field (Kearns and Inouye, 1993; Packer et al. 2018). Each specimen not identified in the field was labelled with the floral species, site, and date. Collected specimens were placed in 70% ethanol (Kearns and Inouye, 1993) and pinned at the end of each sampling day throughout the season.

Pan trap sampling was conducted in the natural areas adjacent to the gardens. Pan traps were placed 500 m away from the garden site at two sites. Small plastic coloured bowls (yellow, blue, and white) were placed on the surface of the ground and filled with soapy water. A 200 m transect was used, and pan traps were placed every 10 m in a repeating order of yellow, blue, and white. Pan traps were set in place for 24 hr periods on sweep sampling days.

Bee Identification

Vouchered collected specimens are stored at Dr. Sheila Colla's laboratory at York University, Toronto, Canada. ~~The 2019 records were identified to genus and include preserved, physical as well as imaged specimens.~~ The records were identified to species or morphospecies and include preserved, physical and some imaged specimens (e.g., *Xenoglossa* Smith ~~*Eucera* Scopoli~~ (Hymenoptera: Apidae), *Bombus* Latreille (Hymenoptera: Apidae)). Pan trap samples were identified to species. Bees were identified to genus (and species for genera that are monotypic in Eastern Canada) using the key of Packer *et al*. (2007). Species-level identifications were made with reference to the keys and taxon concepts of de Silva (2012) for *Coelioxys* Latreille (Hymenoptera: Megachilidae); Gardner & Gibbs (2022) for metallic weak-veined *Lasioglossum* Curtis (Hymenoptera: Halictidae); Gibbs *et al*. (2013) for non-metallic weak-veined *Lasioglossum*; Laverty & Harder (1988) and Williams *et al*. (2014) for *Bombus*; McGinley (1986) for strong-veined *Lasioglossum*; Mitchell (1960) for *Halictus* Latreille (Hymenoptera: Halictidae); Mitchell (1962) for *Melissodes* Latreille (Hymenoptera: Apidae) and *Osmia* Panzer (Hymenoptera: Halictidae); Onuferko (2017, 2018) for *Epeolus* Latreille (Hymenoptera: Apidae); Oram (2018) for *Hylaeus* Fabricius (Hymenoptera: Colletidae); Portman *et al*. (2022) for *Augochlora* Smith (Hymenoptera: Halictidae) and *Augochlorella* Sandhouse (Hymenoptera: Halictidae); Rehan & Sheffield (2011) for *Ceratina* Latreille (Hymenoptera: Apidae); Mitchell (1962) and Rowe (2017) for non-*Osmia* Osmiini; Mitchell (1962) and Sheffield *et al*. (2011) for *Megachile* Latreille (Hymenoptera: Megachilidae); and Stephen (1954) and Mitchell (1960) for *Colletes* Latreille (Hymenoptera: Colletidae). It was not possible to distinguish some females of *Ceratina dupla* Say from *C. mikmaqi* Rehan & Sheffield, so they were treated as a single morphospecies in data analysis, as *Ceratina dupla/mikmaqi*.

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Statistical Analysis

Statistical analysis was conducted in R (R version 3.6.2 (2019-12-12) (R Core Team, 2019). The R package 'vegan' (Oksanen et al. 2020) was used to run species accumulation estimates using the abundance-based data (Gardener, 2014). The R package 'bipartite' (Dormann et al. 2008) was used to run community (function *networklevel*) and species (function *specieslevel*) level network analyses. Community level analysis was conducted including calculations of weighted nestedness and weighted connectance (Tucker and Rehan, 2018). Nestedness refers to the level of overlap between generalist and specialist interactions, where values closer to "1" indicate a high degree of overlap and values closer to 0 indicate a low degree of overlap (Tucker and Rehan, 2018; Delmas et al. 2019). Connectance refers to the proportion of possible interactions that have been realized in the network (Kearns et al. 1998; Tucker and

Rehan, 2018; Delmas et al. 2019) and is a measure of the community's ability to respond to change such as species loss (Dunne et al. 2002; Tucker and Rehan, 2016). Species level analysis included estimation and comparisons of weighted degree and Pollination Service Index (PSI) values (Dormann et al. 2008; Tucker and Rehan, 2018). Degree measures the diet breadth of the pollinators or the number of unique interactions of floral visitors (Tucker and Rehan, 2016). The PSI measures the relative importance of each pollinator species to the functioning of the community (1 = the species is critical to ecosystem functioning, 0 = community could function without the species) (Dormann et al. 2008). PSI is calculated by first determining the proportion to which a pollinator visit's each plant species, second the proportion a plant is visited by each bee species (Dormann and Fründ, 2024). Third, these proportions are multiplied to determine the PSI value for each pollinator species (Dormann and Fründ, 2024).

Results

Abundance and Diversity

~~A total of 286 interactions were observed during the 2019 sampling period (July–August 2019). Eleven bee genera were recorded at all sites combined (Figure 1), with six genera recorded at the eastern ON sites and nine at the Toronto sites. A total of twelve varieties of plants were observed at all sites (Figure 1), with four at the eastern ON sites and ten at the Toronto sites.~~ A total of 310 interactions were observed during the sampling period (July–August 2020) across all sites. Thirty-seven bee species/morphospecies were identified at all sites combined (Figure 1), with fourteen recorded at Plot A, nineteen at Plot B, and twenty-two at Plot C. A total of ten plant varieties were present across all sites combined (Figure 1), with eight varieties at Plot A, five varieties at Plot B, and seven varieties at Plot C. The species accumulation estimate using the Chao 1 and ACE tests was 63 (se.chao1, 18). The observed 37 species represent 59% of the ~63 bee species estimated to occur in the plant community. Bees of the following three families were recorded: Apidae, Megachilidae, and Halictidae. No Andrenidae or Colletedae were recorded in the interaction sampling. The most frequent family was Apidae (200), the most frequent genus was *Bombus* (93), and the most frequent species were *Bombus impatiens* Cresson (88) and the hoary squash bee, ~~*Eucera*~~ *Xenoglossa*~~.~~ *pruinosa* (Say) (81). The Shannon's Diversity for the garden sampling was 2.37.

A total of 397 bee specimens were collected from pan traps sampling. Bees of nineteen genera and 53 species were collected in the pan trap samples. Bees of five families were collected (Apidae, Megachilidae, Andrenidae, Colletidae, Halictidae). Halictidae (228) was the most frequent bee family collected and Andrenidae was the least frequent (Table 2). *Halictus* (88) was the most frequent genus, and *Halictus ligatus* (68) was the most frequent species (Table 2). The species accumulation estimate using the Chao 1 and ACE tests was 62 (se.chao1, 6) The observed 53 species represent 85% of the ~62 bee species estimated to occur at the site. The Shannon's Diversity for the pan trap sampling was 3.22.

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Plant-pollinator Network

~~During 2019 sampling, the most common interaction observed with all sites combined (Figure 1) and at the eastern ON sites was between *Eucera* and *Cucurbita pepo* 'Patty Pan'. The most common interaction during 2019 at the Toronto sites was between *Apis mellifera* and *Cucurbita maxima* 'Gete-Okosomin'. Weighted connectance was 0.12 and weighted nestedness was 0.2. The Pollinator Service Index (PSI) was highest across sites for *Xylocopa* Latreille (Hymenoptera: Apidae) (1.0) and *Agapostemon* Guérin-Méneville (Hymenoptera: Halictidae) (0.86). The PSI was lowest across sites for *Ceratina* (0.05) (Table 3).~~

For the garden sampling, the most common interaction observed across sites was between *Cucurbita pepo* 'Patty Pan' and ~~*Eucera*~~ *X. pruinosa* (53 interactions) and *B.~~ombus~~ impatiens* (54 interactions) (Figure 1). Weighted connectance was 0.08 and weighted nestedness was 0.47. *Bombus impatiens* had the highest degree (6) and ~~*Eucera*~~ *X. pruinosa* (42) and *B.~~ombus~~* *impatiens* (41) had the highest PSI (Table 3).

Cucurbita pepo 'Patty Pan' was associated with the highest number of bees (127), followed by *Nicotiana rustica* 'Hopi', which was associated with the second highest number (69) (Figure 2). *Phaseolus coccineus* 'Scarlet Runner' and *Verbena hastata* were associated with the least number of bees (4) (Figure 2~~3~~). *Nicotiana rustica* 'Hopi' was associated with the highest number of bee species (19), followed by *C.~~ucurbita~~ pepo* 'Patty Pan' (11) (Figure 2~~3~~). *Phaseolus coccineus* 'Scarlet Runner' was associated with only one species of bee (Figure 3~~4~~). The most common bee species recorded were *B.~~Bombus~~ impatiens* (88) and ~~*Eucera*~~ *X. pruinosa* (81) (Figure 4). Seventeen bee species were recorded only once (Figure 4). There were no interactions recorded for corn flowers in any years.

Discussion

The most abundant visitor to the Three Sisters gardens, and the visitor with the highest PSI, was ~~*Eucera*~~ *X. pruinosa*. The most common interactions were between ~~*Eucera*~~ *X. pruinosa* and *C.~~ucurbit~~a pepo* 'Patty Pan' and *B.~~ombus~~ impatiens* and *C.~~ucurbita~~ pepo* 'Patty Pan'. Based on PSI, ~~*E*~~*X. pruinosa* is one of the key species within the Three Sisters Garden system. There were only two records of ~~*E*~~*X. pruinosa* in the pan trap samples, which can be explained by this species' specialization on cucurbits. ~~*Eucera*~~*Xenoglossa pruinosa* had the highest PSI at all sites combined as well as at Plots A and B and was most frequently collected while visiting *C.~~ucurbita~~ pepo* 'Patty Pan' and *C.~~ucurbita~~ pepo* 'Delicata'.

As highlighted by Willis Chan (2020) and Willis~~-~~Chan and Raine (2021a; 2021b; 2023), ~~*E*~~*X. pruinosa* has a close association with *Cucurbita* crops grown for agricultural purposes in Ontario. The range of ~~*E*~~*X. pruinosa* in Ontario is outside of the range of wild *Cucurbita* (López-Uribe et al. 2016). The range of ~~*E*~~*X. pruinosa* has been found to be impacted by agricultural expansion in North America (Pope et al. 2023). The history of the Three Sisters Garden and the remnants of domesticated beans and squash during the woodland period (Boyd et al. 2014), as

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Commented [SW10]: Not all cucurbits. They specialise on the genus Cucurbita well as the close association still found today between ~~*E*~~*X. pruinosa* and *Cucurbita* crops, highlight the importance of continued research focusing on managing threats to ~~*E*~~*X. pruinosa* in a changing environment to ensure continued pollination services by wild bees to this culturally and economically significant food plant. There were also other bee species interacting with *C.* ~~*ucurbita*~~ *pepo* including: *Apis mellifera*, *Augochlora pura*, *Augochlorella aurata*, *Bombus* spp., *Halictus rubicundus*, *Lasioglossum* spp., *Megachile brevis*, *Melissodes* spp.. Future studies ~~may~~ should examine the role of these bee species to pollination of *Cucurbita*.

The diversity of bees collected in the natural was higher than that in the TSG system. The Shannon's Diversity Index for the garden samples was 2.37, and for the pan traps was 3.22. Based on the species accumulation estimates, our results represent 59% of bee species estimated to occur in the ~~Three Sisters gGarden~~TSG system. The interaction sampling recorded three bee families whereas pan trap sampling recorded five bee families. Perhaps increased sampling effort would have recorded Andrenidae and Colletidae in the interaction sampling. The absence of *Andrena* Fabricius (Hymenoptera: Andrenidae) (the largest genus of andrenids in Eastern Canada) from our samples may be explained by our sampling having take place during the summer and *Andrena* being most active in the spring.

Current management recommendation for ~~*E*~~*X. pruinosa* in agroecosystems are as follows: (1) minimize pesticide exposure, (2) provide nesting sites, (3) maintain yearly field proximity, (4) monitor populations, parasites, & pathogens, and (5) limit deep tillage (Brochu et al. 2021). With information about the importance of ~~*E*~~*X. pruinosa* to the ~~Three Sisters Garden~~TSG and current management recommendations, it is possible to both maintain healthy pollinator populations and ensure adequate pollination of culturally significant food plants. In the absence of squash bees, honey bees and bumble bees are known to be effective pollinators of *Cucurbita* crops (McGrady et al. 2021); however, these pollinators can create competition for resources with squash bees (Brochu et al. 2021) and are not as effective due to their foraging activity taking place primarily after the early morning pollination window for *Cucurbit*~~*at*~~ plants (Willis Chan and Raine, 2021). Therefore, using management recommendations for squash bees can increase their populations as well as pollination of *Cucurbita* crops by squash bees. Kovacs-Hostyanszki et al. (2017) recommend managing agricultural areas at the landscape-scale rather than the farm-scale, which is contingent on communication ~~between~~ among those growing the ~~plants~~crops.

Based on abundance, *B. impatiens* and ~~*E*~~*X. pruinosa* were the most common floral visitor bee species in the ~~Three Sisters gGarden~~TSG system. Tucker and Rehan (2016) found *B. impatiens* to be the most abundant species found in their recent study as well. *Bombus impatiens* is a bumble bee species with a broad distribution in North America that is also used as a managed pollinator (including outside its native range) (Ratti and Colla, 2010). It has a wide diet breadth, frequently found on both native and introduced plant species (Williams et al. 2009; Colla and Dumesh, 2010; Richards et al. 2011; Colla et al. 2012; Williams et al. 2014). In this study, *B. impatiens* had the highest overall diet breadth of all bee species, which would explain its ubiquitousness in wild and managed systems. While *B. impatiens* was a common visitor to the

Commented [SW11]: I heartily agree. Especially with respect to pesticide exposure

Commented [SW12]: Again, I agree. Are these species picking up pollen? Are they moving it from flower to flower? Do they groom it off themselves? These are all very interesting questions to consider. Cucurbita pollen is very large, spiney and oily.

Commented [SW13]: environment

Commented [SW14]: That makes sense.

Commented [SW15]: Tell us how the TSG may already be implementing some of these things (no pesticides, no tillage) and how it could be improved. On a different note and for your interest only, my team and I have recently developed a mobile app called SCOUT IPPM that allows Cucurbita growers to monitor pests and pollinators simultaneously. It can be downloaded for free from either google play or the app store. Researchers will have access to the data generated by growers when they use the app.c

Commented [SW16]: I disagree. If present, squash bees outcompete honey bees for Cucurbita floral resources. They simply get to the flowers way ahead of the honey bees.

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Commented [SW17]: There is some very specific and hard hitting data on the effects of exposure to imidacloprid for squash bees in Ontario. It may warrant inclusion here. Doi: 10.1038/s41598-021-83341-7.

Commented [SW18]: This sentence seems to have something missing. Do you mean that growers should communicate with each other?
I simply don't see how you would manage agricultural areas at the landscape level unless the land is not owned privately, which it mostly is. Agriculture is complicated. There may be many crops, many management styles, many personalities within a landscape.
Also, you seem to have drifted away from considering TSG into monoculture production of Cucurbita here.

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~~C~~cucurbits, it is unlikely that this species was contributing to pollination of the *Cucurbita* flowers. Willis~~-~~Chan and Raine (2021) found that bumble bees in Ontario are active after the critical pollination window for ~~C~~cucurbits, and therefore are not significant pollinators of the crop. While bumble bees and honey bees are found visiting *Cucurbita* flowers, it is likely that the pollen has been depleted and the bees are foraging for nectar (Percival 1947, Artz and Nault 2011, Brochu et al. 2020, Willis Chan and Raine, 2021). Pollen supply on staminate flowers has been found to decrease by approximately 60% within the first hour after the flowers open (Brochu et al. 2020 and Willis Chan and Raine, 2021). This information further highlights the importance of *~~E~~X. pruinosa* to the ~~Three Sisters garden~~TSG system. Willis Chan and Raine (2021) found that the pollination window of *Cucurbita* in Ontario to be between 6:00am and 8:00am. Future studies investigating the role of *~~E~~X. pruinosa* to the ~~Three Sisters garden~~TSG should take this into account during experimental design.

Within a pollination network, high connectance and high interaction diversity are associated with stability and resilience (Tscharntke 2021). The overall weighted connectance of 0.08 is the same value found in wild bee pollination networks in northern New England (Tucker and Rehan, 2016). The weighted nestedness of 0.47 is similar to this study as well (0.51) (Tucker and Rehan, 2016). The authors concluded that both connectance and nestedness were low, indicating that the pollination network in their study may not be resilient to change and may be impacted by significant disturbances (Tucker and Rehan, 2016).

Based on the similar results of our study, we ~~can conclude~~deduce that the ~~Three Sisters Garden~~TSG plant-pollinator network may not be resilient to environmental perturbations. Brosi and Briggs (2013) suggest that plant-pollinator networks overestimate the resiliency of pollination networks to perturbations and found that the removal of one pollinator species can affect~~s~~ the quality of reproduction of plant species in the system. Brosi and Briggs (2013) suggest that this is because when a pollinator is removed, the plant-pollinator network finds species within the system that will replace the pollination service to the plants visited by this particular pollinator species, but in doing so the network does not consider the pollinator effectiveness of each species. Pollinator effectiveness refers to how well a species of pollinator moves pollen and sets fruits and seeds for a particular plant (Brosi and Briggs, 2013; McGrady et al. 2021). Therefore, it is important to study not only the plant-pollinator network but also particular interactions between a plant species and its specific pollinators. If *~~E~~X. pruinosa* were not present, *Bombus* species could provide adequate pollination in terms of pollen grains deposited (McGrady et al. 2021)~~, however the pollination~~ as visits would be outside of the pollination window ~~where~~when the plants are most receptive (Willis Chan, 2021).

The second most frequently visited plant in this study was *N. r~~R~~ustica*. Hopi tobacco is a culturally significant plant to many Indigenous peoples (Brokenleg et al. 2013; Sadik, 2014). *Nicotiana rustica* is a monoicous plant reported to produce up to two thirds of its seeds through self-pollination and is pollinated by bees (Mather & Vines, 1952). In this study, *N. rustica* supported the highest diversity of bee species and the second highest abundance of bee visits. *Augochlorella aurata* represented 16% of the records in the pan trap samples from the wild bee

Commented [SW19]: Cucurbita

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Commented [SW20]: Hmmm. I don't think that is what the paper said. I quote from the abstract of that paper "Peak activity of hoary squash bees and bumble bees coincided with the daily crop pollination window, whereas peak activity of honey bees and other wild bees occurred after that window."

Commented [SW21]: My work Willis Chan & Raine, 2021 does not support this statement. See earlier comment.

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families, suggesting growing *N. rustica* may provide important foraging resources to wild bees. While *N. rustica* is self-compatible (Mather & Vines, 1952, Raguso et al. 2003), visits by insects increase its reproductive success (Adler et al. 2012; Gibson et al. 2022). *Augochlorella aurata* belongs to the family Halictidae (sweat bees), which like most other sweat bees nest~~s~~ in bare soil (Buckley et al. 2019), but it is a species that has also been found to be an important pollinator of *N. rustica* (Gibson et al. 2022).

Commented [SW25]: This is very interesting. I am presently working on a project to characterize pesticide hazard to wild bees associated with agriculture. A. aurata ranks very high on the list of wild bee species most at hazard. Unfortunately this work is not yet published but I thought it might interest you.

Management recommendations for Halictidae includes providing appropriate nesting and foraging resources (Buckley et al. 2019). Avoiding tilling the soil is a key factor in providing nesting space for sweat bees (Buckley et al. 2019). Lewandoski (1987) reports that upon observation of European agriculture, the Seneca people who had traditionally grown the Three Sisters Garden were shocked by the "wounding of Mother Earth" occurring, which was a reference to the tillage of the soil. In some cases, however, mild tilling has a positive impact on ground-nesting bee abundance in agricultural areas (Cusser et al. 2023).

Beans, forming a large component of the Three Sisters Garden, had some of the lowest observed rates of bee visitation. ~~. Ibarra-Perez et al. (1999) reports that *P. vulgaris* self-pollinates as the flower opens; thus, the plant does not require insect pollination (Ibarra-Perez et al. 1999). It is also noted, however, that the reproductive success of the plant (seed yield) can be increased by visits by larger bees (carpenter bees, bumble bees) (Ibarra-Perez et al. 1999). Interestingly, *P. coccineus* has been noted to set few pods without the presence of insect visitors (Darwin, 1876, Free, 1966, Free and Racey 1968, Kendall and Smith, 1976).~~ Only *B.~~ombus~~ impatiens* was observed visiting *P. coccineus*, despite the other genera being present and visiting other plants within the plant community. Overall, bee visitation to both bean varieties was quite low. Beans are mostly self-pollinated and with limited floral resources available, and yield is only marginally increased by insect pollination (Ibarra-Perez at al. 1999).

Indigenous and other local communities support pollinator conservation through (1) supporting biocultural (biological and cultural) diversity, (2) landscape management, and (3) diversified farming systems (Hill et al. 2019). Here we examine one of those diversified farming systems. The Three Sisters is a key feature of northeastern North American Indigenous agriculture (Lewandowski, 1987). The intercropping growing method of the Three Sisters Garden has been found to support not just humans physically and spiritually but also a wide diversity of wild pollinators through foraging and nesting provisions. Supporting the cultivation of culturally appropriate foods, and therefore food sovereignty, simultaneously supports and depends on the conservation of wild bee species.

Conclusions

In this study we aimed to determine the plant-pollinator network in a Three Sisters Garden in the Great Lakes Region. The results of this study highlight *Bombus impatiens* and ~~*Eucera*~~ *Xenoglossa pruinosa* as important pollinators in the TSG~~garden~~ system. Three of five bee families found in nearby natural areas were also found in the garden system. One limitation of this study is the lack of sweep net sampling in natural areas which may be an avenue for future research. Future directions may also include more research efforts focused on the critical role of wild pollinators in culturally significant plants and the applied policies and programs towards promoting their conservation and diversity.

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