The Effect of the Three Sisters, the Traditional Indigenous Intercropping Method, on Insect Abundance and Diversity

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ABSTRACT

Habitat loss from intensive agricultural practices is a primary driver of global insect decline. While conservation and restoration efforts in agroecosystems often increase beneficial insect presence, the ecological impact of adopting traditional agricultural techniques as mitigation strategies remains poorly understood. I investigated the effects of the Indigenous Three Sisters intercropping method and alternative planting combinations (monocrop and dicrop) on wild bee and ground beetle communities, as well as crop yield and quality in southern Manitoba and northern Ontario, Canada. The goal of this study was to establish a baseline of beneficial insect communities associated with the Three Sisters by comparing communities observed in alternative crop configurations and in the surrounding natural habitat, while also evaluating harvest metrics to inform future Three Sisters research. I collected 1,882 bees using bee bowls, blue vane traps, and targeted aerial netting, and 11,696 beetles using pitfall traps over two years. Using generalized linear mixed models, I found that the Three Sisters and other sunflower-containing treatments increased bee abundance and richness relative to other crop configurations, whereas beetle communities were unaffected by treatment. Within an exclusively Three Sisters garden, beetle communities were more abundant and diverse than in natural habitats, while the garden edge had no influence on either insect assemblage. Finally, I found crop yield and quality did not differ among Three Sisters, monocrop, and dicrop treatments or by spatial position (exterior vs. interior), but non-parametric testing indicated site location influence on harvest metrics and potential ecosystem service provisioning. These results indicate that the Three Sisters system has the potential to enhance insect assemblage and agricultural performance, but outcomes are highly context dependent. Crop variety and land management techniques should be considered at local scales in collaboration with Indigenous communities to guide the balancing of practical, ecological, and cultural considerations to foster long-term sustainable agriculture.

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CONTRIBUTION OF AUTHORS

Victoria Smelko developed the methodology and experimental design of this study. Victoria Smelko performed all field experiments and collected data for the Manitoba study sites. Data collection and field experiments at The Ontario Crops Research Centre's (Emo and New Liskeard) and the Collège Boréal (Sudbury) were supported by on-site staff. Victoria Smelko examined all specimens and carried out the curation, statistical analysis, interpretation, and the generation of figures for all collected data. Victoria Smelko was the sole author of the literature review, Chapter two, Chapter three, and conclusion with support from Dr. Kyle Bobiwash. Project idea and research objectives conceived by Victoria Smelko and Dr. Kyle Bobiwash.

I would like to dedicate my thesis to my Mom, Dad, Nonna, and little brother.

Thank you for always supporting my dreams…

no matter where they take me.

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INTRODUCTION

Within this study, I examine and compare the influence of intercropping and conventional monocropping on wild bee and ground beetle communities in southern Manitoba and northern Ontario. My thesis is organized into three chapters. The first chapter is a literature review where I explore insights from past publications on the relationships that insects have with current, traditional, and Indigenous agricultural practices. The second chapter is a research manuscript that focuses on quantifying wild bee and ground beetle biodiversity found within the Indigenous intercropping of the Three Sisters and other planting iterations of selected Indigenous crop plants. Concurrently, this chapter evaluates and compares harvest metrics (yield and quality) of select Indigenous crops in multiple planting combinations. The third and final chapter is a second research manuscript that focuses on creating baseline biodiversity metrics for wild bee and ground beetle communities in Three Sisters gardens compared to the surrounding natural landscape and exploring possible edge effects. Similar to Chapter two, the Three Sisters crop harvest metrics are evaluated and compared to one another, including assessing possible edge effects and inferring pollination and pest control provisioning.

The objectives of this thesis are:

i. Document wild bee and ground beetle biodiversity and functional composition associated with Indigenous significant crops grown in monocrop and intercrop systems.
ii. Establish a baseline of wild bee and ground beetle abundance, diversity, and dietary specialization associated with the Three Sisters system.
iii. Evaluate yield and quality of Indigenous significant crops within a variety of planting combinations and Three Sisters systems and determine ecosystem service influence.
iv. Use the findings to inform and guide future Three Sisters focused projects and land management practices for increasing insect biodiversity.

Based on the published literature reviewed in Chapter one, I predict that wild bee and ground beetle abundance, species richness, and Hill-Shannon diversity will be greater and more specialized in planting combinations with increased diversity compared to less diverse mixtures, as well as the surrounding natural landscape. Furthermore, I expect that an exclusively Three Sisters garden will have greatest insect biodiversity and more specialized bees within its interior

compared to its edge. Finally, I anticipate greater overall yield and quality of harvest in more diverse plant mixtures and in the interior of a Three Sisters garden compared to the edge, with provisioning of potential pollination and pest control services being associated with increased harvest yield and quality.

CHAPTER 1: LITERATURE REVIEW

Insects in Agriculture

Insects represent the most dominant and taxonomically diverse group of terrestrial animal life on Earth, with 1 million named species out of an estimated 5.5–6 million, rendering them fundamental to the maintenance, regulation, and stability of ecosystem functioning (Mora et al. 2011, Stork 2018, Noriega et al. 2018, Verma et al. 2023). Human socio-economic, cultural, and health systems are intimately linked to ecosystems as they support a suite of services that directly and indirectly sustain human well-being (Hooper et al. 2005, Noriega et al. 2018, Verma et al. 2023). Provided by insects, these ecosystem services include provisioning resources such as food, fiber, and fuel; regulating services involving climate, pest control, decomposition, and nutrient cycling; and cultural services related to recreation, education, spiritual, and aesthetics (Hooper et al. 2005, Zhang et al. 2007, Noriega et al. 2018, Verma et al. 2023). Agriculture represents humankinds most extensive and intensely managed ecosystem on earth and therefore both relies upon and provides these ecological processes (Zhang et al. 2007, Ritchie and Roser 2019). Within agricultural systems, insects perform a range of essential ecosystem services, including pollination, pest regulation, organic matter decomposition, and soil nutrient cycling (Zhang et al. 2007, Verma et al. 2023). To evaluate these contributions, insects are commonly categorized into functional groups representing a distinct ecological role: pollinators, herbivores, predators, parasitoids, and detritivores (Zhang et al. 2007).

Ecosystem Services - Functional Diversity

Pollinators

The majority of flowering plants worldwide, including economically important food, fibre, and forage crops, rely on pollinators to facilitate important steps in a plant's reproductive cycle by transporting pollen from the anther of one flower to the stigma of another (Ollerton et al. 2011). Most flower-visiting insects belong to the four largest insect orders: beetles (Coleoptera), flies (Diptera), moths and butterflies (Lepidoptera), and bees, wasps, and ants (Hymenoptera) (Wardhaugh 2015). Bees (Apoidea: Anthophila) are considered the most important pollinators of the majority of animal-pollinated crops (approximately 75% of crop species) (Klein et al. 2006, Potts et al. 2010)

Herbivores

Phytophagous insects play a central role in agricultural ecosystems, being both a threat to a plant's survival and a driver of plant abundance and diversity (Schuman and Baldwin 2016, Garcia and Eubanks 2019). Most notable insect orders containing herbivores in descending order are Coleoptera, Lepidoptera, Hemiptera (true bug), Hymenoptera, Orthoptera (grasshopper, locust, crickets), and Diptera (Garcia and Eubanks 2019). Generally, insect herbivores feed on all parts and structures of plants (roots, stems, seeds, etc.), damaging 18% of the world's crop production. (Jankielsohn 2018). However, they also provide beneficial regulatory services by reducing certain pest populations through removing weed communities or signalling plants to produce defensive metabolites (Schuman and Baldwin 2016, Noriega et al. 2018).

Predators and Parasitoids

Predatory and parasitic insects both play a major role in maintaining ecological balance within an ecosystem, acting as natural biological control for phytophagous pest populations (Laxmi Rai et al. 2015, Jankielsohn 2018). Across all life stages, predator insects capture and feed on many different kinds of prey, allowing predators to survive through limited prey conditions (Laxmi Rai et al. 2015, Jankielsohn 2018). Whereas parasitoids are more selective in their prey, killing it or suppressing its growth slowly by laying eggs or living on/in its body (Laxmi Rai et al. 2015). Both contribute to the regulation of pest populations and are responsible for 33% of natural pest control seen in agroecosystems (Chellappan and Ranjith 2023). Predaceous insects are found within approximately 20 insect orders, but parasitoids mainly belong to two orders: Hymenoptera and Diptera (Laxmi Rai et al. 2015, Chellappan and Ranjith 2023).

Detritivores

The decomposition of vegetation and animal matter is an incredibly important ecosystem process that changes the structure of habitats and is largely provided by insects (Louzada and Nichols 2012, Jankielsohn 2018). Insects accelerate the rate organic matter breaks down and is recycled into the soil through both physical (e.g., shredding, burrowing) and chemical mechanisms (e.g., feeding aided by enzymes, spreading frass) (Yang 2006, Louzada and Nichols 2012, Verma et al. 2023). Within an ecosystem, insect detritivores are responsible for processing

95% of all detritus (Yang 2006). Decomposers are found in orders such as Coleoptera, Hymenoptera (ants especially), Diptera, and Blattodea (termites and cockroaches) (Louzada and Nichols 2012, Menta and Remelli 2020, Verma et al. 2023). The presence of these detritivores can also be an indicator of ecosystem health and soil quality (Menta and Remelli 2020).

Key Insect Group - Wild Bees

Bee Diversity

Bees represent more than 20,000 described species across seven families, six of which occur in Canada (Apidae, Andrenidae, Colletidae, Halictidae, Megachilidae, and Melittidae), where about 900 species are known (Michener 2007, Canadian Endangered Species Conservation Council 2022, Gibbs et al. 2023). About half (48%) of the Canadian bee taxa can be found within the Prairie ecozones (Alberta, Manitoba, and Saskatchewan) (Sheffield et al. 2014). Several non-native species, most notably the European honey bee (*Apis mellifera* L.), have colonized Canada (Caron 2021, Canadian Endangered Species Conservation Council 2022). The majority of bees are solitary, raising offspring alone, while only 6% of bee species exhibit eusocial behaviour characterized by division of labour, overlapping generations, and cooperative brood care (Danforth 2007). Bees also exhibit variation in their foraging strategies and floral resource use, which fundamentally shape their ecological roles and interactions with plants.

Diet

Nest-building bees are central place foragers, repeatedly returning to stock their nests with pollen, nectar, and rarely floral oils (Michener 2007, Amaya-Márquez and Wells 2008). Bees typically forage several hundred metres from the nest, but range varies based on body size, energetic cost, and habitat quality, making them particularly susceptible to habitat changes (Greenleaf et al. 2007). Nearly all eusocial bees collect provisions from flowers of many species (polylectic) but have a tendency to have learned fidelity (floral consistency) to flowers of the same species on long flights or under conditions where floral reward such as quality or abundance of provisions is high (Michener 2007, Amaya-Márquez and Wells 2008, Amaya-Márquez 2009, Nicholls and Hempel de Ibarra 2017). The majority of solitary species are inherently specialists (oligolectic) with a narrower diet, preferring a single plant species, genus, or family (Michener 2007, Sheffield et al. 2014). Within Canada, the Prairie ecozone has the

highest percentage of floral specialists, with greatest preference for the daisy family (*Asteraceae*) (54%), specifically sunflower (*Helianthus*) (11%) (Sheffield et al. 2014). The level of specialization in bee diet is referred to as diet breadth or lecty. This species-level functional trait is typically applied to non-parasitic bees, as parasitic or cleptoparasitic species, which make up a minority of bee fauna, do not build or provision their own nests but instead lay their eggs in the nests of other bee species (Michener 2007).

Key Insect Group - Carabid Beetles

Beetle Diversity

Carabid Beetles (Coleoptera: Carabidae), also known as ground beetles, represent a highly diverse insect family of approximately 40,000 species described globally, 900 being found in Canada (Lövei and Sunderland 1996, Goulet 2003). Behavioural mechanisms and physiological modifications have made ground beetles highly sensitive to their surroundings and led to the development of a flexible set of responses to both abiotic and biotic factors (Lövei and Sunderland 1996, Larochelle and Larivière 2003). Consequently, carabids are effective providers of vital ecosystem services and valued ecological indicators for ecosystem health and habitat modification (Larochelle and Larivière 2003, Holliday et al. 2014). Despite their prominence in ecological studies, the natural history and ecology of most carabid species remain poorly understood, with information scattered throughout literature, personal communications, and museum collections, owing to cryptic early life stages, variations in behavioral traits, and contradictory results between laboratory and field observations (Desender et al. 1994, Lövei and Sunderland 1996, Holland 2002, Thomas et al. 2002, Goulet 2003, Kotze et al. 2011).

Habitat

Although carabid beetles are known to occur on almost all continents (except Antarctica) (Desender et al. 1994), it is still undetermined if distributions of specific species are governed by one, several, or a combination of the factors (Lövei and Sunderland 1996, Rainio and Niemela 2003). The most impactful environmental conditions that greatly influence habitat preference include land management, temperature, humidity, vegetation, light, season, altitude, food conditions, presence and distribution of competitors, and soil conditions (Lindroth 1961-1961, Luff and Rushton 1992, Lövei and Sunderland 1996). Carabid habitat choice is so narrow that

assemblages are often used to characterize habitats (Lövei and Sunderland 1996, Holland 2002), thus, making carabids successful bioindicators (Rainio and Niemela 2003, Pearce and Venier 2006).

A bioindicator is a species or assemblage that reflects environmental conditions, signals the impact of change in a community or whole ecosystem, or serves as a proxy for the diversity of other species (Rainio and Niemela 2003). To be considered a good bioindicator, a species taxonomy and ecology should be well-known for easy differentiating between natural trends and those induced by anthropogenic stress, have a cosmopolitan distribution with some specializations towards certain habitats, be able to provide early warning signs of change, be easy and cost effective to survey, and its response should reflect the response of other species (Rainio and Niemela 2003). For instance, in North American agroecosystems, the generalist species *Pterostichus melanarius* (Illiger) functions as a bioindicator of agricultural disturbances (e.g., tilling), with its abundance indicating soil health, vegetation complexity, and altered ecosystem functioning (Pearce and Venier 2006, Müller et al. 2022).

Diet

Carabids have a mixed diet, mainly consuming live prey (insects) or carrion, also plant material either exclusively or interchangeably (Lövei and Sunderland 1996, Holliday et al. 2014, Sreedevi et al. 2023). Similar to the difficulties determining patterns in habitat preference, carabid diets are so varied that preferences are mostly generalizations (Lövei and Sunderland 1996, Holland 2002). Because carabid beetles must consume their body mass in food daily, certain species have long been recognized as important biocontrol agents (Lövei and Sunderland 1996, Sreedevi et al. 2023). Species in the genera *Amara, Bembidion, Carabus, Calosoma, Harpalus,* and *Pterostichus* target a range of aphid species; Dipteran and Coleoptera early life stages; Lepidopterans; and slugs (Holland 2002, Sreedevi et al. 2023). Although early biocontrol efforts primarily focused on specialist predators, a generalist's feeding strategies make them highly efficient in suppressing pest populations, and more importantly, in prolonging the period between pest outbreaks (Lövei and Sunderland 1996). In addition, many seed-feeding species (granivores), such as *Amara* and *Harpalus* spp., are effective biocontrol agents of certain weeds (Lindroth 1961-1961, Sreedevi et al. 2023).

Diversity Equals Function

The hypothesis that biodiversity enhances ecosystem function has become a central paradigm in ecology. While the idea gained popularity in the 1990s, its conceptual origins date back further. Charles Darwin (1859) was among the first to suggest multi-species assemblages leads to improved productivity, with ecologists such as Odum (1953), MacArthur (1955), Margalef (1969), and especially Elton (1958), later echoing these ideas and theorizing that mature, diverse ecosystems were also more stable and resilient (Schulze and Mooney 1994, Tilman et al. 2014). It was not until the studies by Tilman (1994, 1996, 2001) and Naeem et al. (1994) that these long-standing predictions were empirically validated, redefining biodiversity as a functional component of ecosystems.

Mechanisms and Metrics of Diversity

The influential and controversial nature of Tilman's grassland experiments fostered the expansion of biodiversity ecosystem functioning framework research into different areas of study, prompting the formulation of exhaustive lists of statistical criteria, analyses, methods, and mechanisms to assess biodiversity ecosystem functioning and relationships (Tilman et al. 2014, Brown and Williams 2016, Ali 2023). Taxonomic diversity (distribution of individuals among species) is the historical standard of measuring, most commonly through species richness (the number of species), followed by evenness (proportional abundance of each species), and differentiation (differences in species composition in an assemblage) (Chao et al. 2014a, Brown and Williams 2016, Jarzyna and Jetz 2016). There are several basic statistical methods for estimating richness (e.g., rarefaction, bootstrapping, and species-area curves), but because richness is sensitive to sampling effort and size, these methods do not account for variation in species relative abundances, and thus cannot reliably predict true community diversity (Chao et al. 2014b, Brown and Williams 2016, Magurran 2021, Roswell et al. 2021). Diversity metrics, most notably Shannon's and Simpson's indices, incorporate relative abundance by integrating richness and evenness; however, these indices are expressed in incompatible units and often exhibit high correlation, complicating interpretation and comparison (Chao et al. 2014b, 2014a, Roswell et al. 2021). To address these limitations, Mark Hill (1973) developed a unified framework that integrated species richness, Shannon diversity, and Simpson diversity to behave

in logical ways that summarize the relative abundances, differing in how they scale the rarity of a species (Jost 2006, 2007, Chao et al. 2014b, Roswell et al. 2021). This approach is known as Hill diversity, Hill numbers, or the effective number of species. For example, if a part of a community were removed, all Hill numbers would decrease by a certain proportion (Roswell et al. 2021). Species Richness gives high leverage to rare species, Hill-Simpson diversity favours common/dominating species, and Hill-Shannon diversity falls in-between (Roswell et al. 2021).

Analyses focused primarily on taxonomic diversity often fail to capture the differences in ecological roles, evolutionary histories, and trait variation within a community (Brown and Williams 2016, Magurran 2021). Leading to other approaches that quantify species from different perspectives, including functional diversity (differences in species traits and ecological roles) and phylogenetic diversity (evolutionary relatedness) (Chao et al. 2014a, Jarzyna and Jetz 2016, Magurran 2021). Additionally, the influence of random presence/abundance shifts along a time or gradient among a community is lacking from traditional species richness but is also crucial to consider (Brown and Williams 2016, Magurran 2021). For example, two sites can have similar species abundance and distribution but be assembled differently. In an analysis, ignoring species composition, such as variation in abundance of wild or specialist taxa, could be the difference in determining the appropriate way to develop land or select conservation management practices (Chao et al. 2014b, Socolar et al. 2016, Magurran 2021). To address this, the concept of three levels of diversity were introduced: alpha (α) diversity (local diversity of a site or community), beta (β) (compositional difference of these sites), and gamma (γ) diversity (diversity of all the sites in the entire wider assemblage) (Magurran 2021, Roswell et al. 2021). Although the three dimensions of diversity (taxonomic, functional, and phylogenetic) focus on different perspectives of select species, they all can be viewed from the perspective of alpha, beta, and gamma diversity (Magurran 2021, Roswell et al. 2021). Furthermore, measuring diversity at only one or a few spatio-temporal scales may still be insufficient to describe biodiversity gradients in nature; therefore, Hill numbers are commonly used with alpha, beta, and gamma components to compare patterns across different regions and timescales (Magurran 2021, Roswell et al. 2021). Of equal importance, and one of the more challenging measures in biodiversity, is controlling for sampling effort, as the amount of diversity is tied to effort and scale (Chao et al. 2014b, Brown and Williams 2016, Magurran 2021). Colwell et al. (2012) unified the rarefaction and extrapolation method for species richness, allowing for

standardization based on sampling efforts, while Chao and Jost (2012) coverage-based approach standardized based on sample completeness. The integration of these two frameworks and the three dimensions of diversity within the context of Hill numbers, enhanced cross-system comparisons and ecological interpretations (Chao et al. 2010, 2014b). These innovations and others have not only standardized biodiversity measurement in ecologically meaningful ways but also improved researchers' ability to link biodiversity with specific ecosystem functions (e.g., nutrient cycling, carbon storage, decomposition, resistance to disturbances, and erosion control) (Diaz et al. 2006, Jost 2007, Chao et al. 2014a, Roswell et al. 2021).

Disturbances to Insect Biodiversity in Agroecosystems

The expansion and intensification of agriculture during the 20th century paralleled the rapidly growing human population to combat the global food demands, but these gains came with substantial ecological costs (Tilman 1999). The permanent destruction, degradation, and fragmentation of non-agricultural ecosystems are among the main consequences of intensive agricultural practices that now expands over a third of the world's usable land (Tilman 1999, Tilman et al. 2011, Dudley and Alexander 2017). Agriculture intensification can be defined as the modification of natural landscapes and the overtaking of traditional small-scale farming practices to create large monocultures for the purpose of high crop productivity using techniques such as the use of fertilizers, pesticides, and high disturbance agronomic practices (e.g., tillage) (Dudley and Alexander 2017, Raven and Wagner 2021, Hemberger et al. 2021). As a result, agriculture has become the main driver of global biodiversity decline, encompassing the major interacting systematic factors responsible for species losses worldwide: habitat loss, intensive use of pesticides and herbicides, pollution, introduction of invasive species, and climate change (Norris 2008, Tilman et al. 2011, Dudley and Alexander 2017, Sánchez-Bayo and Wyckhuys 2019, Raven and Wagner 2021, Bali and Kaleka 2022, Dar et al. 2022, Grevé et al. 2024).

Insects are no exception to this decline; however, despite their great abundance, richness, and cosmopolitan distribution, recognition and concern of widespread insect decline has emerged only within the past few decades (Dirzo et al. 2014, Sánchez-Bayo and Wyckhuys 2019, Wagner 2020). Long-term studies have revealed striking collapses in insect biomass and richness, including a steady >70% decline in flying insects across agricultural fields over the past three decades (Hallmann et al. 2017), and 78%-98% reductions in ground-dwelling and foraging

arthropod biomass (Sánchez-Bayo and Wyckhuys 2019). Insect declines rarely occur in isolation, with losses extending across entire lineages and trophic levels, triggering cascading effects that compromise ecosystem stability and services (Dirzo et al. 2014, Hallmann et al. 2017, Sánchez-Bayo and Wyckhuys 2019, Wagner 2020). Hallmann et al. (2017) and other studies, such as Benton et al. (2002), document a parallel decrease in insect biomass and insectivorous vertebrates. The recognized declines in insect biomass and diversity highlight how intensive agricultural practices have already reshaped ecosystems, and the forecasted need to double global crop production by 2050 (Tilman et al. 2011) will likely further escalate insect loss.

Habitat Loss

Habitat loss is the greatest current threat to insects, as it can directly result in the extinction of species and long-term disruptions to ecological communities (Laurance 2010, Heinrichs et al. 2016, Bali and Kaleka 2022). It often occurs through human conversion of natural environments and can be categorized into three processes that often happen simultaneously (Heinrichs et al. 2016, Bali and Kaleka 2022). The first being habitat destruction, which involves processes that destroy or damage a natural ecosystem to the point it is no longer capable of supporting species and ecological communities that it originally sustained (Heinrichs et al. 2016, Bali and Kaleka 2022). This occurs mainly through land clearing (e.g., deforestation, tillage) and removal of natural vegetation features (e.g., hedgerows, wind breaks) (Bali and Kaleka 2022, Grevé et al. 2024). An example of this occurring is the Rocky Mountain grasshopper (*Melanoplus spretus* (Walsh)*)*, once a serious agricultural pest with abundances estimated at 15 trillion individuals across western and central North America, which was forced into extinction after dramatic expansion of agricultural activity (Gaston and Fuller 2007). Secondly, habitat degradation is the decline of biological conditions in an ecosystem that further degrades and reduces the quality of the environment, making it difficult for plant and animal communities to thrive (Heinrichs et al. 2016, Bali and Kaleka 2022). This happens through human- and non-human mediated expansion, where land is frequently lost to erosion, desertification, nutrient depletion, pollution (pesticides/spillover), invasive species, and overexploitation of natural resources (Gaston and Fuller 2007, Bali and Kaleka 2022). For instance, neonicotinoid insecticides are widely used in controlling pests found in wheat and

sunflower fields, but also affects non-target beneficials such as bumble and wild bees, both directly through contact with treated plants and indirectly via spillover, with the eventual residue accumulation degrading foraging and nesting habitats, increasing mortality rates and weakening pollination services (Scott-Dupree et al. 2009, Kerr et al. 2015). Thirdly, habitat or land fragmentation is the break-up of land into smaller, disconnected patches, causing restricted movement, reduced range, and isolated populations, leading to decreased genetic diversity (Fahrig 2003, Laurance 2010, Bali and Kaleka 2022). Fragmentation also increases the proportion of habitat influenced by edges, a phenomenon often referred to as the edge effect hypothesis, which states that diversity is higher at an edge than in adjacent interiors (Fahrig 2003, Nguyen and Nansen 2018). For instance, ground beetles often show higher abundances and richness of generalist or disturbance-tolerant species at field margins compared to patch interiors where altered microclimates and interactions occur, potentially reshaping insect communities (Magura et al. 2001).

Climate Change

Since industrialization, climate change has rivalled habitat loss as the major driver of insect decline, as the impact of climactic events on insect abundance, distribution, or phenology are difficult to predict and not usually apparent until after critical survival thresholds are surpassed (Travis 2003, Wagner 2020, Hill et al. 2021, Harvey et al. 2023). Rising temperature alone is a detrimental environmental stressor on insects and interacting species, as it has created gradual long-term changes such as a longer growing season and variable weather patterns (e.g., change in rainfall, snow cover, cloud cover, heatwaves) (Wagner 2020, Harvey et al. 2023). Culminating in shifts in historical ranges and species density or changes to phenology, behaviour, and morphology at a species level (Bali and Kaleka 2022, Harvey et al. 2023). For example, in some insects such as butterflies and moths, elevated temperatures have been found to correlate with quicker development and decreased body size, jeopardizing dispersal capacity and fecundity (Hill et al. 2021). However, in some species like North American and European bumblebees, such plasticity does not occur resulting in permanent range shifts north to higher elevations and complete disappearance from southern range limits, weakening plant pollinator networks (Kerr et al. 2015, Scheffers et al. 2016). The rise in temperature is also responsible for the increase in frequency, duration, and intensity of extreme weather events (e.g., floods, fires,

tornadoes, hurricanes, volcanic activity, earthquakes, and drought), which amplify the severity of the consequences above and create new challenges for insect survival (Skendžić et al. 2021, Harvey et al. 2023). For example, flooding can have direct and indirect effects on soil-dwelling insects by causing displacement or mortality by drowning, dislodging plants used for shelter or food and thereby increasing vulnerability to predators, or altering microclimatic conditions that influence feeding activity or extend development time (Skendžić et al. 2021, Harvey et al. 2023)

Mitigating Insect Decline in Agroecosystems

Based on the impact current drivers of biodiversity loss have on insects, it is speculated that 40% of the world's insect species will be extinct within the next few decades (Sánchez-Bayo and Wyckhuys 2019). To combat this, traditional mitigation approaches such as the expansion of conservation areas, reduction of chemical inputs, organic farming, and restoration of degraded landscapes have become widely adopted in biodiversity protection efforts (Dar et al. 2022). However, applying these strategies within agricultural landscapes presents significant challenges given the need to maintain and eventually increase global food production; simply reducing the intensity or scale of agriculture is not feasible (Pretty 1997, Tilman et al. 2011). Modern agriculture is a dominant and permanent fixture of the landscape. Despite being characterized by depleted resources, fragmentation, and structural simplification, agricultural land is not a featureless or universally unsuitable habitat, but a heterogeneous matrix in which "suitability" varies among species (Norris 2008, Shuey 2013). Recognizing agriculture as a habitat consisting of natural/seminatural and managed components reframes biodiversity mitigation as a challenge of integration rather than exclusion (Norris 2008). With this shift in perspective, it is crucial that biodiversity loss mitigation moves beyond the traditional and often preservationist framework toward approaches that explicitly operate within production systems.

Sustainable Intensification

The sustainable intensification (SI) of agriculture is a concept of increasing food production while simultaneously protecting and minimizing damage to the environment through the integration of management technology and the more efficient utilization of naturally occurring regenerative resources (Pretty 1997). Although traditional mitigation techniques, such as small-scale farming, are presented as equivalent to SI and have been known to mitigate insect

decline, the efficacy of these strategies used separately is often exaggerated and impractical to meet forecasted growing food demands (Tilman et al. 2011, Petersen and Snapp 2015, Tscharntke et al. 2021, Chowdhury et al. 2023). The SI paradigm is purposely without defined technologies, practices, or policies in order to accommodate changing environmental conditions, cultural and agronomic differences, and the context-dependent performance of management strategies across ecosystems (Pretty 1997, Xie et al. 2019). Currently, the range of practices include the diversification of cropping systems (e.g., intercropping, alley cropping, crop rotations, and cover crops), soil and water conservation techniques (e.g., conservation tillage, integrated nutrient management, irrigation, etc.), integrated pest management (IPM), and restrictive use of pesticides (Petersen and Snapp 2015, Wezel et al. 2015, Xie et al. 2019).

Conservation

Conservation remains the primary global strategy for halting environmental degradation and mitigating insect biodiversity loss, facilitated through enhancing landscape connectivity and ecological resilience to environmental change (Shuey 2013, Kearney et al. 2020, Chowdhury et al. 2023). Historically, conservation has been implemented through the spatial separation of protected areas from agricultural production, reinforcing the perception that agriculture and conservation are incompatible (Tscharntke et al. 2005). However, multiple studies highlight a substantial portion (~75%) of threatened species depend on existing within managed landscapes, and over half of those species face one or more threats that require actions conservation areas alone cannot remove (Tscharntke et al. 2005, Kearney et al. 2020).

Within the framework of SI, strategies such as land sparing and land sharing represent alternative conservation planning approaches that address rising food demand while minimizing cost to biodiversity (Xie et al. 2019). Land sparing separates conservation from agriculture by maximizing farmland to facilitate the protection of remaining natural habitat from further expansion (Green et al. 2005, Fischer et al. 2008, Xie et al. 2019). Alternatively, land sharing embeds biodiversity-supporting features within crop fields using wildlife-friendly farming methods (Green et al. 2005, Fischer et al. 2008, Xie et al. 2019). Although land sparing and land sharing are often framed as contrasting conservation strategies, in practice, both approaches rely on ecological restoration as a central mechanism for conserving, recovering, or enhancing habitat quality (Rey Benayas and Bullock 2012). Land sparing restoration involves re-establishing

native vegetation or habitats on abandoned cropland, whereas land sharing restoration focuses on rebuilding or creating specific elements (e.g., hedgerow planting, riparian buffer restoration, or floral strips) to benefit ecological communities and services without competition for agricultural land use (Rey Benayas and Bullock 2012). The perceived dichotomy oversimplifies how biodiversity responds to agricultural intensification, as the ecological outcome of either approach depends strongly on the spatial scale at which they are implemented and the taxa under consideration (Green et al. 2005, Norris 2008, Phalan et al. 2011a, Rey Benayas and Bullock 2012). For example, habitat heterogeneity is perceived differently by insects then by human observers: a structurally, continuous block of natural vegetation protected through land sparing may contain a high diversity of houseplants and microhabitats for herbivorous and canopy-dwelling insects, whereas a purposely heterogenous strip of native vegetation created through land sharing practises may offer a comparatively limited range of resources at a spatial scale relevant to those taxa. (Green et al. 2005, Phalan et al. 2011a). Therefore, rather than representing mutually exclusive alternatives, land-sparing and land sharing should be viewed as complementary components of a multi-scale conservation framework that can be implemented in unison based on biophysical properties, socioeconomic context, and production targets (Green et al. 2005, Fischer et al. 2008, Phalan et al. 2011b, Rey Benayas and Bullock 2012).

Monocultures and Associated Insects

Most mitigation strategies emphasize diversification, a principle embedded in early agricultural practice. To understand how we arrived at today's rate of insect decline, it's necessary to examine the trajectory of agricultural development, particularly the rise of monocultures and the marginalization of traditional, ecologically integrated systems. Agriculture developed independently in many regions with limited recorded accounts prior to 1850, but Nikolai Ivanovich Vavilov's (1926) concept of the centres of origin for cultivated plants points roots almost exclusively to the Global South (Harris 1990, Sauer 1993, Federico 2005, Jacques and Jacques 2012, Ordish et al. 2024, Igamberdiev 2025). Archaeological and genetic evidence later reinforced this framework as a foundation for understanding plant domestication (Harris 1990, Sauer 1993, Fedick 1995, Federico 2005, Igamberdiev 2025).

History of Agriculture

The Americas (Mesoamerica and North America) offer a unique lens through which to understand the foundations of farming ~~i~~n a wide range of environments over time (Hurt 1987, Sauer 1993, Fedick 1995, Federico 2005, Jacques and Jacques 2012, Ordish et al. 2024). Early evidence of plant domestication in Mesoamerica appears in Tamaulipas (northeastern Mexico), Tehuacán Valley (southern-central Mexico), and Oaxaca Valley (southern-central Mexico), beginning around 7000-5000 BCE with key crops including gourds (*Lagenaria siceraria* (Molina) Standl. and *Cucurbita pepo* L.), maize/corn (*Zea mays* L.), runner beans (*Phaseolus coccineus* L.) and chilli peppers (*Capsicum* spp.) (Hurt 1987, Sauer 1993, Lira et al. 2016). Gradually, the cultivation of plants became increasingly important and farming, once naturally fueled by specific landscape features, climate, and environmental events, was manipulated by the Indigenous Peoples through seed selection, simple irrigation techniques, and manual land clearing efforts (Hurt 1987, Reitz et al. 2008, Zizumbo-Villarreal et al. 2012). Between 900 BCE and 200 CE, Mesoamerican agriculture became considered complex as simple techniques developed into reliable farming methods such as slash-and-burn vegetation clearing, field-rotation schedules, drainage canals, raised and stone terraced fields, and intercropping (Hurt 1987, Reitz et al. 2008). The most notable strategy was the milpa system (growing corn, bean, and squash in a mound) as it integrated multiple complex farming strategies (Zizumbo-Villarreal et al. 2012, Fonteyne et al. 2023).

North American agriculture followed a similar trajectory, with earliest evidence of domestication being squash (*C. pepo*) around 5000 BCE and gradual incorporation of other locally important crops (e.g., goosefoot (*Chenopodium* spp.), sumpweed (*Iva annua* L.), maygrass (*Phalaris* spp.), sunflower (*Helianthus annuus* L.) (Hurt 1987). Contrary to the Indigenous Peoples of Mesoamerica, North America's simple and complex agricultural practices were influenced directly from Mesoamerican techniques, as evidenced by a shared squash descent (Hurt 1987, Reitz et al. 2008).

The Green Revolution

European colonization disrupted Indigenous agricultural landscapes through land, dispossession, war, and cultural suppression (Hurt 1987, Reitz et al. 2008, Hart and Winchell-

Sweeney 2023). Colonial policies imposed foreign land-tenure systems and altered long-standing cultivation traditions. Post-war economic pressures and population growth during the 19th and early 20th century accelerated agricultural intensification (Fitzgerald-Moore and Parai 1996). As a result, the United States (US) and the United Nations Food and Agriculture Organization (FAO) initiated international collaboration efforts towards developing high-yield varieties of major grain crops (wheat, rice, and corn) (Rosset et al. 2000, Borlaug 2000, Pingali 2017). These efforts, now coined as the Green Revolution (GR) (Fitzgerald-Moore and Parai 1996, Evenson and Gollin 2003, Feldman and Biggs 2012, Jacques and Jacques 2012), culminated in the Rockefeller Foundation's Mexican Agricultural Program achieving a 250% per-acre increase in yield through the development of short-stemmed, fungus-resistant wheat (Fitzgerald-Moore and Parai 1996, Mann 1997, Rosset et al. 2000, Borlaug 2000, 2007). Replicated success with other major cultivars such as corn, was a pivotal technological advancement that led to the global transition of agriculture into a highly industrialized enterprise, expanding cropland and pastures by 154 million hectares (Mha) between 1985 and 2005 (Mann 1997, Evenson and Gollin 2003, Foley et al. 2005, Feldman and Biggs 2012).

However, the GR's legacy included profound ecological and social trade-offs. High-yield monocultures depend on a combination of high input chemicals (fertilizers, pesticides, fungicides, herbicides), large-scale irrigation infrastructure, and specialized equipment (Fitzgerald-Moore and Parai 1996, Borlaug 2000, 2007, Jacques and Jacques 2012). These capital-intensive requirements disproportionately favour large-scale operations and often displace small-scale landholders practicing more traditional agricultural methods, who lack the resources to adopt the necessary changes, thereby contributing to farm consolidation. (Rosset et al. 2000, Feldman and Biggs 2012, Jacques and Jacques 2012). Monocultures are now the dominant agricultural model occupying approximately 44% of the world's habitable land, with a third exclusively devoted to cropland (Ritchie and Roser 2019). Currently, over 2,500 species of flowering plants have one or more domesticated crop species with just 103 supplying over 90% of the direct or indirect calories consumed by humans (Dirzo and Raven 2003). The remainder of this section will discuss current crops central to this thesis that are both important in agriculture and hold cultural significance in Indigenous systems.

Corn

Corn (*Z. mays*) or maize is a cereal plant belonging to the grass family (Poaceae) (Sauer 1993). Molecular evidence suggests a single domestication event from teosinte (*Zea mays* subsp. *parviglumis* (Iltis & Doebley) Doebley) in Mexico around 5000 BCE (Matsuoka et al. 2002). Corn's ability to easily hybridize facilitated its rapid spread through the Americas, producing thousands of locally adapted varieties (Shultz 2008). Its high productivity and agronomic stability under diverse environmental conditions contributed to its adoption as the dominant staple crop in Indigenous communities, accounting for 50% to >70% of diets (Pleasant 2015, Hart and Winchell-Sweeney 2023).

Maize remains one of the world's most important crops, with global production constantly increasing exponentially. Over a 15-year period, global corn production increased from 590 million tonnes (Mt) to 960 Mt and is estimated to reach approximately 1.2 billion tonnes by 2021 (Wolf et al. 2017, FAO 2023b). The primary use of corn depends on the country/region, but in general, it is grown for human consumption, animal feed, and biofuel (e.g., ethanol) (Wolf et al. 2017, García-Lara and Serna-Saldivar 2018). However, despite its central role in global food and industrial systems, corn production is constrained by biotic stressors that reduce attainable yields and contribute to persistent yield gaps (Azerefegne et al. 2002, Oerke 2006, Mammadov et al. 2018). Pathogens and insect herbivory in particular are major contributors to these losses, with estimated global corn yield loss ranging from ~20-40% (Savary et al. 2019). Therefore, to meet rising global demands and mitigate yield losses from pest insects, continued genetic improvement through hybridization and transgenic approaches focusing on associated insects is crucial (Oerke 2006).

The insect community associated with maize is dominated by numerous herbivorous pests that attack every life stage of the plant, including Lepidopterans (e.g., armyworms, borers, cutworms, earworms, and grain moths), Coleopterans (grain borers, grubs, root worms and weevils), and Hemipterans (e.g., leafhoppers and aphids) (Ortega 1987). Among these, the European corn borer (*Ostrinia nubilalis* (Hübner)) is one of the most destructive and widespread pests, injuring stalks and ears, reducing grain yield and quality, and increasing plant susceptibility to secondary infections by fungus and other pathogens (Kaçar et al. 2023). Associated beneficial insects to maize are few, primarily naturally occurring predators (e.g.,

assassin bugs, lady beetles) and introduced parasitoids (e.g., parasitic wasps (*Trichogramma* spp.)) (Ortega 1987, Kaçar et al. 2023).

Bean

The common bean (*Phaseolus vulgaris* L.) is a legume in the Fabaceae family (Sauer 1993). The genus *Phaseolus* includes ~70 wild species, five of which were domesticated (Bellucci et al. 2014). *P. vulgaris* is the most widely cultivated due to its direct use in human consumption, representing 90% of the agricultural area dedicated to *Phaseolus* production (Singh 1999). Currently, *P. vulgaris* is considered to have two centres of domestication, occurring around 6000 BCE in Mesoamerica (Oaxaca valley) and the Andes (Bellucci et al. 2014). Historically, all parts of the plant were used, but the seed was the main protein source in Indigenous diets, eaten fresh (snap beans) or dried (pulse) (Lira et al. 2016, Celmeli et al. 2018).

Today, the common bean remains a staple food crop valued for its high protein, mineral composition, and bioactive compounds (Jones 1999, Uebersax et al. 2023, Meza-Maicelo et al. 2025). With global production of snap bean and pulse reaching ~1.6 Mt and ~28.5 Mt from ~25.8 Mha and ~37.7 Mha of land in 2023, respectively (FAO 2023a). Extensive breeding programs have been developed to increase yield, adaptability, and agronomic performance in common bean (Singh and Schwartz 2011, Meza-Maicelo et al. 2025). However, productivity is constrained by numerous biotic and abiotic stressors (Singh 1999, Meza-Maicelo et al. 2025). Particularly, insects and other pests cause substantial yield and quality losses, ranging from 35-100% globally (Singh and Schwartz 2011). Although resistance to several major insect pests has been achieved, durable resistance to regionally specific pests or across multiple taxa remains limited, leaving key ecological interactions, such as pest regulation, reliant on beneficial insect communities (Singh and Schwartz 2011, Wyckhuys et al. 2023).

Numerous insect pests attack *P. vulgaris* at various stages of growth, including sap-feeding Hemipterans (e.g., aphids, leafhoppers), stem and pod borers (e.g., bean flies), leaf-chewing Coleopterans (e.g., chrysomelids), and post-harvest bruchids (Singh 1999, Singh and Schwartz 2011). In addition to direct damage, insects (e.g., aphids and whiteflies) also act as vectors of numerous common bean viruses (Singh and Schwartz 2011). Leafhoppers (e.g., *Empoasca kraemeri* Ross & Moore and *E. fabae* (Harris)) are among the most widely distributed

and damaging pests in the Americas, capable of causing 80-100% yield losses (Singh 1999, Singh and Schwartz 2011).

Although *P. vulgaris* is predominantly self-pollinating, floral visitors from multiple insect orders (Hymenoptera, Coleoptera, Lepidoptera, and Diptera) have been recorded to facilitate low-frequency outcrossing (Kingha et al. 2012, OECD 2015). Several studies have identified carpenter bees (e.g., *Xylocopa olivacea* (Fabricius)), bumblebees, and honeybees as the most effective visitors exhibiting floral consistency, increasing pod and seed production (Amaya-Márquez 2009, Kingha et al. 2012, Ramos et al. 2018, Franceschinelli et al. 2022). Additional beneficial taxa include naturally occurring and reared parasitoids (e.g., wasps and flies) that promote biological control (Ramos et al. 2018, Wyckhuys et al. 2023).

Squash

Squash or pumpkin (*C. pepo*) belongs to the *Cucurbitaceae* family, in the genus *Cucurbita* (Sauer 1993). Of cultivated species, *C. pepo* is the most morphologically and ecologically diverse, exhibiting wide adaptability across habitats and climatic zones (Hosen et al. 2021, Dhatt et al. 2024). Archaeological records and molecular studies indicate that *C. pepo* underwent two independent domestication events, one in Mesoamerica (Oaxaca Valley, ~8000 BCE) and one in eastern North America (~5000 BCE) (Sauer 1993, Sanjur et al. 2001). Squash was an integral food plant in Indigenous communities, valued not only for its nutritional content from most parts of the plant and fruit, but also its medicinal properties (Lira et al. 2016, Hosen et al. 2021). Today, squash is of high economic importance, cultivated for fruit (mature and immature), seed, and seed oil, with globally dedicated land in 2023 was estimated to be 1.5 Mha, yielding ~23.6 Mt (Hosen et al. 2021, FAO 2023a, Dhatt et al. 2024). Despite its high nutritional content and medical potential, *C. pepo* remains an underutilized food crop, as a result of being largely excluded from the Green Revolution's priorities (Hosen et al. 2021). Another major constraint to squash cultivation is the numerous chewing and sucking insect pests known to infest crops (Chowdhury et al. 2022).

Physical damage caused by insects has been reported to affect approximately 80% of a squash crop, with the most destructive pest insects reducing seedling survival, damaging leaves, stems, flowers, and fruit, and transmitting viruses, resulting in yield losses ranging from 30-100% (Sharma et al. 2016, Chowdhury et al. 2022). Notable pests include Coleopterans (e.g.,

pumpkin and cucumber beetles), Hemipterans (e.g., squash bug, aphids, whiteflies), Lepidopterans (e.g., squash vine borers), and Dipterans (e.g., fruit flies) (McLeod and Rashid 2016, Sharma et al. 2016)Among these, cucumber beetles are the most common pest causing damage throughout the entire plant's life, and more critically, transmitting bacterial wilt, which results in rapid plant mortality (Diver and Hinman 2008, McLeod and Rashid 2016).

C. pepo's monoecious reproductive system, characterized by the presence of both male and female floral structures on a single vine, combined with its single-day bloom period, renders it highly reliant on diurnal insect pollinators (Rani et al. 2017). In regions heavily focused on squash production, bees are the main beneficial insect visitors to squash crops, with honey bees (*Apis* spp.), bumble bees (*Bombus* spp.), and specialist squash bees (e.g., *Euceras spp.* and *Xenoglossa* spp.) consistently documented as principal squash pollinators (Delaplane and Mayer 2000, Knapp and Osborne 2019, Mani 2022). Also, a significant number of parasitoids (e.g., tachinid flies and lady beetles) have been recorded in squash fields, performing pest control services (Chowdhury et al. 2022). Additional wild bee species and other insect taxa have been recorded as present within squash crop fields; however, community composition greatly varies according to regional species pools and management intensity.

Sunflower

The common sunflower (*H. annuus*) belongs to the *Asteraceae* family and represents one of the most important oilseed crops (Sauer 1993, Blinkov et al. 2022). Domestication of *H. annuus* is linked to a single event in eastern North America (~2900 BCE), making sunflower one of the few globally cultivated crops originating from this region (Heiser 1955, 2008, Smith 2014). In Indigenous communities, sunflowers were a highly prized food source and widely used in medicines and ceremonies (Heiser 1951). Currently, sunflower remains a major multi-purpose crop with an estimated global production of 52.7 million metric tonnes of sunflower seeds across 28.30 Mha as of 2022/2023 (USDA Foreign Agricultural Service 2025). As the fourth most cultivated seed crop across six continents, sunflower accounts for 10% of the global volume of plant oil produced and is grown for everyday uses such as biodiesel, cosmetics, cut flowers, and dyes (Blinkov et al. 2022). Not only do humans take advantage of sunflowers, but hundreds of insects also use sunflowers for food, shelter, and/or as a habitat to ambush prey because of its unique morphological structures and micro-ecosystems (Rogers 1992, Prasifka and Hulke 2012).

Most sunflower pests are considered “occasional” and do not consistently cause great economic damage; however, native insect pests within the northern Plains of the US and the Canadian Prairies that coevolved with *H. annuus* are particularly damaging (Rogers 1992, Prasifka and Hulke 2012). The most destructive attack the flower head and developing seeds, these include Lepidopterans (e.g., sunflower moths), Coleopterans (e.g., seed weevils), and Dipterans (e.g., midges) (Schulz 1978, Rogers 1992, Prasifka and Hulke 2012). Among these, the sunflower moth (*Homoeosoma electellum* (Hulst)) is the most harmful, exceeding 200 larvae per head, resulting in yield losses of > 90% (Schulz 1978, Rogers 1992). Sunflowers also support an abundance of beneficial insects, particularly pollinators, across multiple orders (Shpak et al. 2023). Bees dominate with the highest percentage of visits and overall species abundance, followed by flies, butterflies, and beetles (Robertson 1922, Shpak et al. 2023). Individually, the commercial honey bee (*A. mellifera*) plays the central role in global sunflower pollination (Greenleaf and Kremen 2006). However, regional wild bees, particularly sunflower specialists, are equally if not more efficient because simultaneous foraging by commercial and wild bees enhances pollination efficiency beyond observed individual performance, increasing overall seed yield and quality (Greenleaf and Kremen 2006, Shpak et al. 2023). In North America, the predominant sunflower specialists are sunflower bees (e.g., miner bee (*Andrena helianthin* Robertson)), long-horned bees (e.g., *Melissodes trinodis* Robertson and *M. agilis* Cresson), and bumble bees (*Bombus* spp.) (Mallinger et al. 2019).

Intercropping and The Three Sisters

The global adoption of monocultures as standard agricultural practice was driven by their economic efficiency and compatibility with modern farming techniques, which has led to simplified landscapes, biodiversity loss, and amplified ecological disturbances (Horwith 1985). In response, intercropping, also referred to as mixed cropping, companion planting or polycultures, emerged as a new alternative approach that enhances structural and functional diversity within agroecosystems (Willey 1979, Harwood 2024). Intercropping is defined as the practice of growing two or more crops in a single field that have a significant portion of their growing period occurring in unison (Willey 1979). Although often framed as an innovation in sustainable intensive agriculture, intercropping has historical roots in Indigenous farming practices (Harwood 2024).

Intercropping

Formal scientific investigation of intercropping as an agronomic strategy gained momentum in the mid-20th century, largely in response to concerns of resource efficiency and food security (Harwood 2024). Research focused on the fundamental mechanisms governing intercropping performance centres around two key interactions: competition and complementarity (Willey 1979, Horwith 1985, Brooker et al. 2015, Harwood 2024). Competition in intercropping systems refers to the negative effects that arise when plants of the same species (intraspecific competition) or different species (interspecific competition) contend for shared, limited resources such as sunlight, water, and essential soil nutrients (Willey 1979, Malézieux et al. 2009, Bybee-Finley and Ryan 2018). Willey (1979) identified three broad categories of competitive relationships in the context of yield outcome: mutual inhibition, where both individuals or species yield less than they would in a monoculture; mutualism, where both individuals or species exceed expected yields; or compensation, where a single individual or species' reduced performance is offset by the increased productivity in another.

Although competition could be expected to limit the positive effects from intercropping, known as complementarity, these interactions often operate simultaneously in polycrops (Gebru 2015, Duchene et al. 2017, Bybee-Finley and Ryan 2018). For example, when interspecific competition is weaker than intraspecific competition for a limited resource, complementarity arises as plants differentially exploit available resources (Willey 1979, Malézieux et al. 2009, Brooker et al. 2015). There are two primary mechanisms that contribute to complementarity: resource partitioning and facilitation (Brooker et al. 2015, Duchene et al. 2017, Bybee-Finley and Ryan 2018). The division of resources is the process by which species-specific traits enable the utilization of different ecological niches (e.g., spatial and temporal) and the respective resources (Duchene et al. 2017, Bybee-Finley and Ryan 2018). Spatial complementarity occurs when plant species differ in root architecture or canopy structure, thereby minimizing overlap in light, nutrients, and/or water uptake (Willey 1979, Gebru 2015, Duchene et al. 2017). Temporal complementarity arises when crop growth patterns exhibit significant timing differences, for instance, early- and late-maturing species or those utilizing different photosynthetic pathways (Willey 1979, Gebru 2015, Duchene et al. 2017).

Through these complementarity mechanisms acting alongside competition, a nested cascade of ecological benefits emerge, including the regulation of pests (weeds, insects, and disease), more efficient use of resources (light, water, nutrients), improved soil health, and the creation or enhancement of microhabitats (Willey 1979, Horwith 1985, Malézieux et al. 2009, Gebru 2015, Brooker et al. 2015, Duchene et al. 2017, Bybee-Finley and Ryan 2018). In current practice and research, modern intercropping is largely focused on the agronomic advantages of increased productivity and stability, as it is associated with economic profitability and consistent success documented in numerous studies (Willey 1979, Horwith 1985, Malézieux et al. 2009, Gebru 2015, Brooker et al. 2015, Duchene et al. 2017, Bybee-Finley and Ryan 2018, Sharma et al. 2020, Harwood 2024). For instance, long-term field studies report that maize-based intercrops achieve yields averaging ~22% higher than matched monocultures and exhibit greater year-to-year yield stability, and meta-analyses further suggest polycultures can achieve comparable or greater combined yields while using less land than monoculture systems (Li et al. 2020, 2021, Tilman 2020).

However, in practice, intercropping is predominantly implemented as simple two-species mixtures in low-input fields, as large-scale adoption is constrained by the logistical complexity of cultivation, management, and harvest of multiple crops simultaneously (Malézieux et al. 2009, Brooker et al. 2015, Li et al. 2020). These limitations reflect that modern intercropping rarely capitalizes on its full potential, largely because the ecological and cultural dimensions embedded in the traditional intercropping strategy are overlooked (Malézieux et al. 2009, Brooker et al. 2015, Sharma et al. 2020).

Three Sisters

One of the most ecologically sophisticated and culturally significant examples of traditional intercropping in North America is the Three Sisters, a term coined by the Haudenosaunee, also known as “Diohe’ko”, meaning “these who sustain us” (Lewandowski 1987). This polyculture system commonly consisted of maize (*Z. mays*), bean (*Phaseolus* spp.), and squash (*Cucurbita* spp.) cultivated together in a raised mound (Lewandowski 1987, Pleasant 2006). While the exact origins are unknown, archeological evidence dates the adoption of the Three Sisters complex to 1070 CE Mesoamerica (Hurt 1987, Lewandowski 1987). The system’s high adaptability to different environments enabled it to become the dominant food source and

plant association for numerous Indigenous groups across North America, especially the northeastern US and southern regions of Canadian provinces (Ngapo et al. 2021). The most prominent practitioners included Iroquoian-speaking nations such as the Haudenosaunee and Huron-Wendat, with varying degrees of adoption among neighbouring nations such as Algonquian-speaking communities (e.g., Anishinaabe) (Lewandowski 1987, Pleasant 2006).

Structure

Rather than functioning as an isolated planting technique, the Three Sisters system reflected an integrated worldview in which ecological processes, social responsibilities, and cultural values were inseparable (Lewandowski 1987). Farming practices are guided by principles of reciprocity and long-term responsibility, recognizing that the land provided sustenance, and in turn, required care to remain productive over time (Lewandowski 1987, Kimmerer 2012). These cultural principles were reflected in oral histories emphasizing cooperation among maize, bean, and squash, and were physically manifested through the arrangement of crops and their complementary roles within the polyculture (Lewandowski 1987, Pleasant and Burt 2010). Maize provided vertical support for bean tendrils and was known to be remarkably competitive against weeds, disease and pests, legumes contributed biologically fixed nitrogen to the soil, and squash suppressed weeds and moderated soil conditions through its extensive ground cover (Lewandowski 1987, Pleasant 2006) In some contexts, a complimentary floral plant species, such as sunflower (*H. annuus*), was considered a "fourth sister", cultivated for their edible seeds, role in attracting pollinators, or as an alternative structural support to maize (Pleasant 2006, Kruse-Peeples 2016). Complementing the above-ground interactions, crops were commonly planted in a raised soil mound, which altered the physical and biochemical properties of soil, minimizing erosion and creating localized microclimates (Pleasant 2006). Together, these interactions enhanced productivity and stability of the Three Sisters polyculture (Hurt 1987).

Planting Practices

Although coordinating crop coexistence and resource sharing appeared straightforward within the Three Sisters system, successful implementation of these mechanisms depended on plant species, cultivation date, mound organization, site management techniques, and local

environmental conditions (Pleasant 2006). However, due to most traditional knowledge being communicated orally, specific historical details are sparse and greatly contrast between each reference (Pleasant and Burt 2010). Therefore, to provide a fuller outline of traditional cultivation practices associated with the Three Sisters system, the following information is a combination of the most commonly referenced guidelines, with emphasis on Haudenosaunee techniques.

The Three Sisters complex began with the creation of a mound approximately 8" to 12" high and 24" in diameter, spaced ~1 m from each other (Sanders n.d., Pleasant and Burt 2010, Kruse-Peeples 2016). Planting followed a rough schedule, with the last frost date as the starting point. Maize was planted first as it had the longest growing period, 6" from the centre of the mound (Sanders n.d., Pleasant and Burt 2010, Kruse-Peeples 2016). Once the maize reached at least 4" to 6" in height, approximately 2-3 weeks, beans were planted ~3" to 6" from each corn plant (Sanders n.d., Pleasant and Burt 2010, Kruse-Peeples 2016). After the beans had started climbing, around one week later, squash was planted around the edge of the mound (Kruse-Peeples 2016).

Other aspects of timing and placement varied widely, including but not limited to the nuances regarding the duration before or after the last frost date in which maize was planted, the number of crop plants per mound, seeding number and depth, orientation of crops, and specific varieties used. These decisions heavily relied on the environment in which the Three Sisters were planted and on the Indigenous community. For example, a Three Sisters planting technique in the upper Midwest US included maize and bean planted in alternating rows with sunflowers growing along garden edges, and squash mounds created every fourth row (Kuepper et al. 2016). If this methodology were slightly altered to have bean and maize together in a mound, this would mirror a Three Sisters planting technique traditionally practiced in northeastern US (Kuepper et al. 2016).

Regarding the varieties of the crops used, Yanovski's (1936) publication summarized records of food plants used by Indigenous Peoples, while more recently, Lewandowski (1987) provided an extensive list of combined early documentation of Indigenous crops associated with the Three Sisters complex. Regardless of specifics, continued field maintenance throughout the growing season was critical and often performed by women and children within the community (Hurt 1987). The use of natural fertilizer was rarely recorded, thinning of crops occurred shortly after planting, and weeding was carried out three to four times over the growing season by hand or using hand tools (Hurt 1987, Pleasant and Burt 2010). This variation in the documentation and details regarding

Indigenous ways of knowing reflects not only the diversity of Indigenous agricultural practices but also highlights the broader issue of intentional disregard for agricultural history, resulting in the loss of the disconnect of land and people (Herrighty 2022).

Current Research

While historical research has documented traditional planting strategies, peer-reviewed studies on the Three Sisters or related mound-based intercropping remain limited. A review by Fonteyne et al. (2023), broadly explored mound-based intercropping research from 1955 to 2021 using Scopus and other Mexican journals, finding a total of 176 articles that address various overarching topics: agronomic issues (soil fertility, weed management, and productivity) (61); social and cultural factors (44); environmental issues (29); crop diversity (25); and wild species biodiversity (17). Five of the 176 articles exclusively focused on Three Sisters (Fonteyne et al. 2023), with my own research uncovering only a few additional journal entries, with the majority having the foundation of the paper being yield advantage. Current research generally seeks to provide more support, document underlying mechanisms, or uncover additional interconnected benefits.

The most popular focus in studies has been comparing various yield metrics of the Three Sisters to monocultures. Pleasant and Burt (2010) provide a detailed review of major studies prior to 2005. More recent experiments were conducted by Pleasant (2016) and Cryan et al. (2024). Pleasant (2016) was notable as the first to explore Three Sisters yield through energy and protein per unit land area, finding that the polycrop produced more energy and protein than monocrops, supplying energy for 13.42 people per hectare and protein for 15.86 people per hectare. Cryan et al. (2024) examined Three Sisters yield in terms of biomass, growth rate, and labour requirement. In terms of dry biomass, maize harvest was greater in the polycrop, but sole plantings of bean and squash produced more harvest than the Three Sisters. The growth rates of all plants within the Three Sisters continued to expand stem diameter after the monocrops had stopped, with maize being the fastest-growing crop when compared to its monoculture. Notably, wind damage allowed Cryan et al. (2024) to comment on maize lodging, concluding 73% of maize plants in monocultures fell or leaned after the event, compared to 48% in the Three Sisters treatment. Finally, regarding labour efficiency, monoculture produced 25.78lbs of harvest per of labour, while the Three Sisters produced 20.34 lbs. Although this outcome was heavily

influenced by squash production, which accounted for 89.9% of the harvest, it required less labour than other monocultures or the Three Sisters treatment.

Research into the complementary mechanisms of the Three Sisters structure and related soil health benefits has recently received increased attention. Most recently, Zhang et al. (2014) determined differences in root crown architecture, vertical root distribution, and lateral root density, which enhanced soil exploration and nutrient foraging. These differences contributed positively to the Three Sisters growth and yield production, as well as increased nitrogen and phosphorus uptake compared to monocultures. Although the underlying mechanism remains unclear, Zhang et al. (2014) speculated that reduced competition for space increases local nutrient availability, triggering root proliferation. Regarding soil health, a recent short-term study found that after one year, Three Sisters intercropping increased soil respiration by 24%, decreased salt-extractable nitrate by 54% and increased the carbon-to-nitrogen ratio by 32% (Kapayou et al. 2023).

Studies exploring associated biodiversity are limited but emerging. Three articles published in 2024 are the only records documenting insect associations with the Three Sisters. Gibson et al. (2024) explored plant-bee relationships, identifying *Bombus impatiens* Cresson and *Xenoglossa pruinosa* Cresson to be important visitors. Liao et al. (2024) considered crop chemical defences against herbivorous pest insects, while Grof-Tisza et al. (2024) assessed the broader arthropod community, finding that Three Sisters plots foster higher levels of insect abundances than monocultures.

Research Objectives

While studies on the Three Sisters intercropping system have started initial exploration on comparative yield advantages and root-soil interactions, the current body of research remains remarkably limited in scope. Even within these focal areas, only a handful of papers exist, often only briefly mentioning broader implications without further study. Key areas of inquiry such as the potential role of the Three Sisters in addressing food insecurity, enhancing nutritional output compared to monocultures, or supporting community health through culturally relevant food systems, are largely undeveloped. Additionally, important structural and applied dimensions are missing from the literature. These include strategies for scaling from small-scale gardens to large farming contexts, the need for long-term trials to assess ecological and agronomic stability over

time, and the integration of Indigenous leadership and community-based participatory approach in both research design and implementation. Among these topics, the ecological dimensions of this system, particularly the roles of insect biodiversity and functional interactions within the Three Sisters, remain critically unexplored. These gaps highlight the need for more holistic research that respects the system's cultural origins while rigorously investigating its potential contributions to sustainable agriculture. Given the central role insects have globally on agriculture and the current state of threat both are in, we must determine the current insect community structure and interactions that occur within the Three Sisters to better understand and utilize this intercropping system as a potential sustainable agricultural practice.

References

Ali, A. 2023. Biodiversity–ecosystem functioning research: brief history, major trends and perspectives. Biological Conservation, **285**: 1–17. doi: 10.1016/j.biocon.2023.110210.

Amaya-Márquez, M. 2009. Floral constancy in bees: a revision of theories and a comparison with other pollinators. Revista Colombiana de Entomología, **35**: 206–216. Available from http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-04882009000200017&lng=en&nrm=iso.

Amaya-Márquez, M., and Wells, H. 2008. Social complexity and learning foraging tasks in bees/Complejidad social y aprendizaje de tareas de forrajeo en abejas. Caldasia, **30**: 469–477. Available from www.unal.edu.co/icn/publicaciones/caldasia.htm.

Azerefegne, F., Kitaw, D., and Asayehegne, B. 2002. Major insect pests of maize and their management: a review. *In* Enhancing the Contribution of Maize to Food Security in Ethiopia: Proceedings of the Second National Maize Workshop of Ethiopia: 12-16 November 2001, Addis Ababa, Ethiopia. *Edited by* M. Nigussie, D. Tanner, and S. Twumasi-Afriyie. Central Printing Press PLC, Addis Ababa, Ethiopia. pp. 89–96.

Bali, G.P.K., and Kaleka, A.S. 2022. Potential reasons for insect decline. *In* Global Decline of Insects. *Edited by* H.A. Farag El-Shafie. IntechOpen, London, United Kingdom. pp. 25–42. doi: http://dx.doi.org/10.5772/intechopen.94711.

Bellucci, E., Bitocchi, E., Rau, D., Rodriguez, M., Biagetti, E., Giardini, A., Attene, G., Nanni, L., and Papa, R. 2014. Genomics of origin, domestication and evolution of *Phaseolus vulgaris*. *In* Genomics of Plant Genetic Resources (Volume 1): Managing, Sequencing and Mining Genetic Resources. Springer Netherlands, pp. 483–507. doi: 10.1007/978-94-007-7572-5_20.

Benton, T.G., Bryant, D.M., Cole, L., and Crick, H.Q.P. 2002. Linking agricultural practice to insect and bird populations: a historical study over three decades. Journal of Applied Ecology, **39**: 673–687. doi: 10.1046/j.1365-2664.2002.00745.x.

Blinkov, A.O., Varlamova, N. V., Kurenina, L. V., and Khaliluev, M.R. 2022. The production of *Helianthus* haploids: a review of its current status and future prospects. Plants, **11**: 1–28. doi: 10.3390/plants11212919.

Borlaug, N.E. 2000. The green revolution revisited and the road ahead. Stockholm: Nobelprize.

Borlaug, N.E. 2007. Sixty-two years of fighting hunger: personal recollections. Euphytica, **157**: 287–297. doi: 10.1007/s10681-007-9480-9.

Brooker, R.W., Bennett, A.E., Cong, W.F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L., Mckenzie, B.M., Pakeman, R.J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., and White, P.J. 2015. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytologist, **206**: 107–117. doi: 10.1111/nph.13132.

Brown, E.D., and Williams, B.K. 2016. Ecological integrity assessment as a metric of biodiversity: are we measuring what we say we are? Biodiversity and Conservation, **25**: 1011–1035. doi: 10.1007/s10531-016-1111-0.

Bybee-Finley, K.A., and Ryan, M.R. 2018. Advancing intercropping research and practices in industrialized agricultural landscapes. Agriculture, **8**: 1–24. doi: 10.3390/agriculture8060080.

Canadian Endangered Species Conservation Council. 2022, December 2. Wild species 2020: the general status of species in Canada. Available from https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry/general-status/wild-species-2020.html#toc41.

Caron, D.M. 2021. Honey bee strains. *In* Honey Bee Medicine for the Veterinary Practitioner. *Edited by* T.R. Kane and C.M. Faux. John Wiley & Sons, Inc., pp. 73–80. doi: 10.1002/9781119583417.ch6.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., and Toker, C. 2018. The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. Agronomy, **8**: 1–9. doi: 10.3390/agronomy8090166.

Chao, A., Chiu, C.H., and Jost, L. 2010. Phylogenetic diversity measures based on Hill numbers. Philosophical Transactions of the Royal Society B: Biological Sciences, **365**: 3599–3609. doi: 10.1098/rstb.2010.0272.

Chao, A., Chiu, C.H., and Jost, L. 2014a. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. Annual Review of Ecology, Evolution, and Systematics, **45**: 297–324. doi: 10.1146/annurev-ecolsys-120213-091540.

Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., and Ellison, A.M. 2014b. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs, **84**: 45–67. doi: 10.1890/13-0133.1.

Chao, A., and Jost, L. 2012. Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. Ecology, **93**: 2533–2547. doi: 10.1890/11-1952.1.

Chellappan, M., and Ranjith, M.T. 2023. Insect predators. *In* Insect Predators in Pest Management. *Edited by* O. Omkar. CRC Press, pp. 1–54. doi: 10.1201/9781003370864.

Chowdhury, S., Jennions, M.D., Zalucki, M.P., Maron, M., Watson, J.E.M., and Fuller, R.A. 2023. Protected areas and the future of insect conservation. Trends in Ecology & Evolution, **38**: 85–95. doi: 10.1016/j.tree.2022.09.004.

Chowdhury, Z., Ahmed, J., Shitel, A., and Saha, S. 2022. Status of the major insect pests of squash plants at fruiting stage. Fundamental and Applied Agriculture, **7**: 199–205. doi: 10.5455/faa.114314.

Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L., and Longino, J.T. 2012. Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. Journal of Plant Ecology, **5**: 3–21. doi: 10.1093/jpe/rtr044.

Cryan, T., Musselman, O., Baumgardner, A.W., Osborn, S., Beuscher, C.J., Stehn, C., Burt, A., Chaaban, R., Lopez, A., Lewis, G., Mata, G., Rothenberg, M., Plascencia-Marquez, A., Greer, R., Stremlau, R., Murphy, H., Newman, L.R., Merrill, A., and Wadgymar, S.M. 2024. Yield, growth, and labor demands of growing maize, beans, and squash in monoculture versus the Three Sisters. Plants People Planet, **7**: 204–214. doi: 10.1002/ppp3.10576.

Danforth, B. 2007. Bees. Current Biology, **17**: 156–161. doi: 10.1016/j.cub.2007.01.025.

Dar, S.A., Ansari, M.J., Al Naggar, Y., Hassan, S., Nighat, S., Zahra, S.B., Rashid, R., Hassan, M., and Hussain, B. 2022. Causes and reasons of insect decline and the way forward. *In* Global Decline of Insects. *Edited by* H.A. Farag El-Shafie. IntechOpen, London, United Kingdom. pp. 3–24. doi: http://dx.doi.org/10.5772/intechopen.94711.

Darwin, C. 1859. On the origin of species. Murray, London.

Delaplane, K.S., and Mayer, D.F. 2000. Crop pollination by bees. CABI Publishing.

Desender, E., Dufrêne, M., Loreau, M., Luff, M.L., and Maelfait, J.-P. (*Editors*). 1994. Carabid beetles: ecology and evolution (Volume 51). Springer Science & Business Media. doi: 10.1007/978-94-017-0968-2.

Dhatt, A.S., Pandey, S., Garch, K.S., Verma, N., Sagar, V., and Sharma, M. 2024. Comprehensive review of pumpkin (*Cucurbita* spp.): domestication, global distribution, genetic characterization, breeding strategies, and genomic insights. Vegetable Science, **51**: 196–210. doi: 10.61180/vegsci.2024.v51.i2.01.

Diaz, S., Fargione, J., Chapin, F.S., and Tilman, D. 2006. Biodiversity loss threatens human well-being. PLoS Biology, **4**: 1300–1305. doi: 10.1371/journal.pbio.0040277.g001.

Dirzo, R., and Raven, P.H. 2003. Global state of biodiversity and loss. Annual Review of Environment and Resources, **28**: 137–167. doi: 10.1146/annurev.energy.28.050302.105532.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B., and Collen, B. 2014. Defaunation in the Anthropocene. Science, **345**: 401–406. doi: 10.1126/science.1251817.

Diver, S., and Hinman, T. 2008. Cucumber beetles: organic and biorational integrated pest management. Available from www.attra.ncat.org/attra-pub/cucumberbeetle.html.

Duchene, O., Vian, J.F., and Celette, F. 2017. Intercropping with legume for agroecological cropping systems: complementarity and facilitation processes and the importance of soil microorganisms. a review. Agriculture, Ecosystems & Environment, **240**: 148–161. doi: 10.1016/j.agee.2017.02.019.

Dudley, N., and Alexander, S. 2017. Agriculture and biodiversity: a review. Biodiversity, **18**: 45–49. doi: 10.1080/14888386.2017.1351892.

Elton, C.S. 1958. The ecology of invasions by animals and plants. London, Methuen.

Evenson, R.E., and Gollin, D. 2003. Assessing the impact of the Green Revolution, 1960 to 2000. Science, **300**: 758–762. doi: 10.1126/science.1078710.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics, **34**: 487–515. doi: 10.1146/annurev.ecolsys.34.011802.132419.

FAO. 2023a. FAOSTAT: Statistics database. Available from https://www.fao.org/faostat/en/#home [accessed 3 June 2023].
FAO. 2023b. World food and agriculture – statistical yearbook 2023. *In* World Food and Agriculture – Statistical Yearbook 2023. FAO, Rome, Italy. p. doi: 10.4060/cc8166en.
Federico, G. 2005. Feeding the world: an economic history of agriculture, 1800 - 2000. *Edited by* J. Mokyr. Princeton University Press.
Fedick, S.L. 1995. Indigenous agriculture in the Americas. Journal of Archaeological Research, **3**: 257–303. Available from https://www.jstor.org/stable/41053106.
Feldman, S., and Biggs, S. 2012. International shifts in agricultural debates and practice: an historical view of analyses of global agriculture. *In* Integrating Agriculture, Conservation and Ecotourism: Societal Influences. *Edited by* W.B. Campbell and Ortíz S.L. Springer Netherlands, pp. 107–161. doi: 10.1007/978-94-007-4485-1_2.
Fischer, J., Brosi, B., Daily, G.C., Ehrlich, P.R., Goldman, R., Goldstein, J., Lindenmayer, D.B., Manning, A.D., Mooney, H.A., Pejchar, L., Ranganathan, J., and Tallis, H. 2008. Should agricultural policies encourage land sparing or wildlife-friendly farming? Frontiers in Ecology and the Environment, **6**: 380–385. doi: 10.1890/070019.
Fitzgerald-Moore, P., and Parai, B.J. 1996. The Green Revolution.
Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Stuart Chapin, F., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Colin Prentice, I., Ramankutty, N., and Snyder, P.K. 2005. Global consequences of land use. Science, **309**: 570–574. 10.1126/science.1111772.
Fonteyne, S., Castillo Caamal, J.B., Lopez-Ridaura, S., Van Loon, J., Espidio Balbuena, J., Osorio Alcalá, L., Martínez Hernández, F., Odjo, S., and Verhulst, N. 2023. Review of agronomic research on the milpa, the traditional polyculture system of Mesoamerica. Frontiers in Agronomy, **5**: 1–16. doi: 10.3389/fagro.2023.1115490.
Franceschinelli, E.V., Ribeiro, P.L.M., Mesquita-Neto, J.N., Bergamini, L.L., Madureira de Assis, I., Elias, M.A.S., Fernandes, P.M., and Carvalheiro, L.G. 2022. Importance of biotic pollination varies across common bean cultivars. Journal of Applied Entomology, **146**: 32–43. doi: 10.1111/jen.12951.
Garcia, L.C., and Eubanks, M.D. 2019. Overcompensation for insect herbivory: a review and meta-analysis of the evidence. Ecology, **100**: 1–14. doi: 10.1002/ecy.2585.
García-Lara, S., and Serna-Saldivar, S.O. 2018. Corn history and culture. *In* Corn: Chemistry and Technology, 3rd edition. Elsevier, pp. 1–18. doi: 10.1016/B978-0-12-811971-6.00001-2.
Gaston, K.J., and Fuller, R.A. 2007. Biodiversity and extinction: losing the common and the widespread. Progress in Physical Geography, **31**: 213–225. doi: 10.1177/0309133307076488.
Gebru, H. 2015. A review on the comparative advantages of intercropping to mono-cropping system. Journal of Biology, Agriculture and Healthcare, **5**: 1–13.
Gibbs, J., Hanuschuk, E., Miller, R., Dubois, M., Martini, M., Robinson, S., Nakagawa, P., Sheffield, C.S., Cardinal, S., and Onuferko, T.M. 2023. A checklist of the bees (Hymenoptera: Apoidea) of Manitoba, Canada. The Canadian Entomologist, **155**: 1–38. doi: 10.4039/tce.2022.45.

Gibson, S.D., Onuferko, T.M., Myers, L., and Colla, S.R. 2024. Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes region. PeerJ, **12**: 1–24. doi: 10.7717/peerj.17401.

Goulet, H. 2003. Biodiversity of ground beetles (Coleoptera: Carabidae) in Canadian agricultural soils. Canadian Journal of Soil Science, **83**: 259–264. Available from https://archive.org/details/carabidbeetlesin0010thie/page/n15/mode/2up.

Green, R.E., Cornell, S.J., Scharlemann, J.P.W., and Balmford, A. 2005. Farming and the fate of wild nature. Science, **307**: 550–555. doi: 10.1126/science.1106818.

Greenleaf, S.S., and Kremen, C. 2006. Wild bees enhance honey bees' pollination of hybrid sunflower. Proceedings of the National Academy of Sciences, **103**: 13890–13895. Available from www.pnas.orgcgidoi10.1073pnas.0600929103.

Greenleaf, S.S., Williams, N.M., Winfree, R., and Kremen, C. 2007. Bee foraging ranges and their relationship to body size. Oecologia, **153**: 589–596. doi: 10.1007/s00442-007-0752-9.

Grevé, M.E., Marx, M.T., Eilmus, S., Ernst, M., Herrmann, J.D., Baden, C.U., and Maus, C. 2024. Insect decline—evaluation of potential drivers of a complex phenomenon. Insects, **15**: 1–37. doi: 10.3390/insects15121021.

Grof-Tisza, P., Muller, M.H., Gónzalez-Salas, R., Bustos-Segura, C., and Benrey, B. 2024. The Mesoamerican milpa agroecosystem fosters greater arthropod diversity compared to monocultures. Agriculture, Ecosystems & Environment, **372**: 1–10. doi: 10.1016/j.agee.2024.109074.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., and De Kroon, H. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One, **12**: 1–21. doi: 10.1371/journal.pone.0185809.

Harris, D.R. 1990. 3. Vavilov's concept of centres of origin of cultivated plants: its genesis and its influence on the study of agricultural origins. Biological Journal of the Linnean Society, **39**: 7–16. doi: 10.1111/j.1095-8312.1990.tb01608.x.

Hart, J.P., and Winchell-Sweeney, S. 2023. Resetting archaeological interpretations of precontact Indigenous agriculture: maize isotopic evidence from three ancestral Mohawk Iroquoian villages. American Antiquity, **88**: 497–512. doi: 10.1017/aaq.2023.44.

Harvey, J.A., Tougeron, K., Gols, R., Heinen, R., Abarca, M., Abram, P.K., Basset, Y., Berg, M., Boggs, C., Brodeur, J., Cardoso, P., de Boer, J.G., De Snoo, G.R., Deacon, C., Dell, J.E., Desneux, N., Dillon, M.E., Duffy, G.A., Dyer, L.A., Ellers, J., Espíndola, A., Fordyce, J., Forister, M.L., Fukushima, C., Gage, M.J.G., García-Robledo, C., Gely, C., Gobbi, M., Hallmann, C., Hance, T., Harte, J., Hochkirch, A., Hof, C., Hoffmann, A.A., Kingsolver, J.G., Lamarre, G.P.A., Laurance, W.F., Lavandero, B., Leather, S.R., Lehmann, P., Le Lann, C., López-Uribe, M.M., Ma, C. Sen, Ma, G., Moiroux, J., Monticelli, L., Nice, C., Ode, P.J., Pincebourde, S., Ripple, W.J., Rowe, M., Samways, M.J., Sentis, A., Shah, A.A., Stork, N., Terblanche, J.S., Thakur, M.P., Thomas, M.B., Tylianakis, J.M., Van Baaren, J., Van de Pol, M., Van der Putten, W.H., Van Dyck, H., Verberk, W.C.E.P., Wagner, D.L., Weisser, W.W., Wetzel, W.C., Woods, H.A., Wyckhuys, K.A.G., and Chown, S.L. 2023. Scientists' warning on climate change and insects. Ecological Monographs, **93**: 1–37. doi: 10.1002/ecm.1553.

Harwood, J. 2024. The forgotten history of intercropping. Plants People Planet, **6**: 1121–1128. doi: 10.1002/ppp3.10502.

Heinrichs, J.A., Bender, D.J., and Schumaker, N.H. 2016. Habitat degradation and loss as key drivers of regional population extinction. Ecological Modelling, **335**: 64–73. doi: 10.1016/j.ecolmodel.2016.05.009.

Heiser, C.B. 1951. The sunflower among the North American Indians. Proceedings of the American Philosophical Society, **95**: 432–448. Available from https://www.jstor.org/stable/3143283?seq=1&cid=pdf-.

Heiser, C.B. 1951. The sunflower among the North American Indians. Proc. Am. Philos. Soc., **95**: 432–448. Available from https://www.jstor.org/stable/3143283?seq=1&cid=pdf-.

Heiser, C.B. 1955. The origin and development of the cultivated sunflower. The American Biology Teacher, **17**: 161–167. Available from https://www.jstor.org/stable/4438706.

Heiser, C.B. 2008. The sunflower (*Helianthus annuus*) in Mexico: further evidence for a North American domestication. Genetic Resources and Crop Evolution, **55**: 9–13. doi: 10.1007/s10722-007-9300-z.

Hemberger, J., Crossley, M.S., and Gratton, C. 2021. Historical decrease in agricultural landscape diversity is associated with shifts in bumble bee species occurrence. Ecology Letters, **24**: 1800–1813. doi: 10.1111/ele.13786.

Herrighty, E.M. 2022. Seed sovereignty, rematriation, and Three Sisters intercropping in Native American communities. [M.Sc.] thesis, Iowa State University, Ames, Iowa.

Hill, G.M., Kawahara, A.Y., Daniels, J.C., Bateman, C.C., and Scheffers, B.R. 2021. Climate change effects on animal ecology: butterflies and moths as a case study. Biological Reviews, **96**: 2113–2126. doi: 10.1111/brv.12746.

Hill, M.O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology, **54**: 427–432. doi: 10.2307/1934352.

Holland, J.M. 2002. Carabid beetles: their ecology, survival and use in agroecosystems. *In* The Agroecology of Carabid Beetles. *Edited by* J.M. Holland. Intercept, pp. 1–66. Available from https://www.researchgate.net/publication/262827188.

Holliday, N.J., Floate, K.D., Cárcamo, H., Pollock, D.A., Stjernberg, A., and Roughley, R.E. 2014. Ground beetles (Coleoptera: Carabidae) of the prairie grasslands of Canada. *In* Arthropods of Canadian Grasslands: Biodiversity and Systematics Part 2. *Edited by* D.J. Giberson and Cárcamo H.A. Biological Survey of Canada, pp. 1–85. doi: http://dx.doi.org/10.3752/9780968932179.ch1.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., and Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs, **75**: 3–35. doi: 10.1890/04-0922.

Horwith, B. 1985. A role for intercropping in modern agriculture. Bioscience, **35**: 286–291. Available from http://links.jstor.org/sici?sici=0006-3568%28198505%2935%3A5%3C286%3AARFIIM%3E2.0.CO%3B2-H.

Hosen, M., Rafii, M.Y., Mazlan, N., Jusoh, M., Oladosu, Y., Chowdhury, M.F.N., Muhammad, I., and Khan, M.M.H. 2021. Pumpkin (*Cucurbita* spp.): a crop to mitigate food and nutritional challenges. Horticulturae, **7**. 1–25. doi: 10.3390/horticulturae7100352.

Hurt, D.R. 1987. Indian agriculture in America: prehistory to the present. University Press of Kansas.

Igamberdiev, A.U. 2025. Human-driven evolution of cultivated plants and the origin of early civilizations: the concept of Neolithic revolution in the works of Nikolai Vavilov. BioSystems, **247**: 1–12. doi: 10.1016/j.biosystems.2024.105359.

Jacques, P.J., and Jacques, J.R. 2012. Monocropping cultures into ruin: the loss of food varieties and cultural diversity. Sustainability, **4**: 2970–2997. doi: 10.3390/su4112970.
Jankielsohn, A. 2018. The importance of insects in agricultural ecosystems. Advances in Entomology, **6**: 62–73. doi: 10.4236/ae.2018.62006.
Jarzyna, M.A., and Jetz, W. 2016. Detecting the multiple facets of biodiversity. Trends in Ecology & Evolution, **31**: 527–538. doi: 10.1016/j.tree.2016.04.002.
Jones, A.L. 1999. Phaseolus bean: post-harvest operations. *Edited by* D. Mejia and B. Lewis. Food and Agriculture Organization of the United Nations, Centre Internacional de Agricultura Tropical.
Jost, L. 2006. Entropy and diversity. Oikos, **113**: 363–375. doi: 10.1111/j.2006.0030-1299.14714.x.
Jost, L. 2007. Partitioning diversity into independent alpha and beta components. Ecology, **88**: 2427–2439. doi: 10.1890/06-1736.1.
Kaçar, G., Butrón, A., Kontogiannatos, D., Han, P., Peñaflor, M.F.G.V., Farinós, G.P., Huang, F., Hutchison, W.D., de Souza, B.H.S., Malvar, R.A., Kourti, A., Ramirez-Romero, R., Smith, J.L., Koca, A.S., Pineda, M., and Haddi, K. 2023. Recent trends in management strategies for two major maize borers: *Ostrinia nubilalis* and *Sesamia nonagrioides*. Journal of Pest Science, **96**: 879–901. doi: 10.1007/s10340-023-01595-8.
Kapayou, D.G., Herrighty, E.M., Hill, C.G., Camacho, V.C., Nair, A., Winham, D.M., and McDaniel, M.D. 2023. Reuniting the Three Sisters: collaborative science with Native growers to improve soil and community health. Agriculture and Human Values, **40**: 65–82. doi: 10.1007/s10460-022-10336-z.
Kearney, S.G., Adams, V.M., Fuller, R.A., Possingham, H.P., and Watson, J.E.M. 2020. Estimating the benefit of well-managed protected areas for threatened species conservation. Oryx, **54**: 276–284. doi: 10.1017/S0030605317001739.
Kerr, J.T., Pindar, A., Galpern, P., Packer, L., Potts, S.G., Roberts, S.M., Rasmont, P., Schweiger, O., Colla, S.R., Richardson, L.L., Wagner, D.L., Gall, L.F., Sikes, D.S., and Pantoja, A. 2015. Climate change impacts on bumblebees converge across continents. Science, **349**: 177–180. doi: 10.1126/science.aaa7031.
Kimmerer, R.W. 2012. Searching for synergy: integrating traditional and scientific ecological knowledge in environmental science education. Journal of Environmental Studies and Sciences, **2**: 317–323. doi: 10.1007/s13412-012-0091-y.
Kingha, B.M.T., Fohouo, F.-N.T., Ngakou, A., and Brückner, D. 2012. Foraging and pollination activities of *Xylocopa olivacea* (Hymenoptera, Apidae) on *Phaseolus vulgaris* (Fabaceae) flowers at Dang (Ngaoundere-Cameroon). Journal of Agricultural Extension and Rural Development, **4**: 330–339. doi: 10.5897/JAERD11.151.
Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. 2006. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences, **274**: 303–313. doi: 10.1098/rspb.2006.3721.
Knapp, J.L., and Osborne, J.L. 2019. Cucurbits as a model system for crop pollination management. Journal of Pollination Ecology, **25**: 89–102. Available from https://lup.lub.lu.se/search/files/71996418/535_2098_5_PB.pdf.
Kotze, D.J., Brandmayr, P., Casale, A., Dauffy-Richard, E., Dekoninck, W., Koivula, M.J., Lövei, G.L., Mossakowski, D., Noordijk, J., Paarmann, W., Pizzolotto, R., Saska, P., Schwerk, A., Serrano, J., Szyszko, J., Taboada, A., Turin, H., Venn, S., Vermeulen, R.,

and Zetto, T. 2011. Forty years of carabid beetle research in Europe - from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. Zookeys, **100**: 55–148. doi: 10.3897/zookeys.100.1523.

Kruse-Peeples, M. 2016, May 27. How to grow a Three Sisters garden. Available from https://www.nativeseeds.org/blogs/blog-news/how-to-grow-a-three-sisters-garden.

Kuepper, G., Dodson, M., and Duncan, J. 2016. Companion planting & botanical pesticides: concepts & resources. ATTRA Sustainable Agriculture, 1–20.

Larochelle, A., and Larivière, M.-C. 2003. A natural history of the ground beetles (Coleoptera: Carabidae) of America north of Mexico. Pensoft Publishers, Bulgaria.

Laurance, W.F. 2010. Habitat destruction: death by a thousand cuts. *In* Conservation Biology for All. *Edited by* N.S. Sodhi and P.R. Ehrlich. Oxford University Press, pp. 73–87. doi: 10.1093/acprof:oso/9780199554232.001.0001.

Laxmi Rai, V., Sharma, P., and Kushwaha, R. 2015. Beneficial insects and their value to agriculture. Research Journal of Agriculture and Forestry Sciences, **3**: 25–30.

Lewandowski, S. 1987. Diohe'ko, the Three Sisters in Seneca life: implications for a native agriculture in the finger lakes region of New York State. Agriculture and Human Values, **4**: 76–93. doi: 10.1007/BF01530644.

Li, C., Hoffland, E., Kuyper, T.W., Yu, Y., Zhang, C., Li, H., Zhang, F., and van der Werf, W. 2020. Syndromes of production in intercropping impact yield gains. Nature Plants, **6**: 653–660. doi: 10.1038/s41477-020-0680-9.

Li, X.F., Wang, Z.G., Bao, X.G., Sun, J.H., Yang, S.C., Wang, P., Wang, C.B., Wu, J.P., Liu, X.R., Tian, X.L., Wang, Y., Li, J.P., Wang, Y., Xia, H.Y., Mei, P.P., Wang, X.F., Zhao, J.H., Yu, R.P., Zhang, W.P., Che, Z.X., Gui, L.G., Callaway, R.M., Tilman, D., and Li, L. 2021. Long-term increased grain yield and soil fertility from intercropping. Nature Sustainability, **4**: 943–950. doi: 10.1038/s41893-021-00767-7.

Liao, H., Zhou, Z., Liu, Y., Luo, Y., Zhang, C., Feng, Y., Shu, Y., and Wang, J. 2024. 'The Three Sisters' (maize/bean/squash) polyculture promotes the direct and indirect defences of maize against herbivores. European Journal of Agronomy, **155**: 1–11. doi: 10.1016/j.eja.2024.127118.

Lindroth, C.H. 1961–1969. The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska Parts 1 (1969), 2 (1961), 3 (1963), 4 (1966), 5 (1968), 6 (1969). Opuscula Entomologica Supplementum, 35, 20, 24, 29, 33, 34.

Lira, R., Casas, A., and Blancas, J. (*Editors*). 2016. Ethnobotany of Mexico: interactions of people and plants in Mesoamerica. Springer Nature, New York. Available from http://www.springer.com/series/11551.

Louzada, J., and Nichols, E.S. 2012. Detritivorous insects. *In* Insect Bioecology and Nutrition for Integrated Pest Management. *Edited by* A.R. Panizzi and J.R.P. Parra. CRC Press, pp. 313–342. doi: 10.1201/b11713-20.

Lövei, G.L., and Sunderland, K.D. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology, **41**: 231–256. Available from www.annualreviews.org/aronline.

Luff, M.L., and Rushton, S.P. 1992. Classification and prediction of grassland habitats using ground beetles (Coleoptera, Carabidae). Journal of Environmental Management, **35**: 301–315. doi: https://doi.org/10.1016/S0301-4797(11)80012-5.

MacArthur, R.H. 1955. Fluctuations of animal populations and a measure of community stability. Ecology, **36**: 533–536. doi: 10.2307/1929601.

Magura, T., Tóthmérész, B., and Molnár, T. 2001. Forest edge and diversity: carabids along forest-grassland transects. Biodiversity and Conservation, **10**: 287–300. doi: https://doi.org/10.1023/A:1008967230493.

Magurran, A.E. 2021. Measuring biological diversity. Current Biology, **31**: R1174–R1177. doi: 10.1016/j.cub.2021.07.049.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., de Tourdonnet, S., and Valantin-Morison, M. 2009. Mixing plant species in cropping systems: concepts, tools and models. a review. Agronomy for Sustainable Development, **29**: 43–62. doi: 10.1051/agro:2007057.

Mallinger, R.E., Bradshaw, J., Varenhorst, A.J., and Prasifka, J.R. 2019. Native solitary bees provide economically significant pollination services to confection sunflowers (*Helianthus annuus* L.) (Asterales: Asteraceae) grown across the Northern Great Plains. Journal of Economic Entomology, **112**: 40–48. doi: 10.1093/jee/toy322.

Mammadov, J., Buyyarapu, R., Guttikonda, S.K., Parliament, K., Abdurakhmonov, I.Y., and Kumpatla, S.P. 2018. Wild relatives of maize, rice, cotton, and soybean: treasure troves for tolerance to biotic and abiotic stresses. Frontiers in Plant Science, **9**: 1–21. doi: 10.3389/fpls.2018.00886.

Mani, M. (*Editor*). 2022. Trends in horticultural entomology. Springer Nature.

Mann, C. 1997. Reseeding the Green Revolution. Science, **277**. doi: 10.1126/science.277.5329.1038.

Margalef, R. 1969. Diversity and stability: a practical proposal and a model of interdependence. 1–16.

Matsuoka, Y., Vigouroux, Y., Goodman, M.M., Sanchez, J.G., Buckler, E., and Doebley, J. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proceedings of the National Academy of Sciences, **99**: 6080–6084. doi: 10.1073/pnas.052125199.

McLeod, P.J., and Rashid, T. 2016. Cucurbit insect and related pests. *In* Handbook of Cucurbits Growth, Cultural Practices, and Physiology. *Edited by* M. Pessarakli. CRC Press, Boca Raton. pp. 387–402.

Menta, C., and Remelli, S. 2020. Soil health and arthropods: from complex system to worthwhile investigation. Insects, **11**: 1–21. doi: 10.3390/insects11010054.

Meza-Maicelo, W., Balcázar-Zumaeta, C.R., Santillan Culquimboz, H.W., Oliva-Cruz, M., and Lozano-Isla, F. 2025. Global trends in biotic and abiotic stress mitigation strategies for common bean: a bibliometric study. International Journal of Plant Biology, **16**: 1–49. doi: 10.3390/ijpb16040135.

Michener, C.D. 2007. The bees of the world. *In* 2nd edition. The Johns Hopkins University Press, Baltimore.

Mora, C., Tittensor, D.P., Adl, S., Simpson, A.G.B., and Worm, B. 2011. How many species are there on Earth and in the ocean? PLoS Biology, **9**: 1–8. doi: 10.1371/journal.pbio.1001127.

Müller, P., Neuhoff, D., Nabel, M., Schiffers, K., and Döring, T.F. 2022. Tillage effects on ground beetles in temperate climates: a review. Agronomy for Sustainable Development, **42**: 1–20. doi: 10.1007/s13593-022-00803-6.

Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H., and Woodfin, R.M. 1994. Declining biodiversity can alter the performance of ecosystems. Nature, **368**: 734–737. doi: https://doi.org/10.1038/368734a0.

Ngapo, T.M., Bilodeau, P., Arcand, Y., Charles, M.T., Diederichsen, A., Germain, I., Liu, Q., Mackinnon, S., Messiga, A.J., Mondor, M., Villeneuve, S., Ziadi, N., and Gariépy, S. 2021. Historical Indigenous food preparation using produce of the Three Sisters intercropping system. Foods, **10**: 1–19. doi: 10.3390/foods10030524.

Nguyen, H.D.D., and Nansen, C. 2018. Edge-biased distributions of insects. a review. Agronomy for Sustainable Development, **38**: 1–13. doi: 10.1007/s13593-018-0488-4.

Nicholls, E., and Hempel de Ibarra, N. 2017. Assessment of pollen rewards by foraging bees. Functional Ecology, **31**: 76–87. doi: 10.1111/1365-2435.12778.

Noriega, J.A., Hortal, J., Azcárate, F.M., Berg, M.P., Bonada, N., Briones, M.J.I., Del Toro, I., Goulson, D., Ibanez, S., Landis, D.A., Moretti, M., Potts, S.G., Slade, E.M., Stout, J.C., Ulyshen, M.D., Wackers, F.L., Woodcock, B.A., and Santos, A.M.C. 2018. Research trends in ecosystem services provided by insects. Basic and Applied Ecology, **26**: 8–23. doi: 10.1016/j.baae.2017.09.006.

Norris, K. 2008. Agriculture and biodiversity conservation: opportunity knocks. Conservation Letters, **1**: 2–11. doi: 10.1111/j.1755-263x.2008.00007.x.

Odum, E.P. 1953. Fundamentals of ecology. Saunders, Philadelphia.

OECD. 2015. Consensus document on the biology of common bean (*Phaseolus vulgaris* L.). Series on Harmonisation of Regulatory Oversight in Biotechnology. OECD Publishing, Paris, France.

Oerke, E.-C. 2006. Crop losses to pests. Journal of Agricultural Science, **144**: 31–43. doi: 10.1017/S0021859605005708.

Ollerton, J., Winfree, R., and Tarrant, S. 2011. How many flowering plants are pollinated by animals? Oikos, **120**: 321–326. doi: 10.1111/j.1600-0706.2010.18644.x.

Ordish, G., Crawford, G.W., Fussell, G.E., Rasmussen, W.D., Mellanby, K., Gray, A.W., and Nair, K. 2024. Origins of agriculture. Encyclopedia Britannica. Available from https://www.britannica.com/topic/agriculture.

Ortega, A. 1987. Insect pests of maize: a guide for field identification. CIMMYT.

Pearce, J.L., and Venier, L.A. 2006. The use of ground beetles (Coleoptera: Carabidae) and spiders (Araneae) as bioindicators of sustainable forest management: a review. Ecological Indicators, **6**: 780–793. doi: 10.1016/j.ecolind.2005.03.005.

Petersen, B., and Snapp, S. 2015. What is sustainable intensification? views from experts. Land Use Policy, **46**: 1–10. doi: 10.1016/j.landusepol.2015.02.002.

Phalan, B., Balmford, A., Green, R.E., and Scharlemann, J.P.W. 2011a. Minimising the harm to biodiversity of producing more food globally. Food Policy, **36**: S62–S71. doi: 10.1016/j.foodpol.2010.11.008.

Phalan, B., Onial, M., Balmford, A., and Green, R.E. 2011b. Reconciling food production and biodiversity conservation: land sharing and land sparing compared. Science, **333**: 1289–1291. doi: 10.1126/science.1208742.

Pingali, P.L. 2017. The Green Revolution and crop biodiversity. *In* Routledge handbook of agricultural biodiversity. *Edited by* D. Hunter, L. Guarino, C. Spillane, and P.C. McKeown. Routledge, London. 213–223.

Pleasant, J.M. 2006. The science behind the Three Sisters mound system: an agronomic assessment of an Indigenous agricultural system in the northeast. *In* Histories of Maize: multidisciplinary approaches to the prehistory, linguistics, biogeography, domestication, and evolution of maize. *Edited by* J. Staller, R. Tykot, and B. Benz. Left Coast Press, New York. pp. 529–537. Available from https://www.ebsco.com/terms-of-use.

Pleasant, J.M. 2015. A new paradigm for pre-Columbian agriculture in North America. Early American Studies, **13**: 374–412. Available from https://www.jstor.org/stable/24474894.
Pleasant, J.M. 2016. Food yields and nutrient analyses of the Three Sisters: a Haudenosaunee cropping system. Ethnobiology Letters, **7**: 87–98. doi: 10.14237/ebl.7.1.2016.721.
Pleasant, J.M., and Burt, R.F. 2010. Estimating productivity of traditional Iroquoian cropping systems from field experiments and historical literature. Journal of Ethnobiology, **30**: 52–79. doi: https://doi.org/10.2993/0278-0771-30.1.52.
Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W.E. 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution, **25**: 345–353. doi: 10.1016/j.tree.2010.01.007.
Prasifka, J.R., and Hulke, B.S. 2012, February 27–March 1. Current status and future perspectives on sunflower insect pests. *In* 18th International Sunflower Conference Program and Abstracts, Mar del Plata and Balcarce, Argentina.
Pretty, J.N. 1997. The sustainable intensification of agriculture. Natural Resources Forum, **21**: 247–256. doi: 10.1111/j.1477-8947.1997.tb00699.x.
Rainio, J., and Niemela, J. 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation, **12**: 487–506. doi: https://doi.org/10.1023/a:1022412617568.
Ramos, D. de L., Bustamante, M.M.C., da Silva e Silva, F.D., and Carvalheiro, L.G. 2018. Crop fertilization affects pollination service provision – common bean as a case study. PLoS One, **13**: 1–16. doi: 10.1371/journal.pone.0204460.
Rani, D., Yadav, S., Kaushik, H.D., and Kumar, G.N. 2017. Diversity and abundance of insect pollinators on summer squash. Indian Journal of Entomology, **79**: 17–20. doi: 10.5958/0974-8172.2017.00005.0.
Raven, P.H., and Wagner, D.L. 2021. Agricultural intensification and climate change are rapidly decreasing insect biodiversity. Proceedings of the National Academy of Science, **118**: 1–6. doi: 10.1073/PNAS.2002548117.
Reitz, E.J., Scarry, M.C., and Scudder, S.J. (*Editors*). 2008. Case studies in environmental archaeology. *In* 2nd edition. Springer Science & Business Media, New York, NY.
Rey Benayas, J.M., and Bullock, J.M. 2012. Restoration of biodiversity and ecosystem services on agricultural land. Ecosystems, **15**: 883–899. doi: 10.1007/s10021-012-9552-0.
Ritchie, H., and Roser, M. 2019, February 16. Half of the world's habitable land is used for agriculture. Available from https://ourworldindata.org/global-land-for-agriculture.
Robertson, C. 1922. The sunflower and its insect visitors. Ecology, **3**: 17–21. Available from https://www.jstor.org/stable/1929086
Rogers, C.E. 1992. Insect pests and strategies for their management in cultivated sunflower. Field Crops Research, **30**: 301–332. doi: https://doi.org/10.1016/0378-4290(92)90005-T.
Rosset, P., Collins, J., and Lappé, F.M. 2000. Lessons from the Green Revolution. Third World Resurgence, 11–14.
Roswell, M., Dushoff, J., and Winfree, R. 2021. A conceptual guide to measuring species diversity. Oikos, **130**: 321–338. doi: 10.1111/oik.07202.
Sánchez-Bayo, F., and Wyckhuys, K.A.G. 2019. Worldwide decline of the entomofauna: a review of its drivers. Biological Conservation, **232**: 8–27. doi: 10.1016/j.biocon.2019.01.020.

Sanders, S. (n.d.). Creating a Three Sisters garden. Available from https://www.uaex.uada.edu/counties/white/news/horticulture/20210511Creating_Three_Sisters_Garden.aspx.

Sanjur, O.I., Piperno, D.R., Andres, T.C., and Wessel-Beaver, L. 2001. Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae) inferred from a mitochondrial gene: implications for crop plant evolution and areas of origin. Proceedings of the National Academy of Sciences, **99**: 535–540. doi: 10.1073/pnas.012577299.

Sauer, J.D. 1993. Historical geography of crop plants: a select roster. CRC Press.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nature Ecology & Evolution, **3**: 430–439. doi: 10.1038/s41559-018-0793-y.

Scheffers, B.R., De Meester, L., Bridge, T.C.L., Hoffmann, A.A., Pandolfi, J.M., Corlett, R.T., Butchart, S.H.M., Pearce-Kelly, P., Kovacs, K.M., Dudgeon, D., Pacifici, M., Rondinini, C., Foden, W.B., Martin, T.G., Mora, C., Bickford, D., and Watson, J.E.M. 2016. The broad footprint of climate change from genes to biomes to people. Science, **354**: 719–730. doi: 10.1126/science.aaf7671.

Schulz, J.T. 1978. Insect pests. *In* Sunflower Science and Technology Agronomy Monographs. *Edited by* J.F. Carter. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Wisconsin, USA. pp. 169–223. doi: 10.2134/agronmonogr19.c6.

Schulze, E.D., and Mooney, H.A. (*Editors*). 1994. Biodiversity and ecosystem function. Springer-Verlag.

Schuman, M.C., and Baldwin, I.T. 2016. The layers of plant responses to insect herbivores. Annual Review of Entomology, **61**: 373–394. doi: 10.1146/annurev-ento-010715-023851.

Scott-Dupree, C.D., Conroy, L., and Harris, C.R. 2009. Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). Journal of Economic Entomology, **102**: 177–182. doi: https://doi.org/10.1603/029.102.0125.

Sharma, A., Rana, C., and Shiwani, K. 2016. Important insect pests of cucurbits and their management. *In* Handbook of Cucurbits Growth, Cultural Practices, and Physiology. *Edited by* M. Pessarakli. CRC Press, Boca Raton. pp. 327–359. Available from https://www.researchgate.net/publication/297556360.

Sharma, I.P., Kanta, C., Dwivedi, T., and Rani, R. 2020. Indigenous agricultural practices: a supreme key to maintaining biodiversity. *In* Microbiological Advancements for Higher Altitude Agro-Ecosystems & Sustainability. Springer Singapore, Singapore. pp. 91–112. doi: 10.1007/978-981-15-1902-4_6.

Sheffield, C.S., Frier, S.D., and Dumesh, S. 2014. The bees (Hymenoptera: Apoidea, Apiformes) of the Prairies ecozone, with comparisons to other grasslands of Canada. *In* Arthropods of Canadian Grasslands (Volume 4): Biodiversity and Systematics Part 2. *Edited by* D.J. Giberson and Cácamo H.A. Biological Survey of Canada, pp. 427–467. doi: 10.3752/9780968932179.ch11.

Shpak, Y. V., Leheta, U. V., and Sosnovskyi, K.S. 2023. Importance of pollinator diversity for sunflower seed production. Agrology, **6**: 79–85. doi: 10.32819/021113.

Shuey, J.A. 2013. Habitat re-creation (ecological restoration) as a strategy for conserving insect communities in highly fragmented landscapes. Insects, **4**: 761–780. doi: 10.3390/insects4040761.
Shultz, S. 2008. Corn. Journal of Agricultural and Food Information, **9**: 101–114. doi: 10.1080/10496500802173905.
Singh, S.P. (*Editor*). 1999. Common bean improvement in the twenty-first century. Springer Science & Business Media. doi: 10.1007/978-94-015-9211-6.
Singh, S.P., and Schwartz, H.F. 2011. Review: breeding common bean for resistance to insect pests and nematodes. Canadian Journal of Plant Science, **91**: 239–250. doi: 10.4141/CJPS10002.
Skendžić, S., Zovko, M., Živković, I.P., Lešić, V., and Lemić, D. 2021. The impact of climate change on agricultural insect pests. Insects, **12**: 1–31. doi: 10.3390/insects12050440.
Smith, B.D. 2014. The domestication of *Helianthus annuus* L. (sunflower). Vegetation History and Archaeobotany, **23**: 57–74. doi: 10.1007/s00334-013-0393-3.
Socolar, J.B., Gilroy, J.J., Kunin, W.E., and Edwards, D.P. 2016. How should beta-diversity inform biodiversity conservation? Trends in Ecology & Evolution, **31**: 67–80. doi: 10.1016/j.tree.2015.11.005.
Sreedevi, K., Mahendiran, G., and Sree Chandana, P. 2023. Ground beetles (Coleoptera: Carabidae). *In* Insect Predators in Pest Management. *Edited by* Omkar. CRC Press, pp. 175–186. doi: 10.1201/9781003370864-7.
Stork, N.E. 2018. How many species of insects and other terrestrial arthropods are there on Earth? Annual Review of Entomology, **63**: 31–45. doi: 10.1146/annurev-ento-020117.
Thomas, C.F.G., Holland, J.M., and Brown, N.J. 2002. The spatial distribution of carabid beetles in agricultural landscapes. *In* The Agroecology of Carabid Beetles. pp. 305–344. Available from https://www.researchgate.net/publication/262829348.
Tilman, D. 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences, **96**: 5995–6000. doi: https://doi.org/10.1073/pnas.96.11.5995.
Tilman, D. 2020. Benefits of intensive agricultural intercropping. Nature Plants, **6**: 604–605. doi: 10.1038/s41477-020-0677-4.
Tilman, D., Balzer, C., Hill, J., and Befort, B.L. 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences, **108**: 20260–20264. doi: 10.1073/pnas.1116437108.
Tilman, D., and Downing, J.A. 1994. Biodiversity and stability in grasslands. Nature, **367**: 363–365. doi: https://doi.org/10.1038/367363a0.
Tilman, D., Isbell, F., and Cowles, J.M. 2014. Biodiversity and ecosystem functioning. Annual Review of Ecology, Evolution, and Systematics, **45**: 471–493. doi: 10.1146/annurev-ecolsys-120213-091917.
Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., and Lehman, C. 2001. Diversity and productivity in a long-term grassland experiment. Science, **294**: 843–845. Available from https://www.science.org.
Tilman, D., Wedin, D., and Knops, J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature, **379**: 718–720.
Travis, J.M.J. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. *In* Proceedings of the Royal Society B: Biological Sciences, **270**: 467–473 doi: 10.1098/rspb.2002.2246.

Tscharntke, T., Grass, I., Wanger, T.C., Westphal, C., and Batáry, P. 2021. Beyond organic farming – harnessing biodiversity-friendly landscapes. Trends in Ecology & Evolution, **36**: 919–930. doi: 10.1016/j.tree.2021.06.010.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., and Thies, C. 2005. Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. Ecology Letters, **8**: 857–874. doi: 10.1111/j.1461-0248.2005.00782.x.

Uebersax, M.A., Cichy, K.A., Gomez, F.E., Porch, T.G., Heitholt, J., Osorno, J.M., Kamfwa, K., Snapp, S.S., and Bales, S. 2023. Dry beans (*Phaseolus vulgaris* L.) as a vital component of sustainable agriculture and food security—a review. Legume Science, **5**. 1–13. doi: 10.1002/leg3.155.

USDA Foreign Agricultural Service. 2025. World agricultural production. Washington, DC.

Vavilov, N.I. 1926. Centers of origin of cultivated plants.

Verma, R.C., Waseem, M.A., Sharma, N., Bharathi, K., Singh, S., Anto Rashwin A., Pandey, S.K., and Singh, B.V. 2023. The role of insects in ecosystems, an in-depth review of entomological research. International Journal of Environment and Climate Change, **13**: 4340–4348. doi: 10.9734/ijecc/2023/v13i103110.

Wagner, D.L. 2020. Insect declines in the Anthropocene. Annual Review of Entomology, **65**: 457–480. doi: 10.1146/annurev-ento-011019.

Wardhaugh, C.W. 2015. How many species of arthropods visit flowers? Arthropod-Plant Interactions, **9**: 547–565. doi: 10.1007/s11829-015-9398-4.

Wezel, A., Soboksa, G., McClelland, S., Delespesse, F., and Boissau, A. 2015. The blurred boundaries of ecological, sustainable, and agroecological intensification: a review. Agronomy for Sustainable Development, **35**: 1283–1295. doi: 10.1007/s13593-015-0333-y.

Willey, R.W. 1979. Intercropping - its importance and research needs. part 1. competition and yield advantages. Field Crop Abstracts, **32**: 1–10.

Wolf, V., Dehoust, J., and Banse, M. 2017. World markets for cereal crops: global trends for production, consumption, trade and prices. *In* Biokerosene: Status and Prospects. Springer Berlin Heidelberg, Berlin, Heidelberg. pp. 123–145. doi: 10.1007/978-3-662-53065-8_7.

Wyckhuys, K.A.G., Gonzalez-Chang, M., Lavandero, B., Gc, Y., and Hadi, B.A.R. 2023. Legume integration for biological control deserves a firmer scientific base. Outlook on Agriculture, **52**: 281–293. doi: 10.1177/00307270231194086.

Xie, H., Huang, Y., Chen, Q., Zhang, Y., and Wu, Q. 2019. Prospects for agricultural sustainable intensification: a review of research. Land, **8**: 1–27. doi: 10.3390/land8110157.

Yang, L.H. 2006. Interactions between a detrital resource pulse and a detritivore community. Community Ecology, **147**: 522–532. doi: 10.1007/10.1007/s00442-005-0276-0.

Yanovsky, E. 1936. Food plants of the North American Indians. US Department of Agriculture.

Zhang, C., Postma, J.A., York, L.M., and Lynch, J.P. 2014. Root foraging elicits niche complementarity-dependent yield advantage in the ancient “Three Sisters” (maize/bean/squash) polyculture. Annals of Botany, **114**: 1719–1733. doi: 10.1093/aob/mcu191.

Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., and Swinton, S.M. 2007. Ecosystem services and dis-services to agriculture. Ecological Economics, **64**: 253–260. doi: 10.1016/j.ecolecon.2007.02.024.

Zizumbo-Villarreal, D., Flores-Silva, A., and Colunga-García Marín, P. 2012. The Archaic diet in Mesoamerica: incentive for milpa development and species domestication. Economic Botany, **66**: 328–343. doi: 10.1007/s12231-012-9212-5.

CHAPTER 2: EFFECT OF THE THREE SISTERS AND DIFFERENT INDIGENOUS CROP COMBINATIONS ON WILD BEES, GROUND BEETLES, AND AGRICULTURAL PRODUCTIVITY

Abstract

Background. Intensive agriculture has led to a global decline in insect populations, especially those that support critical ecosystem processes and services. In response, mitigation techniques have reverted to intercropping methods to revive biodiversity, such as Indigenous-focused strategies like the Three Sisters (sunflower, bean, and squash). In this study, I recorded wild bee and ground beetle diversity and abundance associated with the Three Sisters and evaluated how these metrics varied among monocrop and two-crop combinations of the same component crops, relative to the Three Sisters system. I also tested the effect of the different cropping variations on yield and quality of harvest.

Methods. Six small sites were constructed in 2022; four were located in southern Manitoba, and two were in northern Ontario, Canada. Study sites included seven treatments, including a monoculture for each Indigenous crop type, two-crop mixed polycrops (dicrop), and a Three Sisters. Bees were captured using bee bowls, blue vane traps, and targeted aerial netting. Ground beetles were sampled through pitfall traps. I hypothesized that with increased Indigenous plant diversity, insect abundance and diversity would increase. I also expected harvest yield and overall quality would be higher in intercrops with more complex diversity when compared to intercrops of lesser diversity.

Results. I identified 812 bees and 10,156 carabid beetles to species. Bee abundance and species richness were found to be highest in the Three Sisters and other sunflower-containing cropping combinations, capturing approximately 10–25 times more bees than non-sunflower-containing treatments. This suggests that the functional traits of a floral species (e.g., large floral display, extended bloom period, high resource availability) could potentially be more influential than crop diversity alone in shaping pollinator assemblages. Similarly, crop diversity did not influence carabid abundance, species richness, or Hill-Shannon diversity, and no differences in yield or crop quality were detected among the Three Sisters, dicrops, or monocultures. The lack of significant results suggests that the expected ecological outcomes of the Three Sisters intercrop may be masked by local abiotic and biotic factors.

Conclusion. My study demonstrated that the Three Sisters system has the potential to enhance wild bee community structure through including pollinator-attractive species, whereas ground beetle diversity and harvest outcomes were largely unaffected by plant composition, likely reflecting constraints imposed by local environment and management factors. These results suggested that the expression of complementary mechanisms of the Three Sisters potentially depends on the interplay between crop traits and site-specific conditions. Future research should examine the Three Sisters across multiple seasons and environmental gradients, integrating Indigenous knowledge to better understand how crop traits and local conditions influence ecological interactions and crop productivity.

Introduction

Both bees (Apoidea: Anthophila) and beetles (Coleoptera) are among the most important and extensively studied insect groups of both natural and agroecosystems as they provide critical ecosystem services (Lövei and Sunderland 1996, Wardhaugh 2015). Wild bees provide pollination services to more than 87% of flowering plants, including food crops (Klein et al. 2006, Ollerton et al. 2011). Beetles, specifically carabids (Carabidae), exhibit substantial ecological flexibility, primarily functioning as predators that suppress pest populations, with additional roles as herbivores and detritivores supporting other ecosystem services, including organic matter recycling and soil health management (Lövei and Sunderland 1996). Unfortunately, global insect abundance and diversity are documented to be in decline, with accelerated losses over the last 30–40 years attributed to increased anthropogenic activity (Hallmann et al. 2017, Wagner 2020, Grevé et al. 2024).

Habitat loss is considered the main driver of this decline as it can trigger long-term, often irreversible environmental effects, including the extinction of species (Laurance 2010). The dominant contributor to habitat loss is the expansion and intensification of agriculture, with over a third of the world's ice-free land devoted to large-scale agricultural production (Dudley and Alexander 2017). Productivity in intensive agriculture is achieved by deliberately maintaining an ecosystem in a highly simplified and nutrient-rich state, typically through tilling, the removal of natural vegetation features, and the application of chemicals (Tilman 1999, Dudley and Alexander 2017, Raven and Wagner 2021). These practices destroy, degrade, and fragment habitats, reduce plant and structural diversity, disrupt trophic interactions, and ultimately

diminish the availability of ecological niches for insects and other wildlife (Tscharntke et al. 2005, Laurance 2010, Bali and Kaleka 2022).

Historically, monocropping emerged as an economically efficient system compatible with agricultural mechanization, enabling streamlined and standardized planting, fertilization, pest control, and harvesting practices (Horwith 1985). While monocultures boosted short-term productivity, they often fail to account for ecological complexity, resulting in reduced biodiversity, greater vulnerability to pests and diseases, and increased reliance on chemical inputs to maintain yields (Horwith 1985, Malézieux et al. 2009). In response to these ecological and agronomic challenges, intercropping has gained renewed attention as a sustainable strategy to mitigate the negative impacts of intensive agriculture (Harwood 2024). Intercropping is defined as the cultivation of two or more crop plant species within the same field, and is known to enhance structural and functional diversity, promote efficient resource use, and increase yield (Willey 1979, Brooker et al. 2015, Bybee-Finley and Ryan 2018). By strategically combining crops with complementary growth patterns, root structure, or phenologies, intercropping can reduce interspecific competition while facilitating mutual benefits such as nutrient enhancement, microclimate regulation, and pest suppression (Willey 1979, Brooker et al. 2015, Duchene et al. 2017, Bybee-Finley and Ryan 2018).

Before intercropping interactions were formally described, Indigenous Peoples were implementing these systems as common agricultural practices (Kimmerer 2012, Sharma et al. 2020). Developing mixed cropping strategies over generations through place-based knowledge guided by the understanding of species interactions, seasonality, and landscape dynamics as a reciprocal relationship among humans, the earth, and coexisting dimensions, where management prioritized ecological balance and long-term persistence rather than resource maximization (Kimmerer 2012, Sharma et al. 2020). The most common example of traditional Indigenous intercropping systems is the Three Sisters, commonly comprised of maize (*Zea mays* L.), bean (*Phaseolus* spp.), and squash (*Cucurbita* spp.) in a single mound (Lewandowski 1987, Pleasant 2006). In this system, maize provides structural support for climbing beans, beans enrich the soil through nitrogen fixation, and squash shades the soil to conserve moisture and suppress weeds (Lewandowski 1987, Pleasant 2006). Archeological and ethnographic evidence indicate that the Three Sisters system has been cultivated for centuries across diverse environments in North

America, resulting in multiple regional variations (Hurt 1987, Lewandowski 1987, Ngapo et al. 2021). The most common adaptation is the inclusion of pollinator-attracting plants, such as sunflowers, known as the fourth sister (Lewandowski 1987, Pleasant 2006, Pleasant and Burt 2010). These structural and functional arrangements, informed by shared Indigenous knowledge, suggest complementary interactions among crops, yet the ecological implications of these interactions remain largely unexplored.

The majority of studies regarding the Three Sisters system focus on yield metrics, spatial and temporal complementarity among crops, and soil nutrients dynamics (Fonteyne et al. 2023), generally reporting positive effects of the Three Sisters, such as outperforming less diverse plantings (Pleasant and Burt 2010, Zhang et al. 2014, Pleasant 2016, Terry et al. 2020, Kapayou et al. 2023, Cryan et al. 2024). While research to understand other dimensions associated with the Three Sisters, such as ecological interactions, remains limited. Only recently have a few studies begun to address these gaps, documenting arthropod communities, plant-pollinator relationships, and the natural defense provided by the Three Sisters against herbivory (Gibson et al. 2024, Liao et al. 2024, Grof-Tisza et al. 2024) Given the crucial roles of bees and beetles in agroecosystem function coupled with the global threats to their populations, increasing our understanding of how traditional intercropping practices shape insect communities is essential.

In this chapter, I address the following objectives and associated predictions:

i. Examine wild bee and ground beetle biodiversity across monocrop and intercrop treatments containing Indigenous significant crops.
    - Prediction: Bee and beetle abundance and species richness metrics will be highest in the Three Sisters mounds and will decrease with reduced crop plant diversity.
ii. Assess how wild bee dietary specialization varies among monocrop and intercrop treatments of Indigenous significant crops.
    - Prediction: The proportion of dietary specialists will be greater in the Three Sisters intercrop relative to lower-diversity treatments.
iii. Evaluate yield and quality of Indigenous significant crops among planting treatments.
    - Prediction: The Three Sisters intercrop will produce higher yield and higher quality harvests than monocrop or reduced-diversity treatments.

The goal of this study is to advance the understanding of the Three Sisters by examining its functional complementarity through assessing associated beneficial insect community diversity and crop productivity. On a broader scale, my research aims to create space for Indigenous communities to explore Traditional Ecological Knowledge (TEK) centred research, strengthen community connections, and support intergenerational knowledge.

Materials and Methods

Terminology

In this thesis, the term "traditional Three Sisters" refers to an interpretive synthesis of planting principles documented across multiple Indigenous Peoples, informed by a combination of oral knowledge shared by Brokenhead community members and the limited published ethnographic and agronomic literature. The Three Sisters system varies substantially among Indigenous communities in spatial layout, crop varieties, and planting schedules (Hurt 1987, Lewandowski 1987, Pleasant and Burt 2010). This study does not replicate any single Indigenous Peoples' practice in its entirety, but rather, common elements across accounts were identified and recreated. Most commonly, this thesis references Haudenosaunee agricultural practices throughout due to their relatively detailed documentation in the literature and relevance within the broader North American context; however, these examples are not presented as representative of all Haudenosaunee Three Sisters systems.

Study Sites

This study was conducted in six field sites during the 2022 field season (May–October). Four sites were located in southern Manitoba, Canada; Brokenhead Ojibway First Nations Reserve, Scanterbury, Manitoba (50.337151 N, 96.584218 W), Glenlea Research Station, Glenlea, Manitoba (49.64548 N, 97.130381 W), Ian N. Morrison Research Farm, West Carman, Manitoba (49.498656 N, 98.029260 W), and The University of Manitoba "The Point" Field Research Laboratory Winnipeg, Manitoba (49.815051 N, 97.123301 W). The remaining two sites were located in northern Ontario, Canada (western and eastern); The Ontario Crops Research Centre, Emo, Chapple, Ontario (48.38130N, -93.51507 W) and The Ontario Crops Research Centre, New Liskeard, New Liskeard, Ontario (47.521857 N, 79.669402 W) (Figure 1).

Southern Manitoba has a moderately dry climate with a plant hardiness zone of 2b or 3a, characterized as a prairie ecozone with a relatively treeless landscape with level prairies, rolling pastures, and clayey soil (Smith et al. 1998, Government of Canada 2020, McLintock et al. 2024). Due to its central place in continental North America, southern Manitoba receives extreme air temperatures that can range from -40°C in the winter months to 38°C in the summer months, with frost-free days falling between 120 and 140 days (McLintock et al. 2024, Government of Manitoba n.d.). Average annual precipitation across southern Manitoba ranges from 250–700 mm, with average snowfall varying from 30–75" covering the ground from November through April (McLintock et al. 2024, Government of Manitoba n.d.). Northern Ontario is made up of two ecozones, the Canadian Shield being the largest, covering 60% of the province with a predominantly forested landscape and agricultural flat land found toward the southern region (Crins et al. 2009, Ontario Biodiversity Council 2024). Similar to southern Manitoba, the plant hardiness zone is 3a or 2b (Government of Canada 2020). Northern Ontario's climate varies based on distance from the Great Lakes; the northwestern and northeastern sections are a greater distance from the moderating influence of the lakes, experiencing cooler temperatures and higher humidity (Wise et al. 2022). Frost-free days range between 40 to over 100, with mean temperatures ranging from -15°C in the winter months to 21°C in the summer months (Wise et al. 2022, McGillvray 2023, Township of Emo 2025). with annual precipitation averaging 500–700 mm and annual snowfall <85″ (Wise et al. 2022).

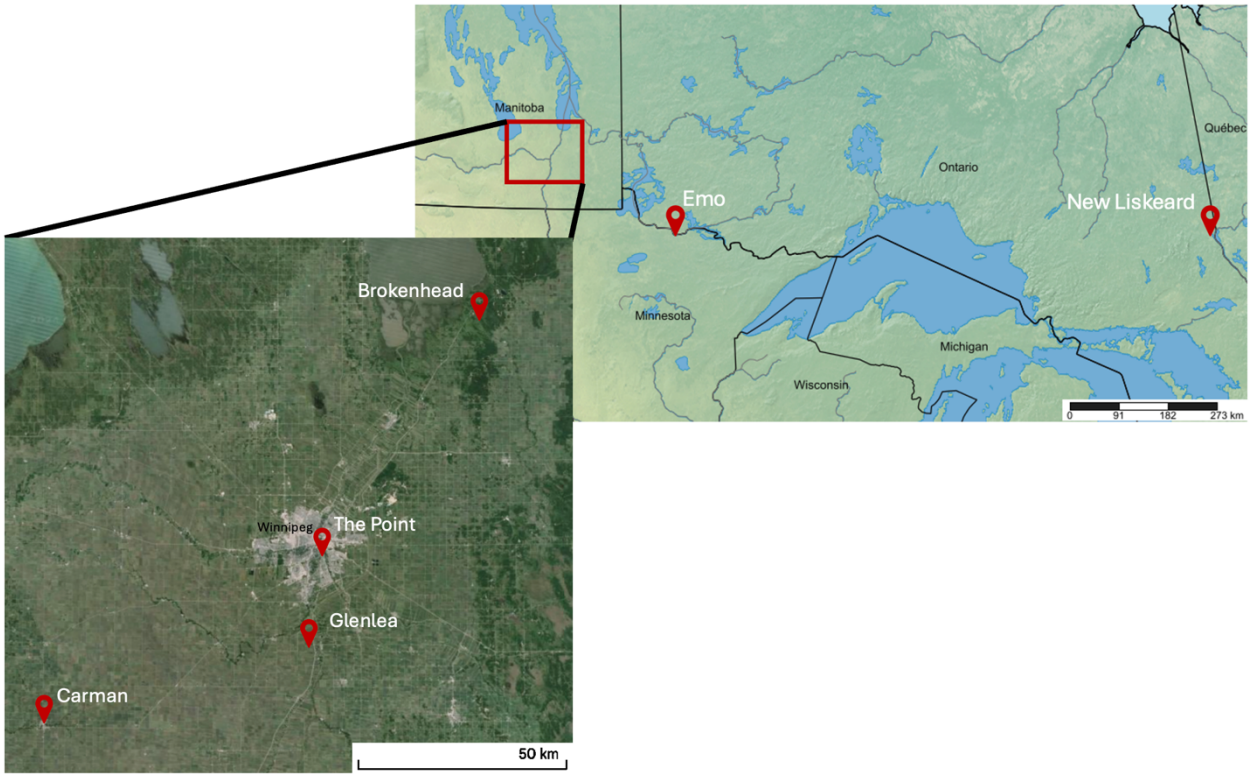

Figure 1. Locations of the study sites across southern Manitoba and northern Ontario. Red points indicate the respective study sites specific location. White text refers to the study sites abbreviated designation. Maps created with SimpleMappr (Shorthouse 2010) and Google Earth (Google Earth 10.55.0.1 2023).

Study Site Selection

Site selection was based on maintenance requirements, proximity to pesticide applications, and the potential to transition sites into permanent biodiversity monitoring stations accessible to all current and future collaborators. These criteria ensured the feasibility of site establishment and maintenance under constraints of limited personnel and equipment access, supported the project parameters of low-input and traditional land management practices, and minimized external disturbances from more intensive farming operations. However, these criteria limited the number of suitable sites, which were located sporadically and varied in management practices and planting histories. The Brokenhead Ojibway First Nations Reserve (Brokenhead) was built on a bison pasture approximated to have been in operation 7–10 years prior to this study, and a controlled burn was performed by the Brokenhead community at the end of the previous fall (September 14, 2021). The Glenlea Research Station (Glenlea), the Ian N. Morrison

Research Farm (Carman), and the University of Manitoba "The Point" Field Research Laboratory (The Point) were all located within a University of Manitoba research station. The Glenlea site was located specifically within the Organic Crop rotation, established in 1992. The Carman site was located on a corner plot adjacent to a prairie grass field and private farmland. The Point site was located within a barbed-wire-fenced area previously planted with Saskatoon berries. The Ontario Crops Research Centre, Emo (Emo) and The Ontario Crops Research Centre, New Liskeard (New Liskeard) were located within University of Guelph research stations.

Study Plot

The overall design of the study plot was developed from knowledge provided by members of the Brokenhead community and by documented traditional Haudenosaunee planting methods (Pleasant and Burt 2010, Kruse-Peeples 2016). The layout was modified to accommodate site-specific parameters and logistical restrictions. Five out of the six field sites were set up in 23 m x 32 m arrays (Figure 2A). Alternatively, The Point was placed in an "L shape" plot occupying a 23 m x 56 m area (Figure 2B). Small-scale study plots were specifically chosen based on the intent to use this layout in future areas where labour and space for cultivation, conservation, or required maintenance equipment would be limited. Study plots were surrounded by a two-layer solar-powered electric fence (Gallagher S40 80 Acres Solar Fence Energizer; Patriot SolarGuard 500 Energizer; Patriot White Poli Wire, Peavey Mart, Winnipeg, Manitoba) or a pre-existing barbed-wire fence (The Point) to deter wildlife from entering the area (Government of Manitoba n.d.).

Within the study plots, seven treatments (10 replicates per treatment) were planted across 70 mounds and consisted of either a monoculture or a variation of intercrop, as described in the Plant Selection and Treatments section. Randomized blocking was used to eliminate bias and prevent identical treatments from being beside one another, diagonally was accepted. Mounds were built by hand to be 1 m in diameter and 6″ in height (Figure 2D). A 1 m x 1 m quadrate (metal) was used for reference, accuracy, and standardization of each mound size. Spacing between each mound and the edge of the study plot was 2 m and always measured from the mound edge (Figure 2C).

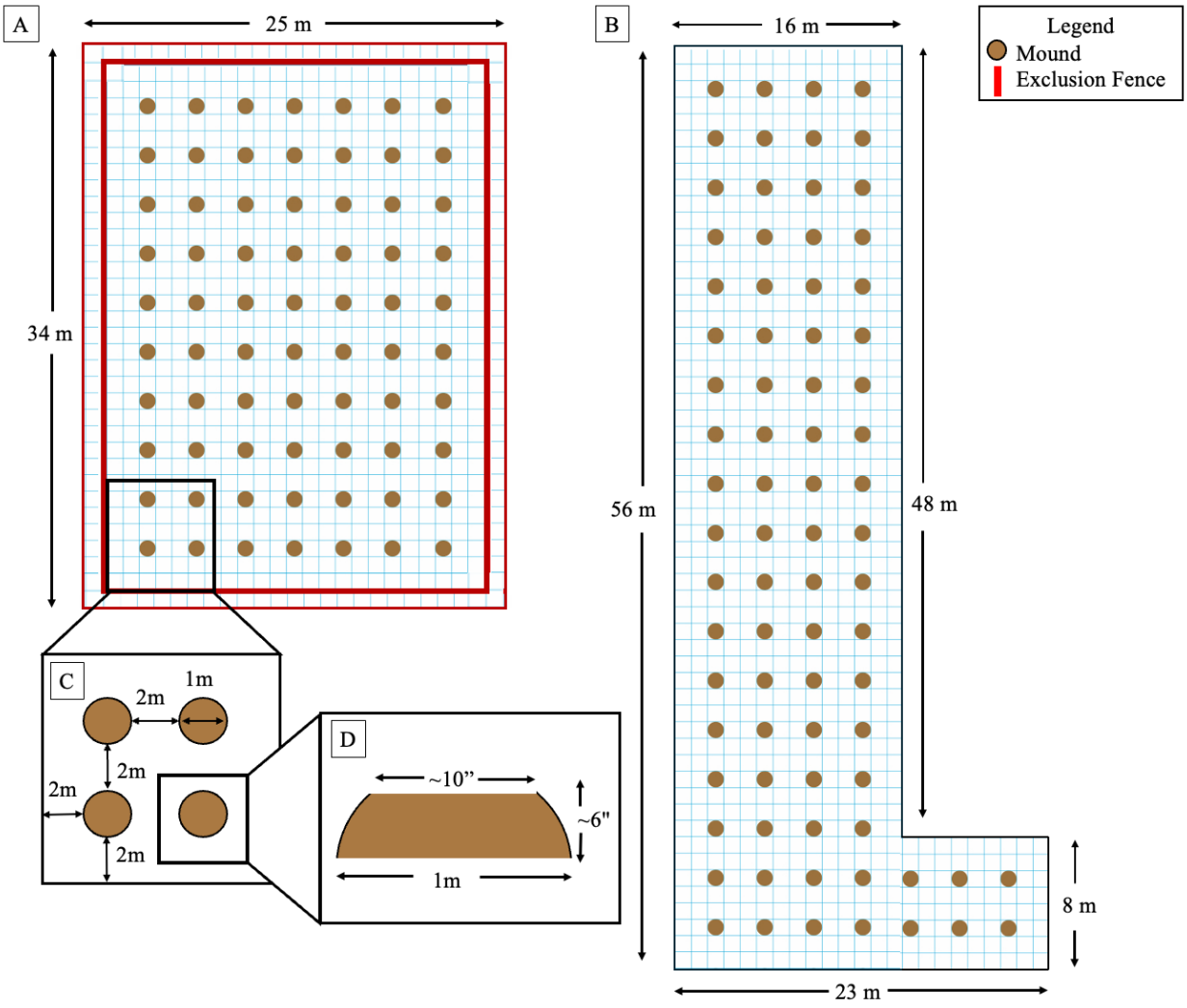

Figure 2. Visual representation of the study plot layouts. Each grid square represents 1 m^2. (A) Study plot layout for five out of six sites (Brokenhead, Carman, Glenlea, Emo, New Liskeard) (B) Study plot layout for single site (The Point). (C) Measurements between mound placement and edge of the study plot. (D) Measurements of a single mound.

Plant Selection and Treatments

In documented traditional Three Sisters systems, maize/corn (*Zea mays* L.), bean (*Phaseolus* spp.), and squash (*Cucurbita* spp.) are commonly planted together within raised mounds, with sunflower (*Helianthus annuus* L.) occasionally included as a companion species (Lewandowski 1987, Pleasant 2006, Pleasant and Burt 2010). For this study, the fourth sister, sunflower, replaced corn within the mound due to corn being wind pollinated. This was deemed

an acceptable adaptation as sunflowers have been historically recorded within traditional Three Sisters mounds, providing equivalent structural support for beans and the added benefit of increased pollinator presence (Pleasant 2006, Kruse-Peeples 2016). The Haudenosaunee Three Sisters planting layout (Figure 3A) served as a guideline for this project's Three Sisters treatment mound (Figure 3B), which was modified to accommodate selected crops and size restrictions (Pleasant and Burt 2010, Kruse-Peeples 2016). The Three Sisters treatment mound design also acted as a template for all other treatment types (monocrop and dicrop) in terms of spacing and seed layout (Figure 3C-D). All plant species were spaced identically, depending on planting pattern, to standardize each mound and eliminate bias. Individual mounds served as the primary unit of replication for this study.

To determine crop species and variety for this study, historical ethnobotanical sources were first consulted to identify the bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita pepo* L.) species most commonly associated with traditional Three Sisters systems (Yanovsky 1936, Lewandowski 1987, Ngapo et al. 2021). Regional Indigenous landraces documented for areas with similar climate and hardiness zones to the selected study sites were examined using seed banks (e.g., USDA National Plant Germplasm System, Seed Savers Exchange, Seeds of Diversity Canada). Due to limited commercial availability of landraces, final cultivars were chosen as the closest accessible analogues based on phenotypic similarities, growth form, and spatial constraints of the study plots. The resulting assemblage included a multi-headed sunflower (*H. annuus* 'Lemon Queen'), a climbing common bean (*P. vulgaris* 'Blue Lake'), and a small pumpkin (*C. pepo* 'Jack-Be-Little'). All seeds were sourced from Ontario Seed Co. Ltd. (Kitchener, Ontario), the largest Canadian-owned and operated seed distributor. The number of seeds purchased included the total required seeds per site (80/crop plant), plus an additional 50% to account for failed seed emergence.

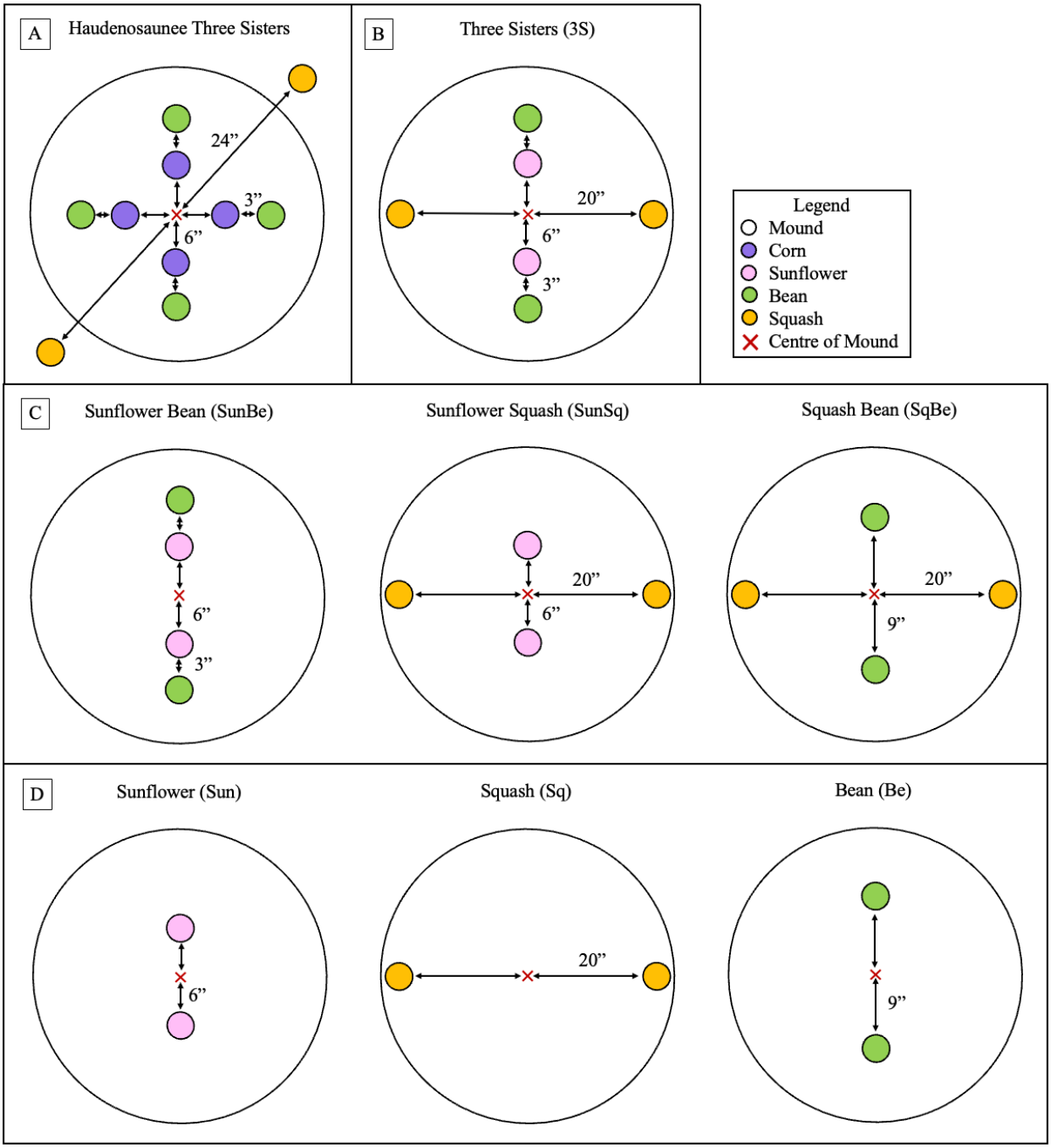

Figure 3. Comparison of a traditional Three Sisters planting layout within a mound to the 2022 field season Three Sisters treatment mound and six corresponding treatment variations. Diagrams include the planting orientation of respective crop plant species and measurements relative to the centre of the mound (1 m diameter). (A) Traditional Haudenosaunee Three Sisters mound. (B) Three Sisters treatment mound used in the 2022 field season. (C) Dicrop treatment mounds containing two plant species. (D) Monocrop treatment mounds containing one plant species.

Planting Schedule and Maintenance

The traditional Three Sisters planting schedule and site maintenance practices were based on published accounts of Haudenosaunee Three Sisters agriculture and were followed as closely as possible across all study sites (Hurt 1987, Pleasant and Burt 2010, Kruse-Peeples 2016). Certain modifications were implemented to accommodate logistical restrictions and availability of resources. Major deviations from traditional practices included the use of greenhouse facilities, timing adjustments (e.g., squash planted prior to beans) (Table S 1), and occasional weeding using small rototillers. Additionally, weeding was restricted to areas outside the mounds to allow for subsequent assessment of non-crop vegetation effects on insect captures, as described in the Non-Crop Plant Surveying section.

Sunflower and squash plants were germinated under controlled conditions to reduce risks associated with late spring frost and early-season herbivory. Manitoba sites utilized a growth chamber (Bally Refrigeration of Canada Limited, Brockville, Ontario) located at the University of Manitoba (Animal Science Building/Department of Entomology), set to a constant temperature of 22°C on a 16-hour light and 8-hour dark cycle. Ontario sites mirrored these conditions at comparable on-site facilities. The number of sunflower and squash plants was over-sown by 45–50% than what was required per site to account for germination failure and transplant loss. Sunflowers were sown five weeks prior to the tentative last frost date, followed three to four weeks later by squash (Table S 1). Both sunflower and squash seeds were planted in Jiffy pucks (42 mm; HJS Wholesale Ltd., Winnipeg, Manitoba) to minimize root disturbance during transplanting. Pole beans were directly seeded into mounds at the time of sunflower transplant to ensure access to structural support. Seed densities and planting depths followed regional agronomic recommendations (Government of Manitoba 2007, n.d., Ontario Ministry of Agriculture Food and Rural Affairs 2022), with minor adjustments to accommodate the volume of Jiffy pucks. Sunflowers were sown 2–3 seeds per hole (0.5” depth), squash at one seed per hole (1" depth), and beans at two seeds per hole (1.5" depth). To ensure consistent planting depth, a stick with marked measures was used to create holes in the Jiffy pucks and soil.

The adjusted planting schedule initially designed was created to foster efficient site setup and allow for sufficient growing time for crop plants; however, extreme weather conditions delayed field establishment at several sites, extending the duration of growth chamber use and

postponing bean planting beyond the intended schedule (Table S 1). Prolonged indoor growth resulted in root-bound and leggy plants that required additional care prior to field establishment. Sunflowers exceeded target transplant height (~6”) and were transferred to larger planting pots (1.28 qt, Dillen Products) filled with Sunshine Mix #4 Aggregate Plus Professional Growing Mix and fitted with wood dowel supports. Squash exhibited low and delayed germination after 1–1.5 months (~25% emergence), particularly at Manitoba sites. Supplemental squash germination was conducted under elevated soil temperatures (30°C) in an incubator (Thermo Scientific Precision Model 818 Incubator, Marietta, Ohio) to induce emergence. Incubator photoperiod mimicked the original growth chamber. As a result of these delays, Ontario sites were established by mid to late June, whereas Manitoba sites were not planted until approximately one month later than originally planned (Table S 1). Prior to insect sampling, beans and sunflowers were thinned to two plants per mound, and failed individuals were replaced.

Insect Surveying Procedures

Four insect sampling methods were conducted to provide data regarding wild bee and ground beetle communities, including timed targeted aerial netting, bee bowls, blue vane traps (BVTs), and pitfall traps. A combination of active and passive sampling techniques was used to survey bees, as each technique targets different taxa and introduces specific biases that influence observed bee community composition (Sircom et al. 2018, Hall 2018, Prendergast et al. 2020, Bell et al. 2023). Although only a single sampling method was selected for collecting ground beetles, it is the most widely used, resilient to different weather conditions, and ideal for long-term deployment (Lövei and Sunderland 1996, Kotze et al. 2011).

All sampling methods were performed or deployed into the field on non-rainy days with temperatures ≥15°C (LeBuhn et al. 2016, O’Connor et al. 2019, Vincze et al. 2025). In addition, bee sampling required wind speeds ≤15 km/h (Vincze et al. 2025). The above meteorological parameters were measured using a handheld weather station (Kestrel 3000). Temperature readings were taken in the shade and wind speeds were taken perpendicular to the wind direction for a total of one minute each. Other daily or weekly meteorological parameters used to account for effects of local weather on insect captures were retrieved from the Government of Canada historical weather archives (Table 1). All insect samples were kept in a freezer at their respective site until the end of the summer field season (September–October).

Table 1. Local weather variables collected with associated insect surveying procedures. Calculated per sampling event and study site.

Insect Sampling Method	Variable	Description
Bee Bowls, Blue Vane Traps, Targeted Aerial Netting	Daily Average Temperature (°C)	Mean air temperature calculated from hourly measurements recorded between 8 a.m. and 5 p.m. local time
	Daily Maximum Temperature (°C)	Highest hourly air temperature recorded within 8 a.m. and 5 p.m. local time
	Daily Minimum Temperature (°C)	Lowest hourly air temperature recorded within 8 a.m. to 5 p.m. local time
	Daily Average Wind Speed (km/hr)	Mean wind speed calculated from hourly measurements recorded between 8 a.m. and 5 p.m. local time
	Daily Maximum Wind Speed (km/hr)	Highest hourly wind speed recorded within 8 a.m. to 5 p.m. local time
	Daily Minimum Wind Speed (km/hr)	Lowest hourly wind speed recorded within 8 a.m. to 5 p.m. local time
Pitfall Traps	Weekly Average Temperature (°C)	Mean air temperature calculated from hourly measurements recorded during the 7-day sampling period
	Weekly Maximum Temperature (°C)	Highest hourly air temperature recorded during the 7-day sampling period
	Weekly Minimum Temperature (°C)	Lowest hourly air temperature recorded during the 7-day sampling period

Wild Bees

Targeted Aerial Netting

To evaluate wild bee biodiversity metrics per treatment, targeted aerial netting was conducted once a week between August 3, 2022–September 9, 2022, for a total of six sampling occurrences per site (Table S 2). Visual surveying for wild bees actively pollinating a flower (crop plant or non-crop plant) was done per mound for a total of one minute. Active pollination was defined as when a wild bee was collecting pollen from a flower and not showing signs of interest or scouting. These behaviours were studied prior to surveying efforts through reference videos to standardize sampling and eliminate bias. When a wild bee was seen actively pollinating, the one-minute timer was paused, and a net or a cyanide jar was used to capture the respective insect. Pollinators caught by net were then transferred directly into a cyanide jar

(separated by plant species) to be euthanized. Once the insect was safely set aside, the collector would resume the one-minute timer until the time ended. When the minute of active sampling was completed and collected insects were no longer moving, bees were transferred into a corresponding freezer-safe Ziplock bag (1 qt, ULINE). Each Ziplock bag was accompanied by a labelled card including the date, site code, mound number, and corresponding plant species. At the end of each sampling day, the sampled bees were left as "dry" specimens and placed in the freezer until processing.

Bee Bowls and Blue Vane Traps (BVTs)

To determine the general bee community of each site, pre-painted 3.5 oz bee bowls (New Horizon Support Services Upper Marlboro, Maryland) and 2 L BVTs (SpringStar Inc, St. Louis, Missouri) were deployed in unison, once a week (depending on weather), totalling seven samples per site (Table S 2). Three bowl colours (blue, yellow, and white) in combination with BVTs were used to capture a range of pollinators since different taxa are attracted to different colours (Droege et al. 2016, Sircom et al. 2018, Hall 2018, Prendergast et al. 2020). Bee bowls were placed on platforms at the average height of the crop and secured to a post located at each corner of the study plot as well as within for a total of 21 bowls per site (Figure 4; Figure 5A) (Droege et al. 2016). BVTs were placed on bare ground along the perimeter of the sampling area at least 8m apart from the bee bowls (Figure 4; Figure 5B) (Droege et al. 2016).

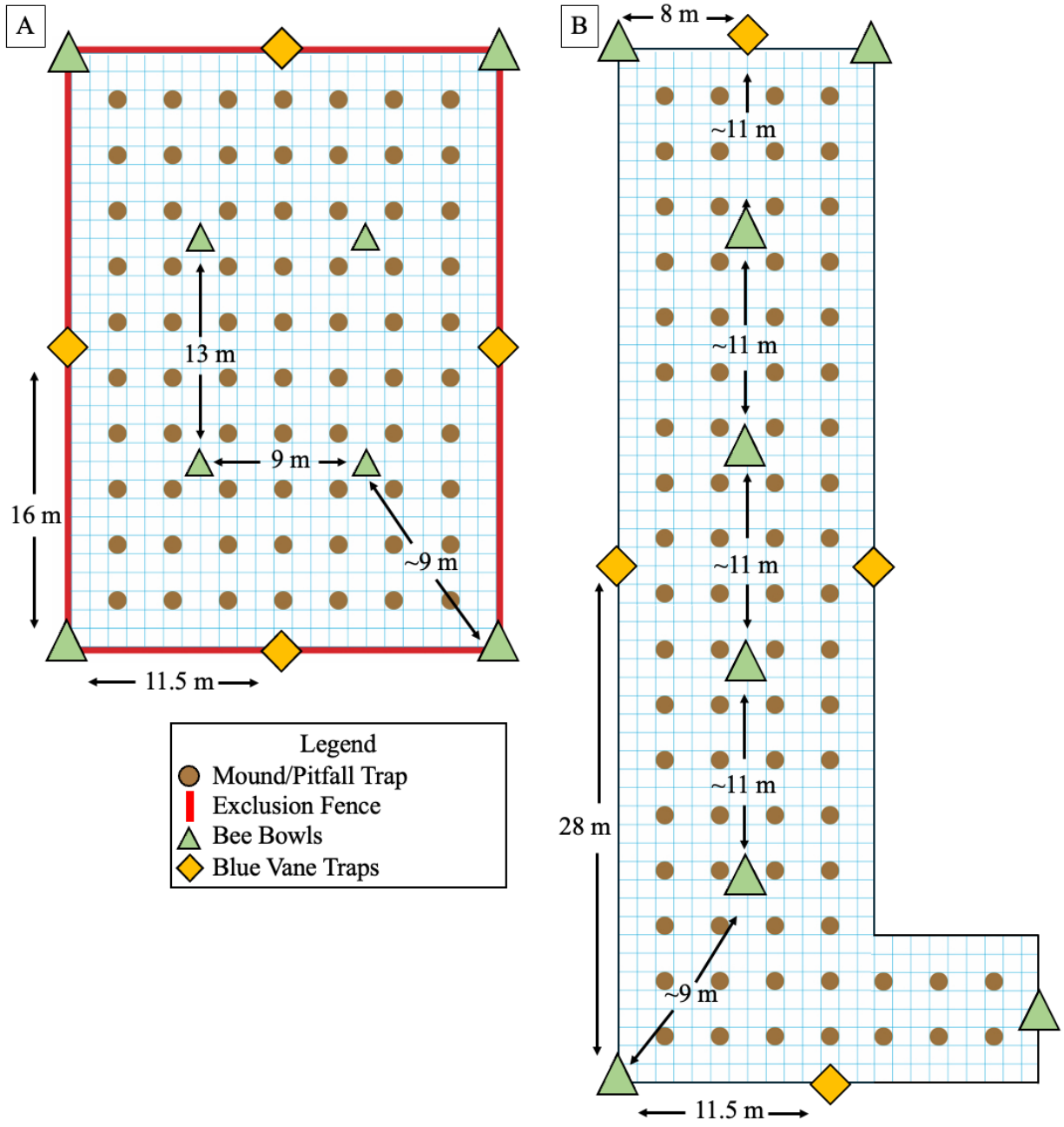

Figure 4. Visual representation of bee bowl, blue vane trap, and pitfall trap placements within the study plots. Each grid square represents 1 m^2. (A) Location of insect sampling methods at five out of six study sites (Brokenhead, Carman, Glenlea, Emo, New Liskeard). (B) Position of insect sampling methods at The Point.

Figure 5. Photos of passive bee sampling methods deployed in the field. (A) Bee bowl sampling method. (B) Blue vane trap sampling method.

Bee bowls and BVTs were filled with a mixture of water and dish soap (4 L water to 1 teaspoon, Dawn Ultra Original Scent®) to approximately ¾ and ⅓ of their total volume, respectively (Droege et al. 2016). Traps were deployed weekly for a total of 8 hours. Traps were collected at the end of the sampling day, no later than 5 p.m., and placed into the corresponding Whirl-Pak containing 70% ethanol (Droege et al. 2016). At the end of each sampling day, the bees were left in their respective Whirl-Paks as "wet" specimens and placed in the freezer until processing.

Processing Bees

Given how the bee samples were stored, dictated the respective processing methods. For bees collected through targeted aerial netting that were left as frozen "dry" specimens, the bees were allowed to thaw for a few minutes before pinning. Bees collected by bee bowls or BVTs that were left as "wet" specimens were separated from the 70% ethanol with a fine sieve and dried using a combination of hairdryer and paper towel before pinning (Droege et al. 2016). All

bee samples were allowed to fully dry before they were labelled and databased following the J.B. Wallis/R.E. Roughley Museum of Entomology guidelines. Honeybees (*Apis mellifera* L.) were excluded from methods as they are a commercial pollinator and not native to Canada (Caron 2021, Portman et al. 2023).

Bees were identified to genus (Packer et al. 2007) and then species using online keys (e.g., DiscoverLife.org), museum voucher specimens, published taxonomic keys, and reference material (Droege et al. n.d., Stephen 1954, LaBerge 1956, 1961, 1967, 1973, 1980, 1985, 1987, Mitchell 1960, 1962, Roberts 1973a, 1973b, Bouseman and LaBerge 1978, McGinley 1986, Laverty and Harder 1988, Romankova 2003, 2007, Coelho 2004, Michener 2007, Rightmyer 2008, Arduser 2009a, 2009b, 2009c, Gibbs 2010, 2011, Colla and Richardson 2011, Rehan and Sheffield 2011, Sheffield et al. 2011, Koch et al. 2012, Silva 2012, Gonzalez and Griswold 2013, Gibbs et al. 2013, 2023, Williams et al. 2014, Droege and Rehan 2016, Portman et al. 2023, Robinson 2023, Hettiarachchi 2024). Jaqueline Bowles, Celine Chevrefils, Tessa Ferch, and Vanessa Siemens (University of Manitoba) assisted with all bee identification. Jason Gibbs (University of Manitoba) assisted in *Lasioglossum* identification and confirmed the identification of all bee species captured. Bee species were further categorized based on their diet breadth, being either oligolectic (plant specialist, narrow diet) or polylectic (plant generalist, broad diet range) (Portman et al. 2023, Gibbs et al. 2023). All vouchers were deposited at the J.B. Wallis/R.E. Roughley Museum of Entomology at the University of Manitoba.

Carabid Beetles

Pitfall Traps

To evaluate ground beetle diversity present within each treatment type, one pitfall trap was placed at the centre of each mound for a total of 70 pitfall traps per site (Figure 4). Due to logistical limitations, pitfall trapping was exclusive to Manitoba study sites. Sampling was done according to Michael Killewald's (University of Manitoba) method, modified to suit the parameters of this project (Killewald 2025). Pitfall traps were a permanent fixture of each mound until the end of the field season (September–October) and were either open for active sampling or closed with a lid, depending on whether sampling was being conducted or not. The pitfall traps were 32 oz deli containers (Richards Packaging, Winnipeg, Manitoba) (Figure 6). When the

pitfall traps were open and actively sampling, the lids of the deli containers were removed, and a 1:1 propylene glycol/water mixture was added to fill about a third of the container (Figure 6) (Hoekman et al. 2017). Pitfall traps were covered with an exclusionary wire grid made from chicken wire (6" x 6") to prevent leaf litter and small animals from falling into the trap. A rain guard was made from a square of corrugated plastic (6" x 6") and four nails placed at least 2" from the ground (Figure 6) (Hoekman et al. 2017). Pitfall traps were deployed for seven days, at biweekly intervals (depending on the weather), totalling four rounds of observations per site (Table S 2). Upon collection, the propylene glycol/water mixture was separated from all trap contents by using a strainer and the remaining content was placed inside Whirl-Paks containing 70% ethanol. At the end of each sampling period, pitfall traps were then covered with a lid until the next sampling event and samples remained in their respective Whirl-Paks as "wet" specimens that were placed in the freezer until processing.

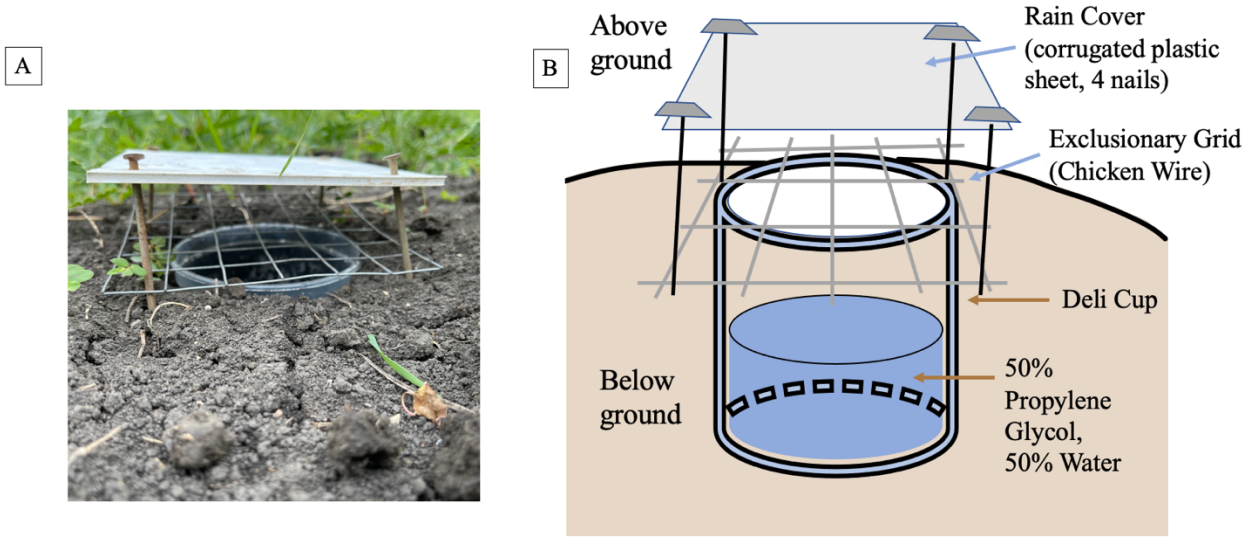

Figure 6. Pitfall trap sampling method. (A) Photo of an open pitfall trap deployed in the field. (B) Illustrated view of above and below ground pitfall trap components.

Processing Ground Beetles

Pitfall trap samples were processed according to Michael Killewald (University of Manitoba) (Killewald 2025). Using a fine sieve, sample contents were separated from the 70% ethanol, large debris was removed by hand or broken down using a gentle rinse of water, and the remaining material was transferred to a large shallow dish. Ground beetles were then separated from other arthropods and non-arthropod material, dried with a paper towel and subsequently pinned, labelled, and databased.

Ground beetles were sorted to morphospecies, identified to genus, and then species using Carl Lindroth's, *The Ground-Beetles of Canada and Alaska* (1961-1969), online sources (e.g., BugGuide.net), and museum voucher specimens. Neil Holliday (University of Manitoba) assisted with initial understanding of the Lindroth key and identifying common genera and species. In cases involving taxonomic revisions or synonym names of a species (e.g., *Harpalus somnulentus* Dejean as a synonym of *Harpalus pleuriticus* Kirby, or *Poecilus lucublandus* (Say) as a synonym of *Pterostichus lucublandus* (Say)), identifications were further verified against Bousquet (2012), Larochelle and Larivière (2003), and curated specimens from the Wallis/R.E. Roughley Museum of Entomology (University of Manitoba). Select *Bembidion* species were identified by Henri Goulet through the Natural Identification Service (NIS) provided by Agriculture and Agri-Food Canada. Two beetles lacked sufficient taxonomic treatments to allow for reliable identification. Vouchers were deposited at the J.B. Wallis/R.E. Roughley Museum of Entomology at the University of Manitoba, and a synoptic set of the most abundant species are held by Victoria Smelko in Milton, Ontario.

Insect Biodiversity Metrics

I measured wild bee and ground beetle communities for their abundance, species richness, and Hill-Shannon diversity (Table 2). I further characterized bees based on functional traits, classifying individuals by species-level diet breadth (lecty) as a plant specialist (oligolectic) or a plant generalist (polylectic) (Gibbs et al. 2023). I used Hill numbers opposed to the traditional species richness indices (Shannon entropy and Gini-Simpsons index) as previous literature suggests problems within traditional diversity calculations, such as sampling stage bias, difficulty with predicting larger size samples, and indices failing to scale intuitively or similarly

with species population fluctuations despite robustness (Jost 2007, Chao et al. 2014). Hill numbers are a unified framework to measure diversity based on the Rényi entropy (Rényi 1961) that considers both the number and the relative abundance of species as interlinked components of diversity (Hill 1973, Chao et al. 2014). The Hill numbers encompass three measures; species richness (q=0) which is the number of species; Shannon number of effective species or Hill-Shannon diversity (q=1) which takes abundance into account but gives more weight to rare species; and the inverse Simpson's number of effective species or Hill-Simpson diversity (q=2) which places more emphasis on dominant species (Hill 1973, Chao et al. 2014, Roswell et al. 2021). The choice of which measure was most appropriate was a decision based on how much leverage rare species were given. For this study, I chose to include the first two measures. Species richness, as it plays a prominent role in foundational ecological models, serves as a cornerstone metric in conservation biology and is frequently used in historical literature (Chao et al. 2014). Hill-Shannon diversity is the middle-of-the-road measure affording leverage to extreme values according to their proportional differences from the mean; thus, it can respond strongly to both very high and very low rarity values, which aligned most with my hypotheses and predictions (Roswell et al. 2021).

Table 2. Insect biodiversity metrics and functional trait variables. Metrics calculated based on insect surveying procedures sampling unit (mound – targeted aerial netting and pitfall traps; trap – bee bowls and BVTs) per sampling event at each study site.

Scale	Variable	Description
Community-Level Metrics	Abundance	Total number of individuals
	Species Richness	Total number of unique species
	Hill-Shannon Diversity	A measure of community diversity that accounts for both species richness and relative abundance, expressed in units of "effective number of species"
Species-Level Trait – Diet Breadth (Bees only)	Oligolectic	Plant specialist, narrow diet range
	Polylectic	Plant generalist, broad diet range

Plant Surveying Procedures

Crop Plant Survey

To evaluate whether crop diversity or density influenced wild bee and ground beetle biodiversity, crop plants were quantified at the individual and species level per mound (Table 3). Mounds were classified by the number of crop species, with counts ranging from one to three, directly corresponding to treatment type (monocrop, dicrop, and Three Sisters). For each individual crop plant, presence was recorded, and growing conditions were assessed as either healthy (growing as expected), dead (discoloured and brittle), or missing (physically absent). Healthy plants present at the time of survey were further assessed for flowering status (actively blooming or not). Plant condition assessments were used to derive insect group-specific mound-level plant count variables for each methods sampling event. Selective incorporation was based on contrasting ecological requirements of wild bees (floral rewards) and ground beetles (vegetation structure, microhabitat, prey availability). For wild bees, plant count was based on flowering potential and included physically present, healthy plants that exhibited an active bloom period at least once during the 2022 field season. In contrast, ground beetle plant count was based on crop plant biomass and included all physically present crop individuals, regardless of condition (healthy or dead). Crop plant surveys were performed once per targeted aerial netting event and twice for pitfall trap sampling (at trap deployment and retrieval).

Table 3. Crop plant variables collected with associated insect surveying procedures. Calculated per mound and sampling event at each study site.

Insect Sampling Method	**Variable**	**Description**
	Cropping	Number of crop plant species in a mound. Reflects the treatment type
Targeted Aerial Netting	Plant Count	Total number of crop plants present and blooming
Pitfall Traps	Plant Count	Total number of crop plants present at end of a sampling round

Non-Crop Plant Survey

To account for local effects of non-crop plant (floral and non-floral weeds) diversity and ground cover characteristics on insect captures, mounds were left unweeded (Grabovska et al. 2020), and vegetation data was collected (Table 4). The percentage of bare ground, grass, and morphologically distinct non-crop plant species was estimated separately using representative scale diagrams provided by Anderson (1986) for quick in-field estimations. Scale diagrams included different orientations of percentages (0–100%) in increments of 5%. This survey was done per mound for a total of 70 surveys per site and conducted only once over the field season due to time restrictions. A measure of floral richness was derived from these percent cover estimates. For each site, a catalogue of all non-crop plant species encountered across the mounds was compiled. For each mound, the incidence of cataloged species that exhibited non-zero percent cover (excluding grasses) was recorded. Each distinct non-grass, non-crop species present was counted as a single occurrence, generating a floral richness value per mound. Non-crop plants were identified in the field using the Apple iPhone feature "Visual Look Up" for quick identification. If a plant could not be identified accurately or at all in the field, the plant was photographed from multiple angles, its defining characteristics were noted (e.g., leaf texture, flower colour, etc.), dry-pressed, and taken back to the lab, where identification was confirmed and brought to the lowest classification using field guides or iNaturalist (iNaturalist n.d., Scoggan 1957, Reaume 2009, Holm 2017).

Table 4. Non-crop plant variables representing local land cover. Calculated per mound at each study site.

Variable	**Description**
Floral Richness	Total number of non-crop plant species (excluding grasses)
Bare Ground (%)	Average bare ground
Grass Cover (%)	Average area covered by grass

Harvest Procedures

To assess if there were differences in monocrop, dicrop, and Three Sisters harvest metrics, mature crops were collected by hand at the end of the growing season prior to each site's

anticipated first frost date of September 18, 2022–September 26, 2022 (Government of Manitoba n.d., Ministry of Agriculture 2024). The first step in processing was to record the total count of each crop's harvest, including the number of whole sunflower heads, bean pods, and squash per mound. Sunflower and bean seeds were then separated from excess plant material (flower head/receptacle, dried petals, bean hull, leaves) by hand, and the "wet" weight (g) of the seeds was recorded per mound to two decimal places. Squash required an additional step before seed processing. For each fruit, the peduncle was removed, and the whole squash was weighed to obtain the "whole squash" weight (g). After this measurement, the fleshy tissue was removed, the seeds were separated, and the seed "wet" weight (g) was recorded per mound to two decimal places. All seeds were then spread across open racks to dry 3-5 days after harvest (FAO 2013, Uddin et al. 2016). The majority of the drying was completed in the Bobiwash lab (University of Manitoba, Winnipeg) using ambient room temperature (~20–25ºC) and constant airflow from an oscillating fan set to high (FAO 2001, Uddin et al. 2016, Gunathilake et al. 2018). Seeds were allowed to dry for up to one month to account for the lack of moisture-testing equipment and varying optimal drying temperatures (Coradi et al. 2015, Uddin et al. 2016, Gunathilake et al. 2018). Seeds were rotated weekly to prevent moisture absorption and reduce the risk of mould or spoilage (Gunathilake et al. 2018). Finally, the "dry" weights (g) of each plant species' seeds were recorded to two decimal places, and seeds were counted.

Harvest Metrics

I measured the yield and quality of sunflower, bean, and squash (Table 5) using the final "dry" weights (g) and seed counts for each plant species. For this study, yield was defined as the average number of seeds per a single harvestable unit (sunflower head, bean pod, or squash) and quality as the average weight (g) of a single seed per single harvestable unit (Table 5). For squash, seed samples were lost before weighing; therefore, squash quality was estimated using a proxy: the average weight of a single whole squash, based on the positive association between seed size and fruit size (Paris and Nerson 2003, Nerson 2005).

Table 5. Harvest metrics. Calculated per treatment type (monocrop, dicrop, Three Sisters) and single unit of harvest (sunflower head, bean pod, or squash) at each study site.

Variable	Description
Sunflower Yield	Average number of seeds per single sunflower head
Sunflower Quality (g)	Average weight of a single seed per single sunflower head
Bean Yield	Average number of beans per single bean pod
Bean Quality (g)	Average weight of a single bean per single bean pod
Squash Yield	Average number of seeds per single squash
Squash Quality (g)	Average whole weight of a single squash, used as a proxy for single seed weight

Statistical Analysis

The following explorations and statistical analyses were performed in R (R Core Team 2024). Prior to all model interpretation, the 'DHARMa' package (Hartig 2024) was used to visually assess residuals for deviation from the expected distribution, dispersion, and outliers using quantile-quantile plots and histograms.

Pre-Analysis Data Screening

All collected data were first assessed for consistency, accuracy, and accompanying metadata. Records were excluded from analysis if they contained missing or incorrect labels; if mound conditions did not reflect the assigned treatment (e.g., absence of plants, loss of all individuals of a crop species within a polycrop, or severe stunted plant growth that precluded flowering or biomass contribution); if sampling rounds were misaligned (e.g., preformed outside the planned sampling window); if complimentary data was missing; or the outlined procedure was not followed correctly. Sites with inadequate sample sizes for comprehensive analyses were also excluded. Consequently, all bee bowl and BTV sampling from Manitoba sites, local land cover variables collected from non-crop plant surveys (floral richness, bare ground and grass percentages), as well as all data from the Ontario sites (Emo and New Liskeard), were omitted from analysis.

Modelling Insect Biodiversity Metrics

I constructed generalized linear mixed models (GLMMs) using the glmmTMB package (Brooks et al. 2017) to test the effects of Indigenous crop plant combinations (treatments) on wild bee and ground beetle abundance, species richness, Hill-Shannon diversity, and wild bee breadth (lecty) (Table 2). In addition, the variables "cropping" and "plant count" (Table 3) were included as additional covariates for treatment to assess whether variation in plant composition or density within treatments might confound the effect seen on insect responses. I also examined several meteorological variables (Table 1). All models included a nested random variable of mound within site, and a crossed random factor of sampling round to account for the evolution of bee and beetle communities over time.

I ran a total of seven global models: four for wild bees and three for ground beetles. The following represents the general structure of the global models used in wild bee (1) and ground beetle analyses (2):

$$Y \sim treatment + cropping + plant\ count + daily\ average\ temperature + daily\ maximum\ temperature + daily\ minimum\ temperature + daily\ average\ wind\ speed + daily\ maximum\ wind\ speed + daily\ minimum\ wind\ speed + (1|site/mound) + (1|sampling\ round) \quad (1)$$

$$Y \sim treatment + cropping + plant\ count + weekly\ average\ temperature + weekly\ maximum\ temperature + weekly\ minimum\ temperature + (1|site/mound) + (1|sampling\ round) \quad (2)$$

Diet breadth was modelled as the proportion of specialist and generalist species using a subset of the wild bee model structure (1) that excluded weather-related variables, as this response reflected a functional trait rather than an abundance metric influenced by sampling conditions.

Global models were initially checked for rank deficiency, convergence issues, and goodness-of-fit. Predictor variables that resulted in rank deficiency were dropped from the model unless they were one of the additional focal variables ("cropping" or "plant count"), in which case, the respective problem variable was run as a separate model. Predictor variables were then assessed for multicollinearity using the 'easystats' package (Lüdecke et al. 2022). Variables found to be moderately or highly correlated (e.g., VIF score of >5) were dropped from consideration as additional covariates (Craney and Surles 2002). Again, if either of the focal

variables to treatment exhibited collinearity, they were evaluated separately. If a global model failed to converge or could not be fit after addressing rank deficiency and collinearity, the model was simplified further by removing non-essential covariates and running the three primary variables of interest as separate models. Although the treatment-focused model was always chosen for final analysis as it was the point of interest for this study, separate models including the additional focal variables that could be successfully fit were formally compared using an analysis of deviance (LaZerte 2021). If a model including a focal variable differed significantly ($p<0.05$) from the treatment-focused model, results were reported.

Count data metrics (abundance and species richness) were run using a Poisson distribution first, and if fit was not achieved, a negative binomial distribution was used to account for overdispersion. The most appropriate family was determined through an analysis of deviance, choosing the model that was significantly different ($p<0.05$) (LaZerte 2021). If no differences were detected, the model with the lowest Akaike's Information Criterion (AIC) score was chosen, indicating optimal balance between model fit (likelihood) and complexity (Akaike 1998, Warren and Seifert 2011). Hill-Shannon diversities were created using the 'hillR' package (Li 2018), and models were first run using a Gaussian distribution; however, if model convergence failed due to variability, Hill-Shannon diversity values were aggregated at the treatment level by averaging across mound, models were then refit with site and sampling round as crossed random factors, and run accordingly. Finally, the diet proportion models, exclusive to wild bee analyses, were run using a binomial distribution. In the final chosen models, if significance was found ($p<0.05$), a pairwise post-hoc test was performed using the 'emmeans' package (Lenth 2024), with p-values adjusted using the False Discovery Rate (FDR) method to control for multiple testing.

Modelling Harvest Metrics

GLMMs were run to evaluate the effect of treatment on sunflower, bean, and squash harvest metrics (Table 5). All models were run using a Gaussian distribution, including treatment as a fixed effect and site as a random variable. If significance was found ($p<0.05$), a pairwise post-hoc test was performed using the 'emmeans' package (Lenth 2024), with FDR adjusted p-values to control for multiple testing.

Results

Insect Biodiversity Metric Analysis

The initial global models for each insect response variable were attempted; however, the inclusion of "cropping" resulted in rank deficiency, prompting two separate models to be run using the respective general structure for each insect biodiversity metric (3a, 3b) and wild bee diet breadth (4) response models:

$$Y \sim \textit{treatment} + \textit{plant count} + \textit{daily average temperature} + \textit{daily maximum temperature} + \textit{daily minimum temperature} + \textit{daily average wind speed} + \textit{daily maximum wind speed} + \textit{daily minimum wind speed} + (1|\textit{site/mound}) + (1|\textit{sampling round}) \quad (3a)$$

$$Y \sim \textit{cropping} + \textit{plant count} + \textit{weekly average temperature} + \textit{weekly maximum temperature} + \textit{weekly minimum temperature} + (1|\textit{site/mound}) + (1|\textit{sampling round}) \quad (3b)$$

$$Y \sim \textit{treatment} + \textit{plant count} + (1|\textit{site/mound}) + (1|\textit{sampling round})$$
$$Y \sim \textit{cropping} + \textit{plant count} + (1|\textit{site/mound}) + (1|\textit{sampling round}) \quad (4)$$

Moreover, "plant count" was correlated with the respective focal variables (Table S 3); therefore, a total of three separate models were run per insect biodiversity metric. Of the weather predictor variables used in the biodiversity metric models, all showed moderate to high levels of correlation, except the minimum daily temperature used in wild bee analysis (Table S 4). Ultimately, this variable was dropped from the final model structure (5) based on biological relevance.

$$Y \sim \textit{treatment} + (1|\textit{site/mound}) + (1|\textit{sampling round})$$
$$Y \sim \textit{cropping} + (1|\textit{site/mound}) + (1|\textit{sampling round}) \quad (5)$$
$$Y \sim \textit{plant count} + (1|\textit{site/mound}) + (1|\textit{sampling round})$$

For wild bee diet breadth and ground beetle Hill-Shannon diversity global models, both required aggregating at the treatment level, simplifying the structure of random effect. Furthermore, I found that the inclusion of additional variables resulted in consistent violation of

assumptions and/or convergence issues. Therefore, Hill-Shannon diversity models used for analysis were run using the simplest model:

$$Y \sim treatment + (1|site) + (1|sampling\ round)$$
$$Y \sim cropping + (1|site) + (1|sampling\ round)$$
$$Y \sim plant\ count + (1|site) + (1|sampling\ round)$$

Wild Bees

Captures

I collected 812 bees utilizing all three collection methods, identifying 56 species in 19 genera (Gibbs et al. 2023). After excluding data that did not meet the project's parameters (e.g., non-representative treatments, missing companion data, early/late sampling periods, etc.), potentially 739 bees could be used for statistical analysis (Table S 5). Further exclusions resulted in bee bowl and blue vane trap data to be omitted from parametric testing, leaving only the 124 bees caught through targeted aerial netting to be used in formal statistical analyses (Table S 5). Bee bowl and blue vane trap data will be taken no further than presenting total counts and relative abundances (expressed as a percentage of total individuals) of select genera and species in the results discussed below (Table S 5; Figure S 1).

Bee Bowls and Blue Vane Traps (BVTs)

Collectively, passive bee sampling resulted in 615 bees: 481 through BVTs and 134 using coloured bee bowls. BVTs captured the highest diversity with 16 genera and 42 species, 11 of which were unique to this method (Table S 5; Figure S 1A). The majority of BVT captures were dominated by the genera *Melissodes* (246 specimens, 51.1%), *Bombus* (102 specimens, 21.2%), and *Lasioglossum* (49 specimens, 10.2%) (Figure S 1A). *Melissodes (Eumelissodes) agilis* Cresson was the most abundant species with 117 specimens, followed by *Melissodes (Eumelissodes) trinodis* Robertson with 68 specimens, both considered sunflower specialists (Table S 5) (Gibbs et al. 2023). Additionally, I found two accounts of a rare provincial record (*Melissodes (Melissodes) bimaculatus* (Lepeletier)) and a single account of a rare Canadian record (*Melissodes (Eumelissodes) bidentis* Cockerell) (Gibbs et al. 2023). Bee bowls caught 15

genera and 34 species, with seven species unique to this technique (Table S 5; Figure S 1B). The majority of bee bowl captures belonged to the genera *Lasioglossum* (45 specimens, 33.6%), *Melissodes* (25 specimens, 18.7%), and *Halictus* (14 specimens, 10.4%) (Figure S 1B). One bee species I caught was a rare provincial record, first found in Manitoba in 2018, *Triepeolus helianthi* (Robertson) parasitizes nests of *Melissodes (Eumelissodes)* spp. and members of the subfamily *Nomiinae* (Gibbs et al. 2023).

Targeted Aerial Netting

Targeted aerial netting yielded the fewest specimens in only 9 genera and 23 species, 4 of which were exclusive to this method (Table S 5; Figure S 2). Dominant genera are presented with total count and relative abundance (expressed as a percentage of total individuals). The majority of bee captures belonged to the genera *Protandrena* (38 specimens, 31%), *Bombus* (26 specimens, 21%), *Melissodes* (26 specimens, 21%), and *Megachile* (18 specimens, 14.5%) (Figure S 2). Within the seven treatments, the ones containing sunflowers were seen to have captured the most bees for all community metrics (Table S 6) Treatments containing sunflowers accounted for ~87% of species, and total specimens (90.3% of individuals) collected through netting, with the remaining treatments (bean, squash, and squash bean dicrop) yielding ≥6 specimens, genera, and species each. Mirroring a similar pattern, *Protandrena (Pterosarus) perlaevis* (Cockerell) was the most abundant species collected in sunflower-containing treatments.

Effect of Treatment

The subsequent biodiversity analyses were conducted using wild bee captures via targeted aerial netting, as this method provided a direct link between wild bees and plants they visit.

Abundance

Bee abundance models were fit using a negative binomial distribution. Model comparison indicated that the treatment-focused model provided the best fit for the observed bee abundance data (Table 6). Cropping (GLMM, χ^2=4.9222, df= 1, p=0.02651) and plant count (GLMM, χ^2=7.0438, df= 1, p=0.007954) focused models both showed a significant relationship with the

number of bees; however, only the plant count model displayed evidence of adequately describing the observed bee abundance patterns among the non-treatment models (Table 6). Treatment was found to have a highly significant effect on wild bee abundance (GLMM, χ^2=45.611, df= 6, p=3.538e^{-08}). I performed a post-hoc analysis to see how these treatments differed from each other (Table 7).

Bee abundance differed strongly among treatments (Figure 7). The Three Sisters treatment was found to catch approximately 24, 10, and 9 times more bees than the monocrop bean, squash, and squash bean dicrop treatment, respectively (Figure 7A–D). Treatments containing sunflowers, regardless of plant diversity, had no difference in the number of bees captured when compared to the Three Sisters (Figure 7A, E–G). When comparing monocrop treatments, bean and squash monocrops did not differ in the number of bees captured from one another but caught approximately 97% and 93% less bees than the sunflower monocrop, respectively (Figure 7B–C, E). Comparisons between monocrops and dicrop treatments revealed a similar pattern. Bean monocrops observed 92% and 95% fewer bees than sunflower bean and sunflower squash dicrops; no differences were seen when compared to the squash bean treatment (Figure 7B, D, F–G). The squash monocrop treatment caught ~81% and ~90% less bees than sunflower bean and sunflower squash dicrops, respectively. Again, no difference in bee captures was observed when compared to the squash bean dicrop (Figure 7C–D, F–G). Finally, the sunflower monocrop caught approximately 2.5 times more bees than sunflower bean dicrops; no difference was seen between sunflower squash mounds and the squash bean treatment observed 92% less bees than the monocrop (Figure 7D–G). When comparing dicrop treatments to one another, two out of the three combinations saw a difference, the squash bean treatment captured approximately 78% and 88% less bees than sunflower squash and sunflower and bean mounds (Figure 7D, F–G).

Table 6. Results of wild bee abundance model selection based on lowest AIC score. Significant p-values are shown in bold (α=0.05).

Model Focus	df	AIC	BIC	LogLik	Deviance	p
Cropping	6	758.68	789.74	-373.34	746.68	
Plant Count	6	756.17	787.23	-372.08	744.17	**<0.0001**
Treatment	11	700.64	757.59	-339.32	678.64	**<0.0001**

Table 7. Results (response scale) of wild bee abundance post-hoc pairwise comparison. The ratio represents an odds ratio comparing the two respective treatments listed and the p-value reflects whether or not that ratio is different from 1. The following abbreviations are used for treatment: 3S., Three Sisters; Be., Bean monocrop; Sq., Squash monocrop; Sun., Sunflower monocrop; SunBe., Sunflower Bean dicrop; SunSq., Sunflower Squash dicrop; and SqBe., Squash Bean dicrop. Significant p-values are shown in bold (α=0.05).

Treatment Pairs	Ratio	Std. Error	z	p
3S - Be	24.3379	19.1958	4.047	**0.0003**
3S - Sq	10.7273	6.9493	3.663	**0.0007**
3S - SqBe	9.3363	5.6004	3.724	**0.0007**
3S - Sun	0.7816	0.2869	-0.671	0.5550
3S - SunBe	1.9964	0.8622	1.601	0.1642
3S - SunSq	1.1067	0.4363	0.257	0.8368
Be - Sq	0.4408	0.4071	-0.887	0.4376
Be - SqBe	0.3836	0.3393	-1.083	0.3657
Be - Sun	0.0321	0.0247	-4.472	**0.0002**
Be - SunBe	0.0820	0.0654	-3.136	**0.0036**
Be - SunSq	0.0455	0.0356	-3.953	**0.0003**
Sq - SqBe	0.8703	0.6335	-0.191	0.8487
Sq - Sun	0.0729	0.0454	-4.203	**0.0002**
Sq - SunBe	0.1861	0.1209	-2.589	**0.0172**
Sq - SunSq	0.1032	0.0653	-3.591	**0.0008**
SqBe - Sun	0.0837	0.0479	-4.336	**0.0002**
SqBe - SunBe	0.2138	0.1278	-2.581	**0.0172**
SqBe - SunSq	0.1185	0.0688	-3.674	**0.0007**
Sun - SunBe	2.5542	1.0092	2.373	**0.0285**
Sun - SunSq	1.4160	0.5093	0.967	0.4120
SunBe - SunSq	0.5544	0.2330	-1.404	0.2245

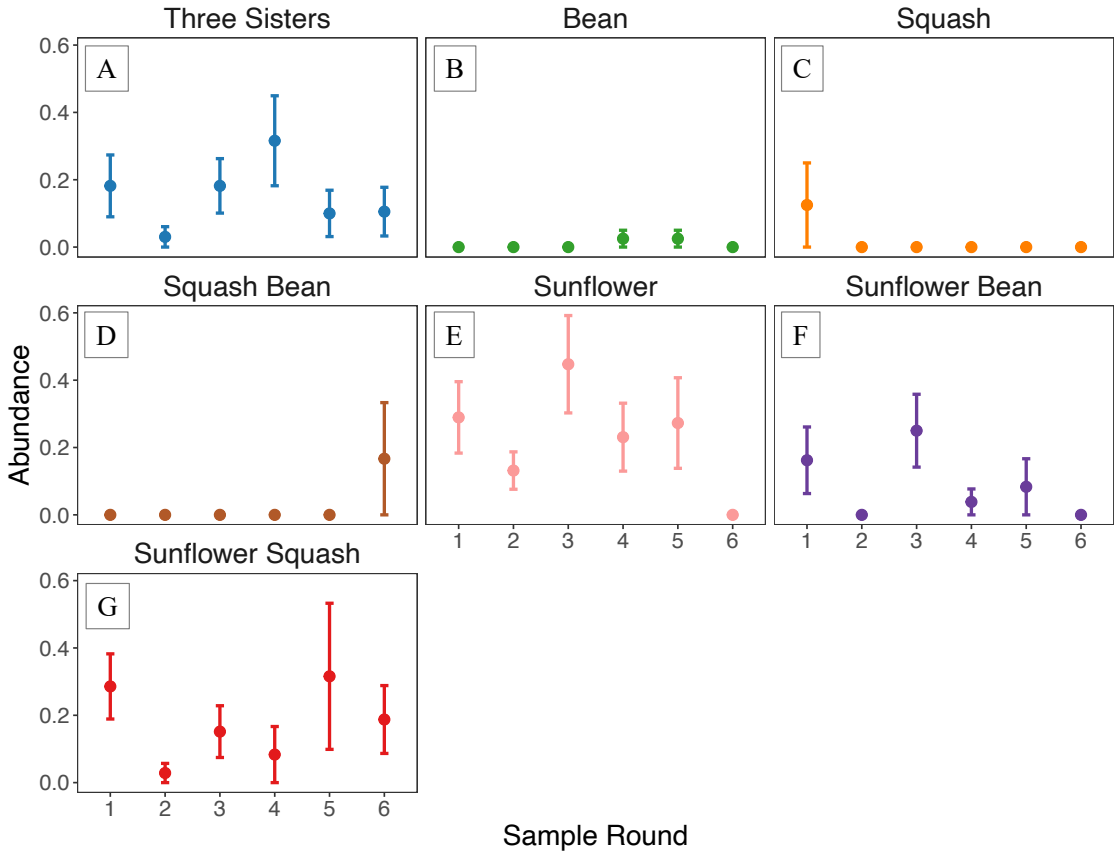

Figure 7. Effect of treatment on wild bee abundance. Points represent the average number of wild bees captured through targeted aerial netting per sampling event. Error bars represent the 95% confidence intervals of the mean.

Species Richness

Wild bee species richness models were fit using a negative binomial distribution. Among the three models, the treatment-focused model was found to best describe the data (Table 8). Both cropping (GLMM, χ^2=5.2908, df= 1, p=0.02144) and plant count (GLMM, χ^2=8.0361, df= 1, p=0.004585) models were significantly associated with species richness when evaluated independently, although only the plant count model captured meaningful patterns in the observed data. Treatment had a highly significant impact on wild bee species richness (GLMM, χ^2=51.704, df= 6, p=2.138e^{-09}). I conducted a post-hoc analysis to evaluate how the treatments differed from one another (Table 9).

Wild bee species richness varied significantly among treatments (Figure 8). The Three Sisters treatment captured approximately 20, 10, and 9.5 times more bee species than the monocrop bean, squash, and squash bean dicrop treatments, respectively (Figure 8A–D). In contrast, no differences in richness were detected between the Three Sisters or any of the sunflower-containing treatments (Figure 8A, E–G). Among the monocrop treatments, bean and squash showed no difference in the number of bee species caught between one another, but both supported significantly fewer species than sunflower, approximately 96% and 92% respectively (Figure 8B–C, E). When comparing monocrops to dicrop treatments, bean monocrops showed ~90% fewer bee species than the sunflower bean dicrop and ~95% fewer species than sunflower squash dicrop, while squash monocrops showed ~81% and 90% less species relative to those same treatments (Figure 8B–C, F–G). Both bean and squash monocrops observed no difference in the number of species when compared to the squash bean treatment (Figure 8B–D). The sunflower monocrop caught approximately 2.4 times more bee species than sunflower bean dicrops, no difference was seen between sunflower squash mounds and the squash bean treatment observed 92% fewer bee species than the monocrop (Figure 8D–G). When comparing dicrop treatments, two of the three combinations saw a significant difference in species richness. Squash bean treatment mounds captured approximately 79% and 88% less species than the sunflower bean and sunflower squash treatments (Figure 8D, F–G). The sunflower bean compared to the sunflower squash treatments, observed no differences in the number of bee species captured (Figure 8F–G).

Table 8. Results of wild bee species richness model selection based on lowest AIC score. Significant p-values are shown in bold (α=0.05).

Model Focus	df	AIC	BIC	LogLik	Deviance	p
Cropping	6	714.61	745.67	-351.3	702.61	
Plant Count	6	711.55	742.62	-349.78	699.55	**<0.0001**
Treatment	11	653.24	710.19	-315.62	631.24	**<0.0001**

Table 9. Results (response scale) of a wild bee species richness post-hoc pairwise comparison. The ratio represents an odds ratio comparing the two respective treatments listed and the p-value reflects whether or not that ratio is different from 1. The following abbreviations are used for treatment: 3S., Three Sisters; Be., Bean monocrop; Sq., Squash monocrop; Sun., Sunflower monocrop; SunBe., Sunflower Bean dicrop; SunSq., Sunflower Squash dicrop; and SqBe., Squash Bean dicrop. Significant p-values are shown in bold (α=0.05).

Treatment Pairs	Ratio	Std. Error	z	p
3S - Be	20.2228	15.3000	3.976	**0.0003**
3S - Sq	10.3655	6.6000	3.673	**0.0006**
3S - SqBe	9.5355	5.4200	3.967	**0.0003**
3S - Sun	0.8078	0.2520	-0.685	0.5452
3S - SunBe	1.9630	0.7510	1.763	0.1168
3S - SunSq	1.0535	0.3530	0.155	0.9145
Be - Sq	0.5126	0.4740	-0.723	0.5452
Be - SqBe	0.4715	0.4140	-0.857	0.4834
Be - Sun	0.0399	0.0296	-4.348	**0.0001**
Be - SunBe	0.0971	0.0750	-3.019	**0.0053**
Be - SunSq	0.0521	0.0392	-3.926	**0.0003**
Sq - SqBe	0.9199	0.7160	-0.107	0.9145
Sq - Sun	0.0779	0.0482	-4.122	**0.0003**
Sq - SunBe	0.1894	0.1240	-2.533	**0.0183**
Sq - SunSq	0.1016	0.0643	-3.616	**0.0007**
SqBe - Sun	0.0847	0.0464	-4.508	**0.0001**
SqBe - SunBe	0.2059	0.1210	-2.679	**0.0141**
SqBe - SunSq	0.1105	0.0623	-3.909	**0.0003**
Sun - SunBe	2.4300	0.8520	2.533	**0.0183**
Sun - SunSq	1.3041	0.3960	0.875	0.4834
SunBe - SunSq	0.5367	0.2010	-1.660	0.1357

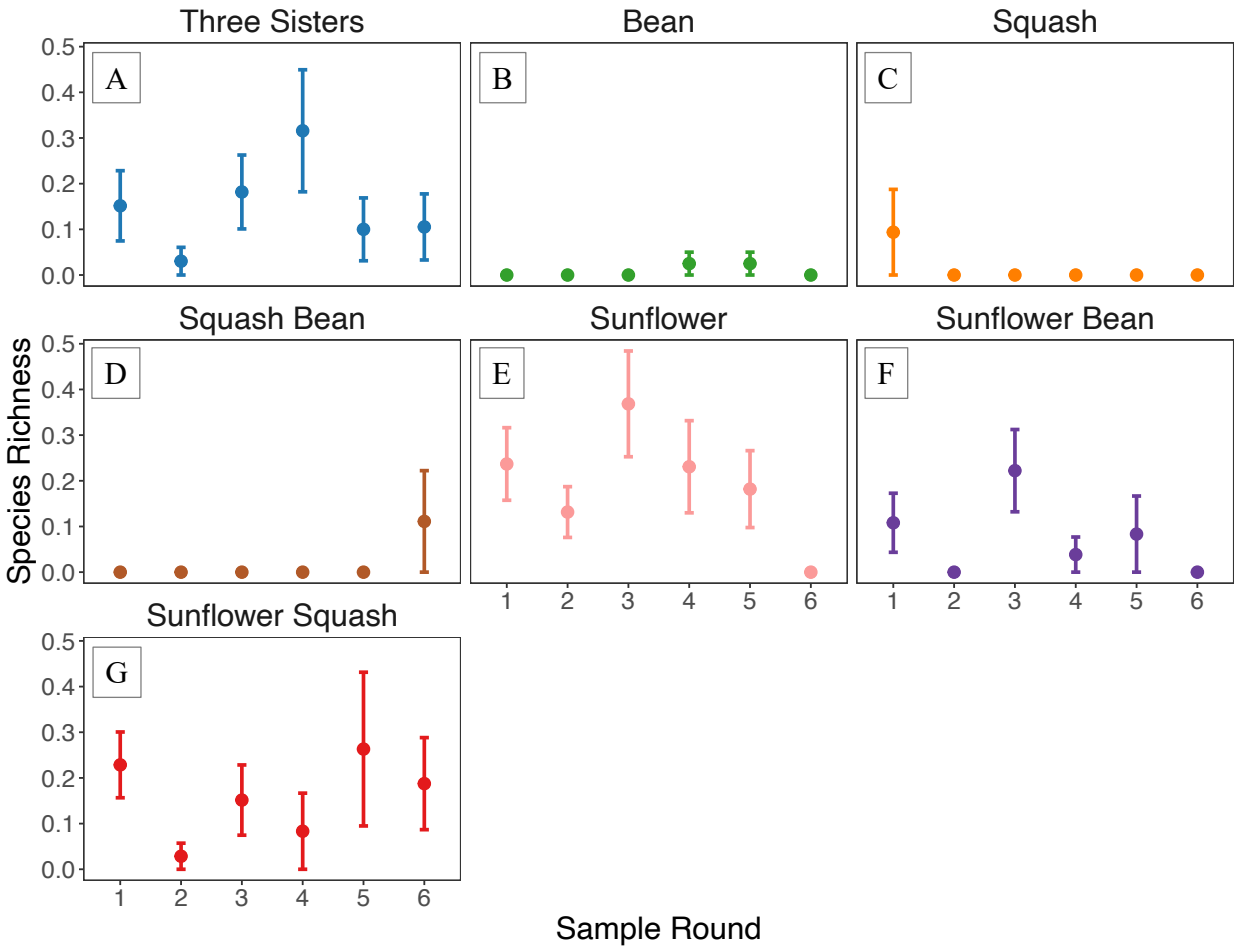

Figure 8. Effect of treatment on wild bee species richness. Points represent the average number of wild bee species captured through targeted aerial netting per sampling event. Error bars represent the 95% confidence intervals of the mean.

Hill-Shannon Diversity

Wild bee Hill-Shannon diversity could not be evaluated using any of the three simplified GLMMs after aggregating, due to low bee capture frequencies across sampling rounds. As a result, Hill-Shannon diversity was examined descriptively by aggregating wild bee captures across the full 2022 field season. Although this data simplification did not resolve analytical limitations, seasonal patterns in Hill-Shannon diversity visually paralleled abundance and species richness, with treatments containing sunflowers exhibiting higher apparent diversity relative to

non-sunflower treatments (Figure 9). These patterns are presented for exploratory purposes only and were not subjected to formal statistical testing.

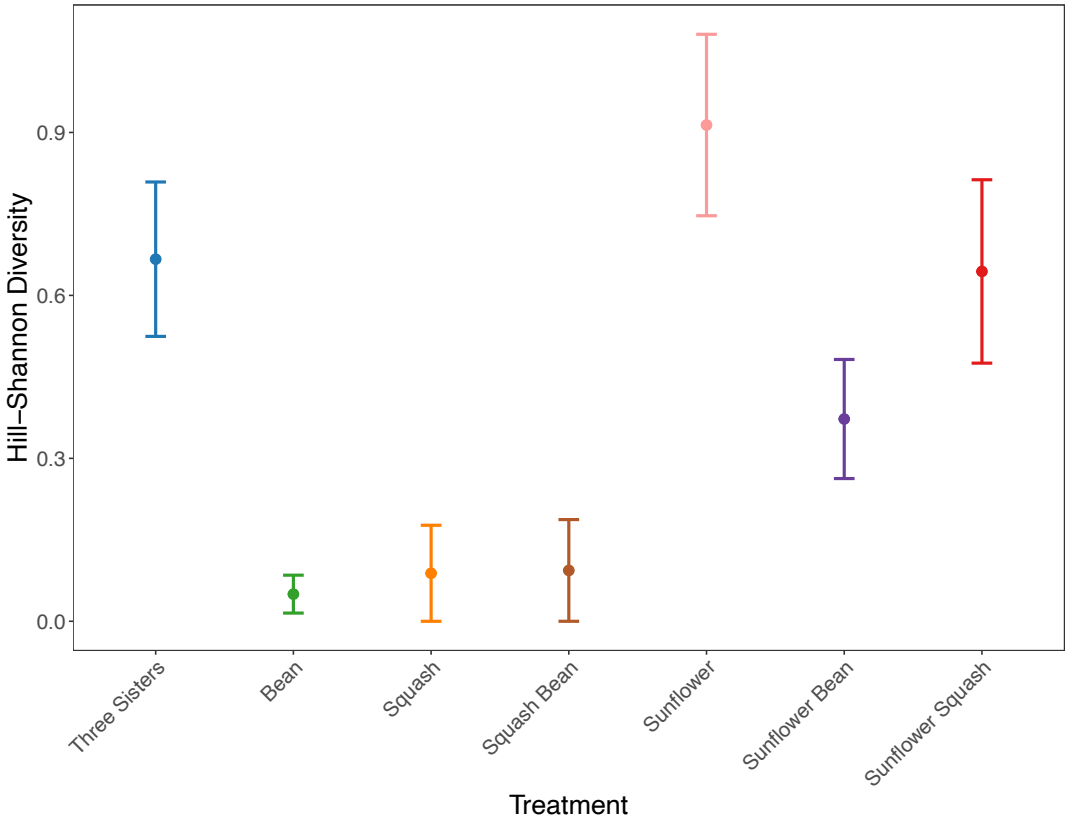

Figure 9. Average Hill-Shannon diversity of wild bees per treatment. Points represent the average Hill-Shannon diversity of wild bees captured through targeted aerial netting during the 2022 field season. Error bars represent the 95% confidence intervals of the mean.

Diet Proportion

Wild bee diet proportions could not be formally analyzed due to an insufficient number of bee captures within sampling events. Consequently, diet breadth was summarized descriptively across the full 2022 field season. Visual inspection of diet proportion indicated a relatively even distribution of generalist and specialist bees in treatments containing sunflowers, whereas bean monocrop and squash bean dicrop treatments were dominated by generalist bees, and, the squash monocrop treatment showed a higher representation of specialist bees (Figure

10). These observations are descriptive and intended to provide ecological context rather than statistical conclusions.

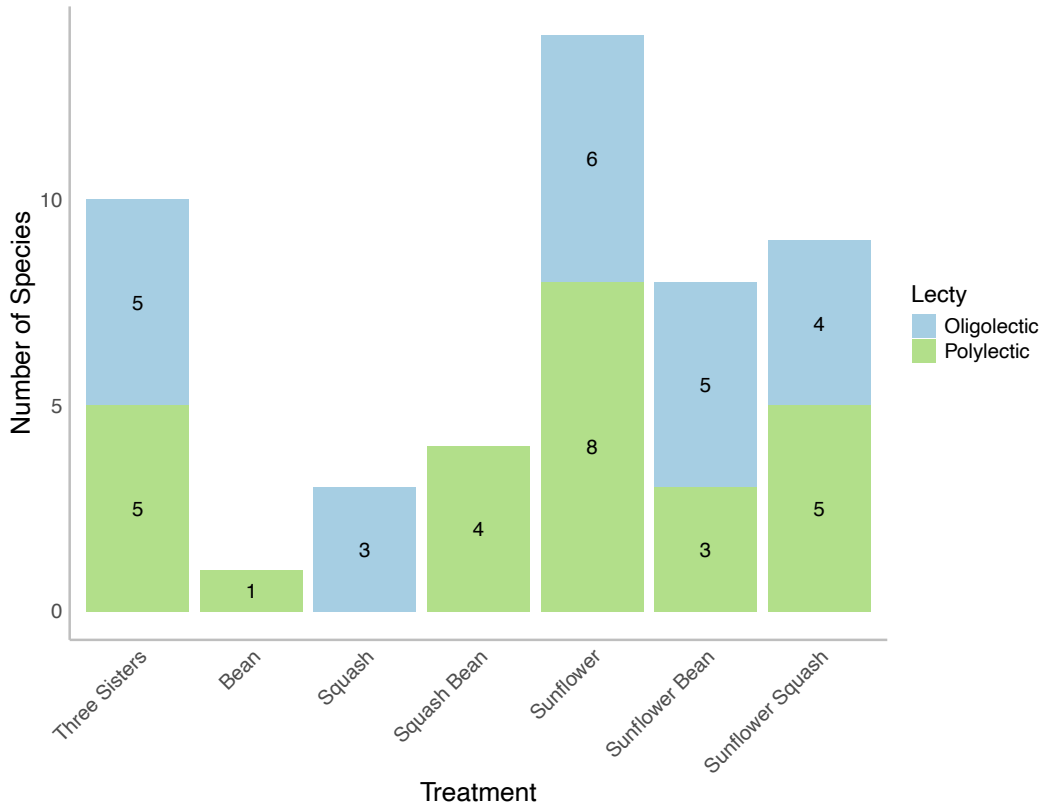

Figure 10. Distribution of specialist and generalist wild bee species per treatment. Bars represent the total number of oligolectic and polylectic species captured through targeted aerial netting within each treatment during the 2022 field season. Graph does not include parasitic bee species.

Carabid Beetles

Captures

I collected 10,158 carabid beetles, identifying 10,156 specimens to 57 species from 21 genera. The remaining two beetles were unable to be identified further than being within the carabid family. After excluding data that did not meet the project's parameters (e.g., non-representative treatments, missing companion data, early/late sampling periods, etc.), a total of 8,557 beetle specimens within 20 genera and 53 species were used for statistical analysis (Table S 7; Figure S 3). Pitfall trap samples were dominated by the genus *Harpalus* in terms of

abundance (~61% of all individuals) and richness (~26% of all species). The *Amara* and *Pterostichus* genera were also abundant and accounted for ~15% and ~10% of all individuals collected, respectively (Figure S 3). While the majority of carabid species were rare (<25 individuals), seven species stood out as highly abundant (>200 individuals). They were, in decreasing order (relative abundance in parentheses, rounded up to the first decimal place), *Harpalus (Pseudoophonus) pensylvanicus* (De Geer) (42%), *Harpalus (Harpalus) amputatus* Say (7.6%), *Amara (Percosia) obesa* Say (6.3%), *Amara (Curtonotus) carinata* LeConte (6.3%), *Harpalus (Plectralidus) erraticus* Say (2.7%), *Poecilus lucublandus* (2.3%), and *Harpalus (Anamblystus) herbivagus* Say (2.2%).

Effect of Treatment

Abundance

Carabid beetle abundance models were fit using a negative binomial distribution. Upon comparison of the three models, no significant differences were found, indicating that no model was clearly supported over the others based on the observed data. Therefore, the treatment model was chosen for analysis. Treatment was found not to influence beetle abundance (GLMM, χ^2=4.3378, df= 6, p=0.631070) (Figure 11).

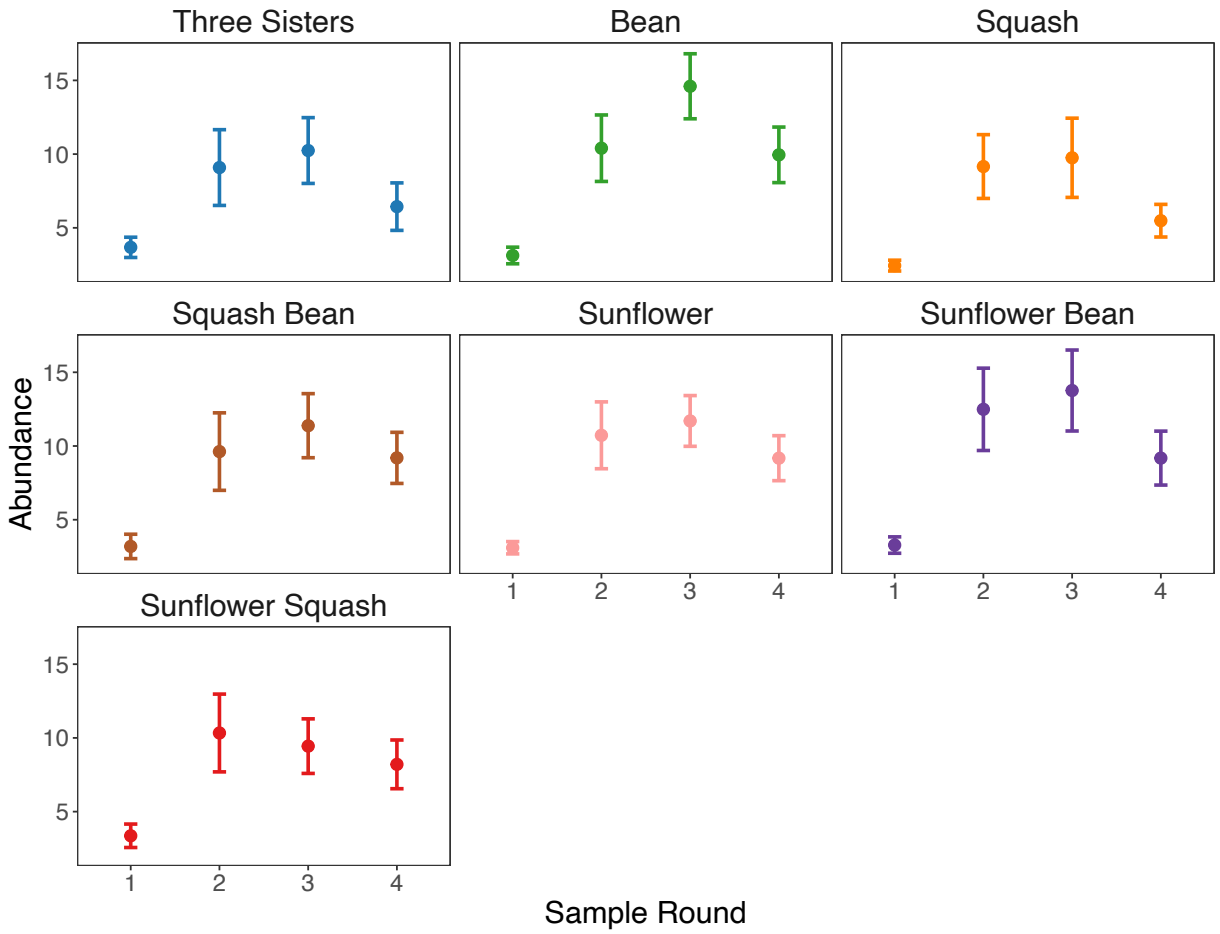

Figure 11. Effect of treatment on ground beetle abundance. Points represent the average number of ground beetles captured per sampling event. Error bars represent the 95% confidence intervals of the mean.

Species Richness

Ground beetle species richness models were fit with a Poisson distribution. The alternative models focusing on cropping or plant count did not differ significantly from the treatment model, suggesting that these predictors provided comparable fits to the observed beetle species richness data. Treatment was found not to influence beetle species richness (GLMM, χ^2=2.6753, df= 6, p=0.848356) (Figure 12).

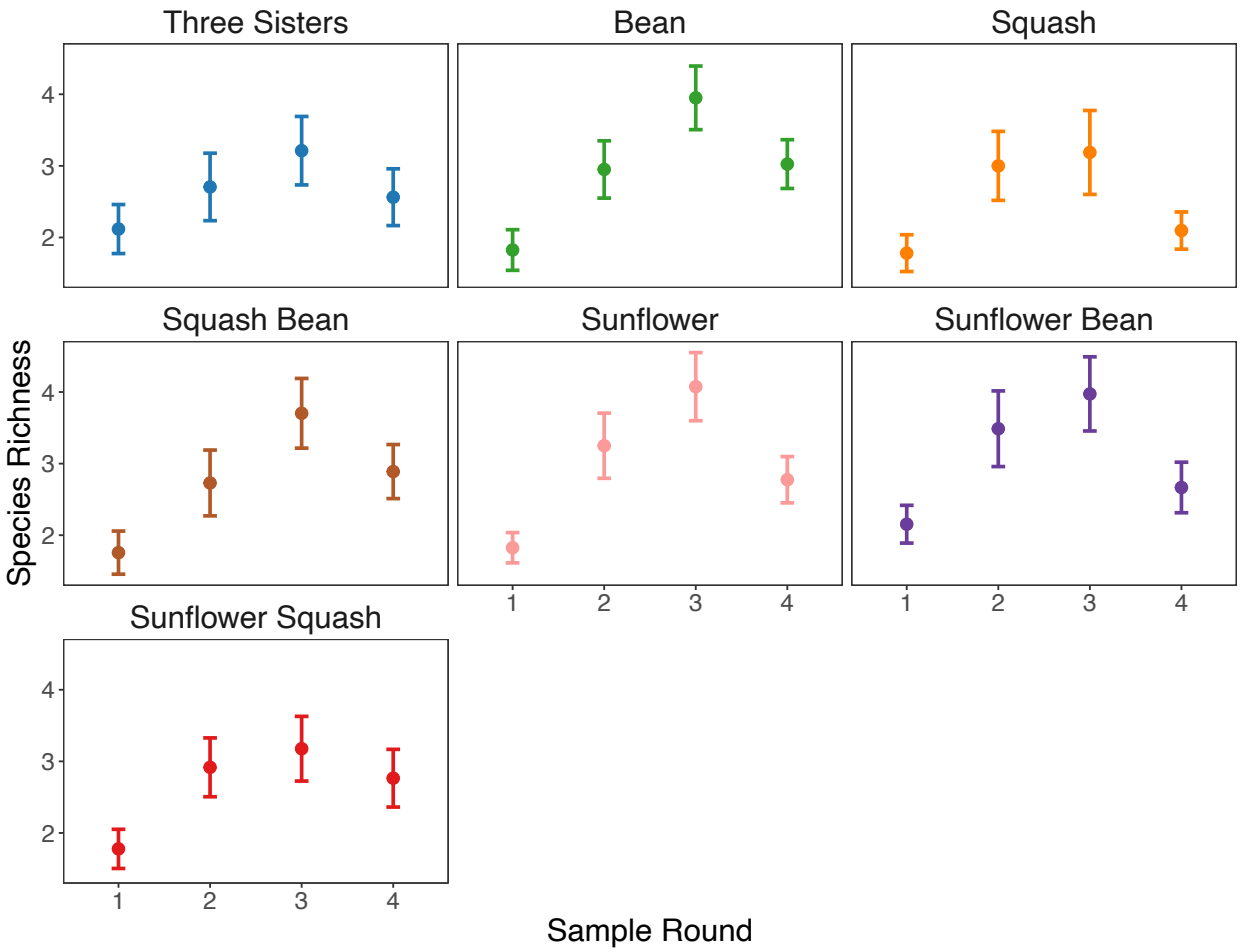

Figure 12. Effect of treatment on ground beetle species richness. Points represent the average number of ground beetle species captured per sampling event. Error bars represent the 95% confidence intervals of the mean.

Hill-Shannon Diversity

Carabid beetle Hill-Shannon diversity model fit best using a Gaussian distribution after simplifying the data (averaging by mound). Comparison of the three models revealed no significant differences between them, indicating the predictors provided comparable fits to the observed data. Treatment was found not to influence beetle Hill-Shannon diversity (GLMM, χ^2=1.9915, df= 6, p=0.9205) (Figure 13).

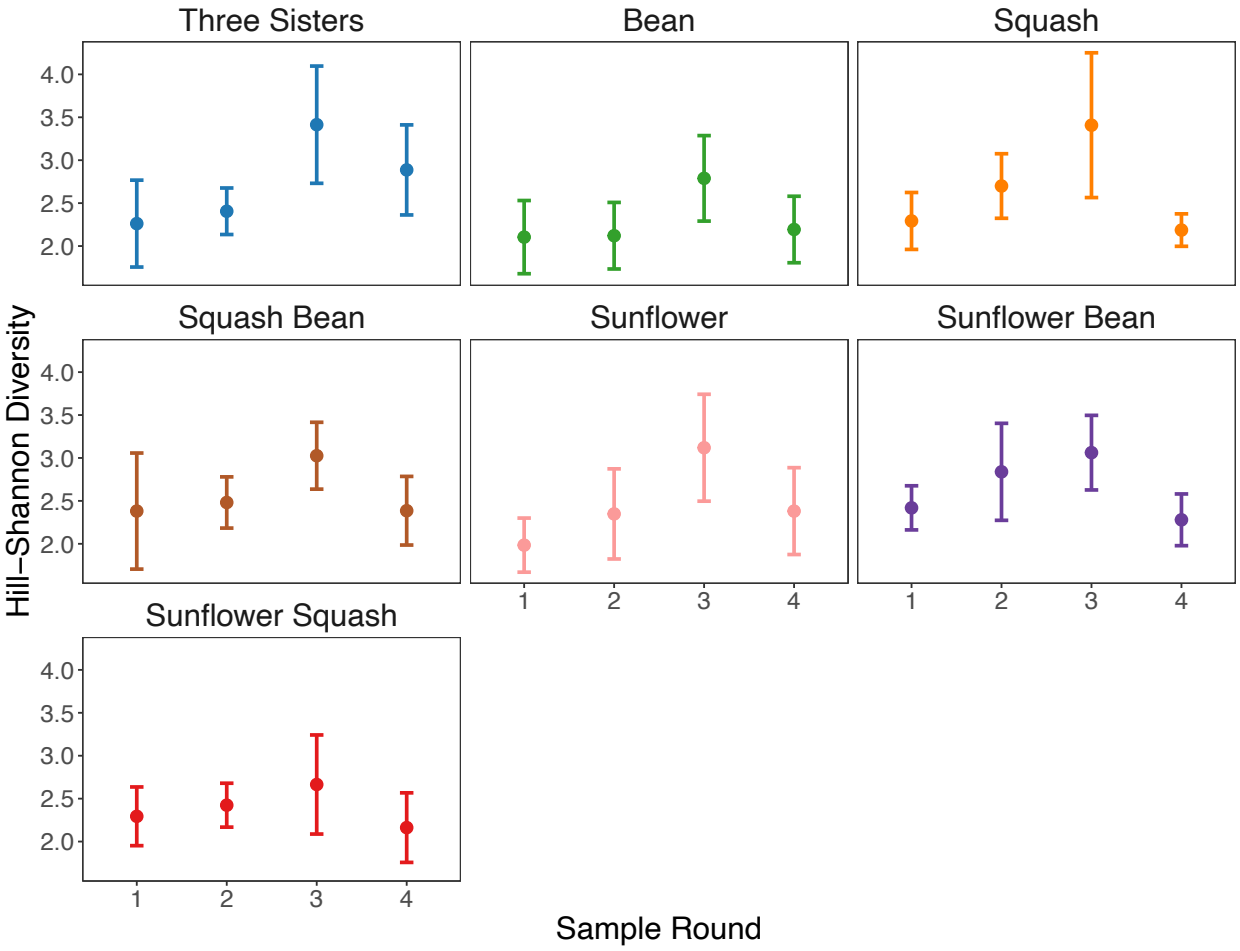

Figure 13. Effect of treatment on ground beetle Hill-Shannon diversity. Points represent the average Hill-Shannon diversity of ground beetles captured per sampling event. Error bars represent the 95% confidence intervals of the mean.

Harvest Metric Analysis

In the 2022 growing season, I sowed a total of 960 plants (not including replacing plants early in the season) across 4 sites (240 plants/site). The end harvest was collected from 264 sunflower plants (969 sunflower heads), 275 bean stalks (1,010 bean pods), and 232 squash plants (78 whole squash). The survival rate for each plant species was less than 30%: 27.5%, 28.6% and 24.2%, respectively. Despite a poor growing season, a total of 35,250 sunflower seeds, 2,275 beans, and 9,251 squash seeds were collected.

Effect of Treatment

Yield

All harvest yields for sunflower, bean, and squash were fit using a Gaussian distribution. Treatment was found to have no significant effect on the average sunflower yield (GLMM, χ^2=0.6430, df= 3, p=0.88652) or bean yield (GLMM, χ^2=2.2447, df= 3, p=0.523196) (Figure 14). Although a marginally significant effect of treatment was seen for squash seed yield (GLMM, χ^2=6.7867, df= 3, p=0.07901), the post-hoc was unable to detect any difference (Table 10).

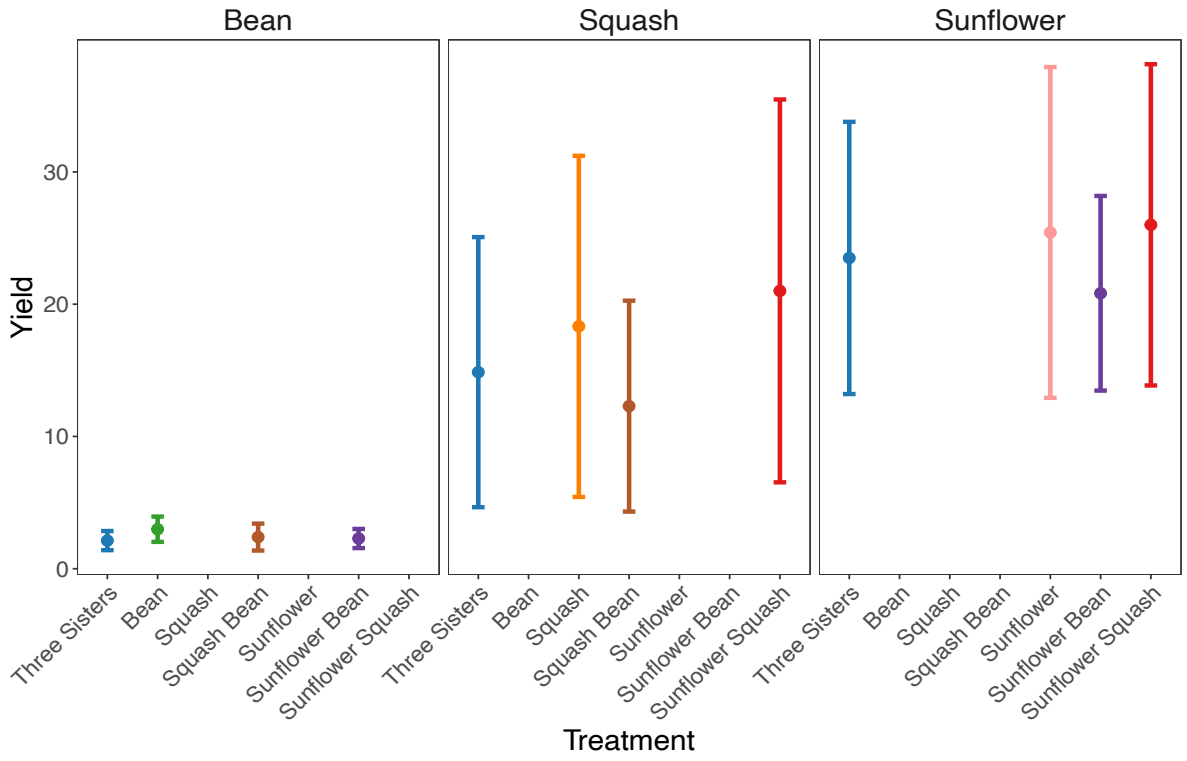

Figure 14. Effect of treatment on crop plant yield. Points represent mean seed yield (average number of seeds per individual harvestable unit: sunflower head, bean pod, or squash). Error bars represent the 95% confidence intervals of the mean.

Table 10. Results (response scale) of the squash seed yield post-hoc pairwise comparison. The ratio represents an odds ratio comparing the two respective treatments listed and the p-value reflects whether or not that ratio is different from 1. The following abbreviations are used for treatment: 3S., Three Sisters; Sq., Squash monocrop; SunSq., Sunflower Squash dicrop; and SqBe., Squash Bean dicrop. Significant p-values are shown in bold (α=0.05).

Treatment Pairs	Ratio	Std. Error	df	z	p
3S - Sq	-3.46	3.6	10	-0.962	0.4916
3S - SqBe	2.57	3.6	10	0.714	0.4916
3S - SunSq	-6.14	3.6	10	-1.707	0.2493
Sq - SqBe	6.03	3.6	10	1.676	0.2493
Sq - SunSq	-2.68	3.6	10	-0.745	0.4916
SqBe - SunSq	-8.71	3.6	10	-2.421	0.2164

Quality

Harvest quality models for individual crop species were analyzed using a Gaussian distribution. No effect of treatment was observed on the sunflower seed quality (GLMM, χ^2=1.5101, df= 3, p=0.6799), but a significant effect of treatment was detected for bean quality (GLMM, χ^2=9.6433, df= 3, p=0.02185). However, post-hoc analysis was unable to detect any significant differences (Table 11). The squash quality model was unable to be run due to convergence issues, likely stemming from a combination of extremely low harvest yields and using whole-fruit average mass as a proxy for individual seed weight. Despite these limitations, descriptive examination indicated modest variation among treatments, with the Three Sisters exhibiting slightly higher quality than the other treatments (Figure 15).

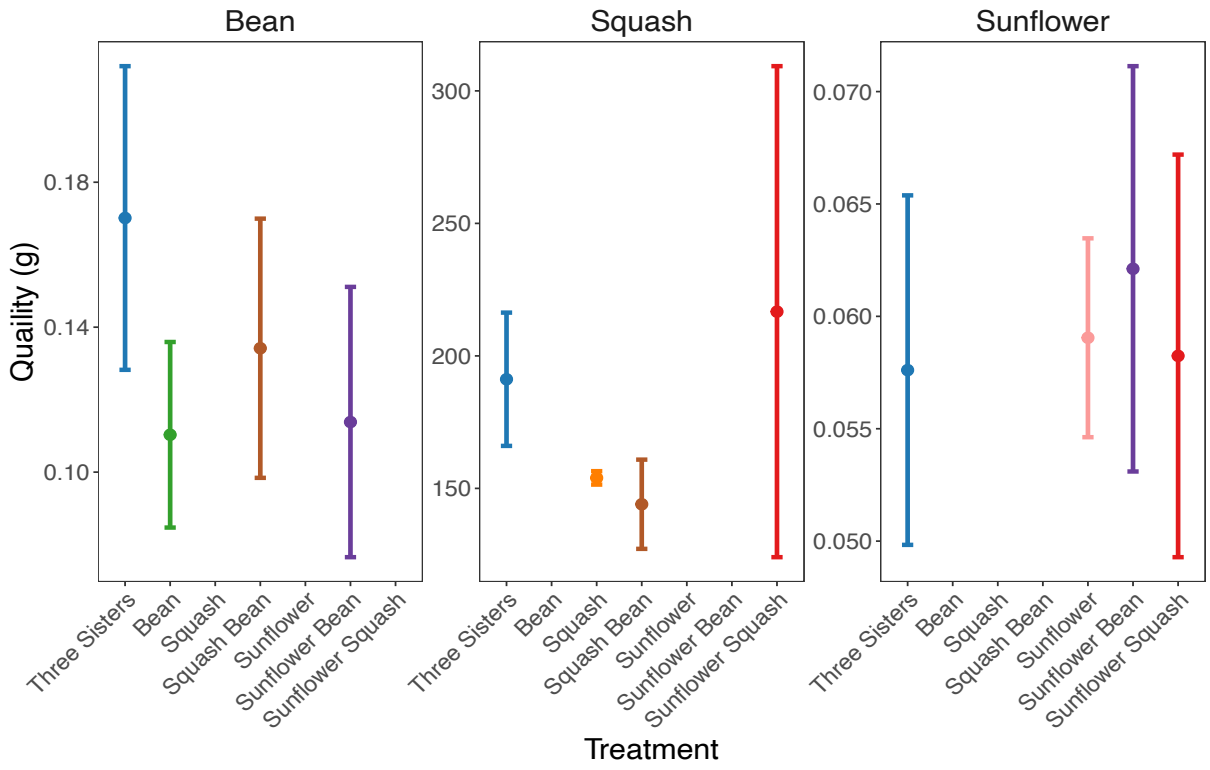

Figure 15. Effect of treatment on crop plant quality (g). Points represent mean seed quality (average weight (g) of a single seed per individual harvestable unit: sunflower head, bean pod, squash). Squash seed quality is represented by a proxy measure based on the average weight (g) of a single whole fruit. Error bars represent the 95% confidence intervals of the mean.

Table 11. Results (response scale) of the bean quality (g) post-hoc pairwise comparison. The ratio represents an odds ratio comparing the two respective treatments listed and the p-value reflects whether or not that ratio is different from 1. The following abbreviations are used for treatment: 3S., Three Sisters; Be., Bean monocrop; SunBe., Sunflower Bean dicrop; and SqBe., Squash Bean dicrop. Significant p-values are shown in bold (α=0.05).

Treatment Pairs	Ratio	Std. Error	df	z	p
3S - Be	0.05983	0.0216	10	2.763	0.0793
3S - SqBe	0.03595	0.0216	10	1.660	0.2556
3S - SunBe	0.05631	0.0216	10	2.601	0.0793
Be - SqBe	-0.02388	0.0216	10	-1.103	0.4429
Be - SunBe	-0.00352	0.0216	10	-0.162	0.8742
SqBe - SunBe	0.02036	0.0216	10	0.941	0.4429

Discussion

Wild Bees

I found that the Three Sisters system and all other sunflower-containing treatments consistently supported higher wild bee abundance and species richness than treatments that did not contain sunflowers. Graphical interpretation of Hill-Shannon diversity suggested that the overall community evenness was consistent with this general trend. The Three Sisters, sunflower bean dicrop, and sunflower squash dicrop likely benefited from the complementary floral traits of the Indigenous significant crops, offering a wider temporal and morphological range of floral resources, effectively creating increased niche space for pollinators (Brooker et al. 2015, Bybee-Finley and Ryan 2018). Staton et al. (2022) similarly found higher bee abundance and species richness in polyculture systems than in monocultures and further demonstrated that functional trait diversity increased as additional bee species filled previously unoccupied areas of trait niche space in monocultures. Although functional traits in their study included characteristics, such as sociality and tongue length, rather than diet, this mechanism provides a conceptual parallel for interpreting patterns in diet proportions in my study. Trait variation (e.g., tongue length) constrains potential resource use, whereas diet reflects realized resource partitioning; therefore, as floral diversity increases, it can support a wider range of both potential and realized resource use, allowing more species with different foraging strategies to coexist. Based on this expectation, I predicted a higher representation of specialist bees in the more diverse treatments, but diet proportions instead appeared relatively balanced. This suggests that increased resource diversity may support the coexistence of multiple foraging strategies rather than favouring a single functional group. However, because these patterns were based on visual interpretation and a limited sample size, they should be taken with caution, as finer-scale niche differentiation may not have been fully captured.

When comparing the Three Sisters polyculture, sunflower monocrop, and sunflower-based dicrops, no differences in wild bee abundance or species richness were observed, suggesting that functional traits of particular crops, rather than crop diversity alone, may shape pollinator assemblages (Ghazoul 2006). In these treatments, it is most likely that the sunflower was the primary driver of bee presence based on its attractive traits (e.g., large floral head, extended bloom period, and high pollen and nectar production) and the observation that the

sunflower monocrop was the treatment that captured the most bees over the whole season (Table S 6). This finding aligns with studies showing that pollinator abundance can be disproportionately influenced by a few highly attractive or resource-rich species in a community, even with otherwise diverse assemblages (Jones and Gillett 2005, Guzman et al. 2019).

These patterns may also reflect asymmetric pollinator competition or distraction, in which sunflower-dominated treatments outcompete nearby flowering crops by attracting the majority of foraging bees. Given the close proximity of treatments and short foraging range of many wild bees, it is possible that the sunflowers acted as a magnet, reducing visitation rates to adjacent non-sunflower treatments (Laverty 1992). However, the influence of floral non-crop weed species within treatment mounds may have also played a critical role. Many non-target flowering plants were observed in bloom during sampling, and incidental observations revealed wild bees and non-target insects foraging, potentially diluting the signal of crop-specific pollination (Riggi et al. 2024). Moreover, competitive displacement by introduced honey bee colonies potentially reduced wild bee presence (Geslin et al. 2017, Iwasaki and Hogendoorn 2022), particularly at The Point and Glenlea sites, where managed honey bee colonies were known to be in close proximity and observed squash flowers contained 8–10 honey bees pollinating at once on multiple sampling dates. Bee captures for squash and bean monocrop and dicrop treatments may have also been underrepresented because of their relatively early and short flowering periods, coupled with the primarily self-pollinating nature of beans (OECD 2015, Bomfim et al. 2016). Therefore, the insect sampling window may not have allowed for corresponding alignment.

Carabid Beetles

Ground beetle abundance, species richness, and Hill-Shannon diversity did not differ significantly across treatments; therefore, my results did not support the hypothesized concurrent increases in carabid and crop diversity. Ground beetle response to crop diversification is mediated not only by plant richness and evenness but also by canopy structure, soil conditions, and other biotic and abiotic factors not investigated in this study (Eyre 1994, Thiele 1997, Kromp 1999, Hummel et al. 2012). These factors may have a greater effect on species diversity than the mixed planting combinations. In a study based on repeated pitfall trapping in a previously sampled field 30 years earlier, species abundance (carabids and formicids) was

reduced by 85–48%, and species dominance shifted by 81–50% depended on soil type (Kromp 1999).

Low plot replication, small plot size, the random movement of carabids, and methodological factors could have masked possible effects of treatments (Cárcamo et al. 1995, Butts et al. 2003, Hummel et al. 2012). However, a meta-analysis by Bommarco and Banks (2003) found that the effects of increased plant diversity may be enhanced in smaller-scale plot designs due to the freedom of movement between control and treatment plots; as spatial scale increased, the ability to detect insect selectivity between simple and diverse plots diminished (Bommarco and Banks 2003). Additionally, research conducted in Alberta using plots of similar size successfully detected significant treatment effects on carabids (Cárcamo et al. 1995, Butts et al. 2003). Together, these studies suggest that the size of my study plots was likely adequate for detecting carabid response to treatment, and it alone is unlikely to explain the absence of significant differences.

The implementation of traditional site maintenance methods (e.g., low-input tilling, hand weeding between treatment mounds), coupled with extreme environmental conditions influencing ground cover, may have further limited the ability to detect treatment effects (Manitoba Agriculture 2022). To facilitate insect sampling, weeding was conducted exclusively between treatment mounds, a process that allowed non-crop plants to establish uniformly across mounds and likely homogenized habitat conditions. Weekly weed removal may have temporarily suppressed weed growth, but it may also have contributed to a forced increase in abundance of *B. quadrimaculatus* Say within the study plot, as they are typically abundant in areas of sparse ground cover (Lindroth 1961-1961, Andersen 1989).

Extreme flooding prior to field establishment further exacerbated these conditions. In 2022, the majority of Canadian provinces experienced historic levels of snowfall, snowstorms, rainfall, and rapid spring thaw, causing delayed site construction, resulting in early establishment of non-crop plants across all study sites (Government of Manitoba 2018, 2019, 2020, 2021a, 2021b, 2022a, 2022b, 2022c, n.d., Statistics Canada 2022a, 2022b, Manitoba Agriculture 2022, Ketonen 2023). Tilling and mound formation displaced newly emerged weed seeds throughout the mounds, creating a near-uniform distribution of non-crop plants. The higher weed coverage most likely contributed to the high abundance of predominant granivores and predators:

Harpalus spp., *Pterostichus* spp., and *Amara* spp. (Kromp 1999). However, in studies including *Pterostichus melanarius* (Illiger) in particular, this species is either unresponsive to crop factors or captured more frequently under largely enclosed, shady canopies (Hummel et al. 2012). The apparent homogenization of ground cover and high abundance of a few dominant ground beetle species likely limited the ability to detect treatment-related effects in carabid beetle abundance, richness, and Hill-Shannon diversity.

Harvest

I predicted that as treatment diversity increased, the yield and quality of crops would increase, but I found no clear production advantage. Although intercropping and functional complementarity are widely associated with improved productivity, resource-use efficiency, and reduced interspecific competition, these benefits are highly context dependent (Liebman and Dyck 1993, Pleasant 2006, Brooker et al. 2015). My results parallel those of Ramirez and Wright (2025), who found the Three Sisters polyculture did not produce more than other monocrop iterations, attributing the absence of effects to early-season environmental and agronomic conditions suppressing crop performance and preventing complementarity from emerging.

An extreme flooding event at the start of the season significantly delayed tillage and transplanting efforts beyond the optimal window. In some systems, such as sunflower or soybean cultivars, orchestrated timing delays of field preparation can reduce weed pressure by disturbing pre-seeding emergence (Liebman and Dyck 1993, Daramola 2020). However, this effect depends on hitting a critical phenological window of vulnerability, also called the critical period of weed control (CPWC), generally occurring during the dry, early spring conditions (Liebman and Dyck 1993, Baker et al. 2021). In some situations, flooding is utilized to impose anaerobic soil conditions to suppress the growth of select seeds; however, this is only applied in conjunction with cultivars that can tolerate such conditions (Blackshaw et al. 2007). In my study, persistent high soil moisture likely reduced the CPWC, making the use of sustainable intensive agricultural methods such as single-pass low-impact tilling ineffective, and as a result, allowed for early weed establishment.

Early weed dominance may have undermined the ecological advantages of crop complementarity associated with the Three Sisters and between Indigenous significant crops on

an individual level. The magnitude of weed competition depends on the timing of weed emergence relative to crops, with weeds emerging prior to or in unison with crops causing increased competition and ultimately resulting in greater yield losses (Swanton et al. 1999). Complementarity relies on the assumption that each crop occupies distinct resource niches (Willey 1979, Gebru 2015, Brooker et al. 2015, Duchene et al. 2017, Bybee-Finley and Ryan 2018), but the use of additional sustainable intensive agricultural maintenance strategies (e.g., zero chemical use and exclusive hand weeding between treatment mounds) allowed weeds within the mounds to persist, intensifying localized plant competition (Rühlemann and Schmidtke 2015). This combination likely contributed to crops being outcompeted before facilitation interactions between crop and non-crop plants could develop.

Resource acquisition favoured weeds, which contributed to the crowding out of crop seedlings and the occupation of critical above- and below-ground niches (Rühlemann and Schmidtke 2015). While certain crops can tolerate some level of early weed competition, these thresholds were likely exceeded in my study sites (Oluoch 2012, Swanton et al. 2015). Redroot pigweed (*Amaranthus* spp.*)* was commonly found at high abundances within all my study sites and is known to compete with crop plants for nutrients, water, and light, significantly reducing yield and impairing harvest quality (Mirshekari and Valizadeh 2010). Studies have shown that pigweeds coupled emergence with sunflower or bean can reduce high-value crop traits (e.g., height, stem diameter, leaf size) (Mirshekari and Valizadeh 2010, Amini et al. 2014). Although squash is often cited as a weed-suppressive crop, most demonstrations of this effect rely on additional management inputs such as herbicides, mulch or cover crops (Fujiyoshi et al. 2007, Soloneski and Larramendy 2011, Ronald and Charles 2012). Several studies have shown that utilizing high-herbicide-residue, evenly distributed mulches from cover crops, particularly winter wheat or rye, can enhance weed suppression in no-till pumpkin systems (Soloneski and Larramendy 2011). However, in the presence of redroot pigweed and other broadleaf weeds, only early-season suppression was reported (Soloneski and Larramendy 2011). Intercropping, particularly maize and squash, is also noted as a strategy that enhances squash weed suppression, but experiments using a variety of crop combinations report a negative correlation between weed growth and intercrop yield advantage (Liebman and Dyck 1993). These dynamics suggest that under high early-season weed pressure, traditional management practices can neutralize the

benefit of intercropping, which may explain the absence of a yield and quality advantage observed in this study.

Saturated soils, especially clayey soil, have been documented to introduce a cascade of below-ground challenges, such as nutrient leaching and anaerobic conditions that restrict oxygen availability to roots, and impair microbial activity, all of which may degrade soil structure and nutrient cycling (Fausey and Lal 1990, Oluoch 2012, Seddaiu et al. 2016). Upon transplant, squash seedlings were already compromised from prolonged growth chamber use, resulting in inadequate hardening and an inability to establish properly in these conditions. Sunflowers in particular were affected, frequently found dislodged from the soil at the roots, most likely from a combination of wind and soil conditions (Cryan et al. 2024). Other authors have reported similar occurrences of constrained sunflower emergence and poor establishment under waterlogged conditions in low- or no-tillage systems across varying soil textures, concluding that yields are significantly reduced as a consequence (Seddaiu et al. 2016). Furthermore, animal pests were not observed, but their presence was evident, especially in Carman, as approximately 25% of whole squash plants were removed from the ground a week after transplant.

Additional ecological mechanisms may have played subtle but reinforcing roles. Treatments with higher plant diversity were also observed to experience greater pest insect herbivory, as evidenced by sunflowers snapping at the midpoint of the stem, which likely delayed or prevented subsequent flowering, reducing the potential mid- to late-season yield potential. Possible hidden stressors, such as soil-borne diseases (e.g., root rot) from long-term moisture or low seed viability, could contribute to low harvest yields (Manitoba Agriculture 2022). Ultimately, the strong early-season competition and multiple abiotic factors appear to have overwhelmed the potential for niche differentiation or facilitation, resulting in uniformly low yields and harvest quality across plants and treatments. This outcome aligns with studies that caution against assuming the automatic benefits of intercropping under suboptimal conditions and instead highlight how context-dependent stressors and the variety of crops chosen can shift the system from one favouring facilitation to one dominated by competition (Hooper et al. 2005, Herrighty et al. 2021).

Conclusion

In this study, insect communities and crop performance in the Three Sisters system were more influenced by functional traits of individual crop plants, environmental conditions, and management practices as opposed to increased plant diversity. Wild bee abundances and community composition were particularly responsive to niches provided by pollinator-attractive crops, but this did not facilitate increased bee presence in the other crop plants within the Three Sisters intercrop, suggesting that crop variety selection should consider floral complementarity in addition to the crop's individual ecological roles. Whereas the ground beetle community and crop yields were not influenced by crop plants but seemed to respond to site conditions, such as early-season weed competition and abiotic stressors. Collectively, these results suggest that the ecological and agronomic outcomes of Three Sisters systems are highly context-dependent, emphasizing the need to consider land management techniques on a more localized scale tailored to abiotic conditions. Future research should examine these interactions across longer temporal scales, broader environmental gradients, and in collaboration with Indigenous communities to better understand how niche complementarity and functional diversity can be harnessed to optimize ecosystem services and system stability in sustainable intensive agricultural practices.

References

Akaike, H. 1998. Information theory and an extension of the maximum likelihood principle. *In* Selected Papers of Hirotugu Akaike. *Edited by* E. Parzen, K. Tanabe, and G. Kitagawa. Springer, New York, NY. pp. 199–213. https://doi.org/10.1007/978-1-4612-1694-0_15.

Amini, R., Alizadeh, H., and Yousefi, A. 2014. Interference between red kidneybean (*Phaseolus vulgaris* L.) cultivars and redroot pigweed (*Amaranthus retroflexus* L.). European Journal of Agronomy, **60**: 13–21. doi: 10.1016/j.eja.2014.07.002.

Andersen, J. 1989. Photoresponse of carabid beetles depends on experimental design. Oikos, **54**: 195–200. Available from http://www.jstor.com/stable/3565266.

Anderson, W.E. 1986. A guide for estimating cover. Rangelands, **8**: 236–238. Available from http://www.jstor.org/stable/3901027.

Arduser, M. 2009a. *Hoplitis* of ENA (east of 100th Meridian). Unpublished.

Arduser, M. 2009b. Key to *Hylaeus* species known from MO (males and females). Unpublished.

Arduser, M. 2009c. Key to *Osmia* females known from eastern North America (east of 100th meridian). Unpublished.

Baker, C., Modi, A.T., and Nciizah, A.D. 2021. Weeding frequency effects on growth and yield of dry bean intercropped with sweet sorghum and cowpea under a dryland area. Sustainability, **13**: 1–15. doi: 10.3390/su132112328.

Bali, G.P.K., and Kaleka, A.S. 2022. Potential reasons for insect decline. *In* Global Decline of Insects. *Edited by* H.A. Farag El-Shafie. IntechOpen, London, United Kingdom. pp. 25–42. http://dx.doi.org/10.5772/intechopen.94711.

Bell, C., Tronstad, L., and Hotaling, S. 2023. Tailoring your bee sampling protocol: comparing three methods reveals the best approaches to capturing bees. Agricultural and Forest Entomology, **25**: 477–488. doi: 10.1111/afe.12569.

Blackshaw, R.E., Anderson, R.L., and Lemerle, D. 2007. Cultural weed management. *In* Non-chemical Weed Management: Principles, Concepts and Technology. *Edited by* M.K. Upadhyaya and R.E. Blackshaw. CAB International, Wallingford, UK. pp. 35–47.

Bomfim, I.G.A., Freitas, B.M., Souza de Aragão, F.A., and Walters, S.A. 2016. Pollination in cucurbit crops. *In* Handbook of Cucurbits Growth, Cultural Practices, and Physiology. *Edited by* M. Pessarakli. CRC Press, Boca Raton. pp. 181–200.

Bommarco, R., and Banks, J.E. 2003. Scale as modifier in vegetation diversity experiments: effects on herbivores and predators. Oikos, **102**: 440–448. doi: 10.1034/j.1600-0579.2003.12578.x.

Bouseman, J.K., and LaBerge, W.E. 1978. A revision of the bees of the genus *Andrena* of the Western Hemisphere. Part IX. subgenus *Melandrena*. Transactions of the American Entomological Society, **104**: 275–389.

Bousquet, Y. 2012. Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico. Zookeys, **245**: 1–1722. doi: 10.3897/zookeys.245.3416.

Brooker, R.W., Bennett, A.E., Cong, W.F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L., Mckenzie, B.M., Pakeman, R.J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., and White, P.J. 2015. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytologist, **206**: 107–117. doi: 10.1111/nph.13132.

Brooks, M.E., Kristensen, K., van Benthem, J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., and Bolker, B.M. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal, **9**: 378–400. doi: 10.32614/RJ-2017-066.

Butts, R.A., Floate, K.D., David, M., Blackshaw, R.E., and Burnett, P.A. 2003. Influence of intercropping canola or pea with barley on assemblages of ground beetles (Coleoptera: Carabidae). Community and Ecosystem Ecology, **32**: 535–541. https://doi.org/10.1603/0046-225X-32.3.535.

Bybee-Finley, K.A., and Ryan, M.R. 2018. Advancing intercropping research and practices in industrialized agricultural landscapes. Agriculture, **8**: 1–24. doi: 10.3390/agriculture8060080.

Cárcamo, H.A., Niemalä, J.K., and Spence, J.R. 1995. Farming and ground beetles: effects of agronomic practice on populations and community structure. The Canadian Entomologist, **127**: 123–140. doi: 10.4039/Ent127123-1.

Caron, D.M. 2021. Honey bee strains. *In* Honey Bee Medicine for the Veterinary Practitioner. *Edited by* T.R. Kane and C.M. Faux. John Wiley & Sons, Inc., pp. 73–80. doi: 10.1002/9781119583417.ch6.

Chao, A., Chiu, C.H., and Jost, L. 2014. Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through hill numbers. Annual Review of Ecology, Evolution, and Systematics, **45**: 297–324. doi: 10.1146/annurev-ecolsys-120213-091540.

Coelho, B.W.T. 2004. A review of the bee genus *Augochlorella* (Hymenoptera: Halictidae: *Augochlorini*). Systematic Entomology, **29**: 282–323.
Colla, S., and Richardson, L.L. 2011. Bumblebees of the eastern United States. USDA Forest Service and the Pollinator Partnership.
Coradi, P.C., Fernandes, C.H.P., Peralta, C.C., and Pereira, T.L. 2015. Effects of drying and storage conditions in the quality of sunflower seeds. Pesquisa Agropecuária Pernambucana, **20**: 26–35. doi: 10.12661/pap.2015.005.
Craney, T.A., and Surles, J.G. 2002. Model-dependent variance inflation factor cutoff values. Quality Engineering, **14**: 391–403. doi: 10.1081/QEN-120001878.
Crins, W.J., Gray, P.A., Uhlig, P.W., and Wester, M.C. 2009. Ecosystems of Ontario. part 1, ecozones and ecoregions. Ontario Ministry of Natural Resources, Inventory, Monitoring and Assessment Section, Peterborough, Ontario.
Cryan, T., Musselman, O., Baumgardner, A.W., Osborn, S., Beuscher, C.J., Stehn, C., Burt, A., Chaaban, R., Lopez, A., Lewis, G., Mata, G., Rothenberg, M., Plascencia-Marquez, A., Greer, R., Stremlau, R., Murphy, H., Newman, L.R., Merrill, A., and Wadgymar, S.M. 2024. Yield, growth, and labor demands of growing maize, beans, and squash in monoculture versus the Three Sisters. Plants People Planet, **7**: 204–214. doi: 10.1002/ppp3.10576.
Daramola, O.S. 2020. Timing of weed management and yield penalty due to delayed weed management in soybean. Planta Daninha, **38**: 1–7. doi: 10.1590/S0100-83582020380100072.
Droege, S., Engler, J., Sellers, E., and O'Brien, L. 2016. National protocol framework for the inventory and monitoring of bees. US Fish and Wildlife Service, Fort Collins, Colorado.
Droege, S., Jean, R., and Orr, M. (n.d.). Draft bee genera of eastern North America. Available from https://www.discoverlife.org/mp/20q?guide=Bee_genera [accessed 22 July 2023].
Droege, S., and Rehan, S. 2016. Guide to the *Ceratina* of eastern North America. Available from https://www.discoverlife.org/mp/20q?guide=Ceratina [accessed 25 July 2023].
Duchene, O., Vian, J.F., and Celette, F. 2017. Intercropping with legume for agroecological cropping systems: complementarity and facilitation processes and the importance of soil microorganisms. a review. Agriculture, Ecosystems & Environment, **240**: 148–161. doi: 10.1016/j.agee.2017.02.019.
Dudley, N., and Alexander, S. 2017. Agriculture and biodiversity: a review. Biodiversity, **18**: 45–49. doi: 10.1080/14888386.2017.1351892.
Eyre, M.D. 1994. Strategic explanations of carabid species distributions in northern England. *In* Carabid Beetles: Ecology and Evolution. *Edited by* K. Desender, M. Dufrêne, M. Loreau, M.L. Luff, and J.-P. Maelfait. Springer, pp. 267–275.
FAO. 2001. Grain crop drying, handling and storage. *In* Rural structures in the tropics. Design and development. Rome, Italy. pp. 363–411.
FAO. 2013. Genebank standards for plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.
Fausey, N.R., and Lal, R. 1990. Soil wetness and anaerobiosis. *In* Advances in Soil Science (Volume 11): Soil Degradation. *Edited by* R. Lal and B.A. Stewart. Springer, New York, NY. pp. 173–186. https://doi.org/10.1007/978-1-4612-3322-0_5.
Fonteyne, S., Castillo Caamal, J.B., Lopez-Ridaura, S., Van Loon, J., Espidio Balbuena, J., Osorio Alcalá, L., Martínez Hernández, F., Odjo, S., and Verhulst, N. 2023. Review of

agronomic research on the milpa, the traditional polyculture system of Mesoamerica. Frontiers in Agronomy, **5**: 1–16. doi: 10.3389/fagro.2023.1115490.
Fujiyoshi, P.T., Gliessman, S.R., and Langenheim, J.H. 2007. Factors in the suppression of weeds by squash interplanted in corn: research paper. Weed Biology and Management, **7**: 105–114. doi: 10.1111/j.1445-6664.2007.00242.x.
Gebru, H. 2015. A review on the comparative advantages of intercropping to mono-cropping system. Journal of Biology, Agriculture and Healthcare, **5**: 1–13.
Geslin, B., Gauzens, B., Baude, M., Dajoz, I., Fontaine, C., Henry, M., Ropars, L., Rollin, O., Thébault, E., and Vereecken, N.J. 2017. Massively introduced managed species and their consequences for plant–pollinator interactions. *In* Advances in Ecological Research. Academic Press, pp. 147–199. doi: 10.1016/bs.aecr.2016.10.007.
Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology, **94**: 295–304. doi: 10.1111/j.1365-2745.2006.01098.x.
Gibbs, J., Hanuschuk, E., Miller, R., Dubois, M., Martini, M., Robinson, S., Nakagawa, P., Sheffield, C.S., Cardinal, S., and Onuferko, T.M. 2023. A checklist of the bees (Hymenoptera: Apoidea) of Manitoba, Canada. The Canadian Entomologist, **155**: 1–38. doi: 10.4039/tce.2022.45.
Gibbs, J., Packer, L., Dumesh, S., and Danforth, B.N. 2013. Revision and reclassification of *Lasioglossum* (*Evylaeus*), L. (*Hemihalictus*) and L. (*Sphecodogastra*) in eastern North America (Hymenoptera: Apoidea: Halictidae). Zootaxa, **3672**: 1–117. doi: 10.11646/zootaxa.3672.1.1.
Gibbs, J. 2010. Revision of the metallic species of *Lasioglossum* (*Dialictus*) in Canada (Hymenoptera, Halictidae, Halictini). Zootaxa, **2591**: 1–382. https://doi.org/10.11646/zootaxa.2591.1.1.
Gibbs, J. 2011. Revision of the metallic *Lasioglossum* (*Dialictus*) of eastern North America (Hymenoptera: Halictidae: Halictini). Zootaxa, **3073**: 1–216. http://doi.org.uml.idm.oclc.org/10.11646/zootaxa.3073.1.1.
Gibson, S.D., Onuferko, T.M., Myers, L., and Colla, S.R. 2024. Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes region. PeerJ, **12**: 1–24. doi: 10.7717/peerj.17401.
Gonzalez, V.H., and Griswold, T.L. 2013. Wool carder bees of the genus *Anthidium* in the Western Hemisphere (Hymenoptera: Megachilidae): diversity, host plant associations, phylogeny, and biogeography. Zoological Journal of the Linnean Society, **168**: 221–425. doi: 10.1111/zoj.12017.
Google Earth 10.55.0.1. 2023. Manitoba, Canada. Available from https://earth.google.com/web/. [accessed 13 October 2023].
Government of Canada. 2020, December 18. Canada's plant hardiness site. Available from https://planthardiness.gc.ca/?lang=en&m=24&speciesid=1000000&s=p&phz=phz1991-2020&bc=1.
Government of Manitoba. 2007. Gardening basics. Available from https://www.gov.mb.ca/inr/pdf/pubs/mafri-gbasic.pdf.
Government of Manitoba. 2018. 2018 Water availability and drought conditions report. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2018/annual_summary.pdf.
Government of Manitoba. 2019. Water availability and drought conditions report: July 2019. Available from

https://www.gov.mb.ca/sd/pubs/water/drought/2019/drought_conditions_report_july_2019.pdf.
Government of Manitoba. 2020. Water availability and drought conditions report: July 2020. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2020/drought_conditions_report_july_2020.pdf.
Government of Manitoba. 2021a. Water availability and drought conditions report: June 2021. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2021/drought_conditions_report_june_2021.pdf.
Government of Manitoba. 2021b. Water availability and drought conditions report: September 2021. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2021/drought_conditions_report_sep_2021.pdf.
Government of Manitoba. 2022a. Water availability and drought conditions report: April 2022. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2022/drought_conditions_report_april_2022.pdf.
Government of Manitoba. 2022b. Water availability and drought conditions report: May 2022. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2022/drought_conditions_report_may_2022.pdf.
Government of Manitoba. 2022c. Water availability and drought conditions report: June 2022. Available from https://www.gov.mb.ca/sd/pubs/water/drought/2022/drought_conditions_report_june_2022.pdf.
Government of Manitoba. (n.d.). Agricultural climate of Manitoba. Available from https://www.gov.mb.ca/agriculture/weather/agricultural-climate-of-mb.html.
Government of Manitoba. (n.d.). Manitoba drought monitor: drought impacts. Available from https://www.gov.mb.ca/sd/water/drought_condition/index.html.
Government of Manitoba. (n.d.). Sunflower production and management. Available from https://www.gov.mb.ca/agriculture/crops/crop-management/sunflowers.html.
Government of Manitoba. (n.d.). Wildlife deterrents. Available from https://www.gov.mb.ca/nrnd/fish-wildlife/wildlife/wildlife_human/wd.html.
Grabovska, T., Lavrov, V., Rozputnii, O., Grabovskyi, M., Mazur, T., Polishchuk, Z., Prisjazhnjuk, N., and Bogatyr, L. 2020. Effect of organic farming on insect diversity. Ukrainian Journal of Ecology, **10**: 96–101. doi: 10.15421/2020_174.
Grevé, M.E., Marx, M.T., Eilmus, S., Ernst, M., Herrmann, J.D., Baden, C.U., and Maus, C. 2024. Insect decline—evaluation of potential drivers of a complex phenomenon. Insects, **15**: 1–37. doi: 10.3390/insects15121021.
Grof-Tisza, P., Muller, M.H., Gónzalez-Salas, R., Bustos-Segura, C., and Benrey, B. 2024. The Mesoamerican milpa agroecosystem fosters greater arthropod diversity compared to monocultures. Agriculture, Ecosystems & Environment, **372**: 1–10. doi: 10.1016/j.agee.2024.109074.
Gunathilake, D.M.C.C., Senanayaka, D.P., Adiletta, G., and Senadeera, W. 2018. Drying of agricultural crops. *In* Advances in Agricultural Machinery and Technologies. *Edited by*

G. Chen. CRC Press, pp. 331–365. https://doi-org.uml.idm.oclc.org/10.1201/9781351132398.
Guzman, A., Chase, M., and Kremen, C. 2019. On-farm diversification in an agriculturally-dominated landscape positively influences specialist pollinators. Frontiers in Sustainable Food Systems, **3**: 1–9. doi: 10.3389/fsufs.2019.00087.
Hall, M. 2018. Blue and yellow vane traps differ in their sampling effectiveness for wild bees in both open and wooded habitats. Agricultural and Forest Entomology, **20**: 487–495. doi: 10.1111/afe.12281.
Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., and De Kroon, H. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One, **12**: 1–21. doi: 10.1371/journal.pone.0185809.
Hartig, F. 2024. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. Available from https://github.com/florianhartig/dharma.
Harwood, J. 2024. The forgotten history of intercropping. Plants People Planet, **6**: 1121–1128. doi: 10.1002/ppp3.10502.
Herrighty, E., Kapayou, D., Camacho, V., Nair, A., Hill, C., McDaniel, M., and Winham, D. 2021. Reuniting the Three Sisters: Native American intercropping, seed saving, and plant health. Iowa State University Research and Demonstration Farms Progress Reports 4.
Hettiarachchi, T. 2024. Taxonomic studies of western North American *Lasioglossum* (*Hemihalictus*) and a problematic L. (*Sphecodogastra*) species complex. [M.Sc.] thesis, University of Manitoba, Winnipeg, Manitoba.
Hill, M.O. 1973. Diversity and evenness: a unifying notation and its consequences. Ecology, **54**: 427–432. doi: 10.2307/1934352.
Hoekman, D., Levan, K.E., Ball, G.E., Browne, R.A., Davidson, R.L., Erwin, T.L., Knisley, C.B., LaBonte, J.R., Lundgren, J., Maddison, D.R., Moore, W., Niemela, J., Ober, K.A., Pearson, D.L., Spence, J.R., Will, K., and Work, T. 2017. Design for ground beetle abundance and diversity sampling within the National Ecological Observatory Network. Ecosphere, **8**: 1–17. doi: 10.1002/ecs2.1744.
Holm, H.N. 2017. Bees: an identification and native plant forage guide. Pollination Press LLC, Minnetonka, Minnesota.
Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., and Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs, **75**: 3–35. doi: 10.1890/04-0922.
Horwith, B. 1985. A role for intercropping in modern agriculture. Bioscience, **35**: 286–291. Available from http://links.jstor.org/sici?sici=0006-3568%28198505%2935%3A5%3C286%3AARFIIM%3E2.0.CO%3B2-H.
Hummel, J.D., Dosdall, L.M., Clayton, G.W., Harker, K.N., and O'Donovan, J.T. 2012. Ground beetle (Coleoptera: Carabidae) diversity, activity density, and community structure in a diversified agroecosystem. Environmental Entomology, **41**: 72–80. doi: 10.1603/EN11072.
Hurt, D.R. 1987. Indian agriculture in America: prehistory to the present. University Press of Kansas.
iNaturalist. (n.d.). iNaturalist observation. Available from https://www.inaturalist.org/observations?place_id=63780&taxon_id=47126.

Iwasaki, J.M., and Hogendoorn, K. 2022. Mounting evidence that managed and introduced bees have negative impacts on wild bees: an updated review. Current Research in Insect Science, **2**: 1–10. doi: 10.1016/j.cris.2022.100043.

Jones, G.A., and Gillett, J.L. 2005. Intercropping with sunflowers to attract beneficial insects in organic agriculture. Florida Entomologist, **88**: 91–96. https://doi.org/10.1653/0015-4040(2005)088[0091:IWSTAB]2.0.CO;2.

Jost, L. 2007. Partitioning diversity into independent alpha and beta components. Ecology, **88**: 2427–2439. doi: 10.1890/06-1736.1.

Kapayou, D.G., Herrighty, E.M., Hill, C.G., Camacho, V.C., Nair, A., Winham, D.M., and McDaniel, M.D. 2023. Reuniting the Three Sisters: collaborative science with Native growers to improve soil and community health. Agriculture and Human Values, **40**: 65–82. doi: 10.1007/s10460-022-10336-z.

Ketonen, K. 2023, March 10. Report on "historic" 2022 Rainy Lake-area flooding calls for watershed access app, emergency floodway study. CBC News, 1–4. Available from https://www.cbc.ca/news/canada/thunder-bay/rainy-lake-flood-report-1.6773593.

Killewald, M.F. 2025. The use of floral enhancements to support beneficial insects in prairie agroecosystems. [PhD] thesis, The University of Manitoba, Winnipeg, Manitoba.

Kimmerer, R.W. 2012. Searching for synergy: integrating traditional and scientific ecological knowledge in environmental science education. Journal of Environmental Studies and Sciences, **2**: 317–323. doi: 10.1007/s13412-012-0091-y.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. 2006. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences, **274**: 303–313. doi: 10.1098/rspb.2006.3721.

Koch, J., Strange, J., and Williams, P.H. 2012. Bumble bees of the western United States. US Department of Agriculture, Forest Service.

Kotze, D.J., Brandmayr, P., Casale, A., Dauffy-Richard, E., Dekoninck, W., Koivula, M.J., Lövei, G.L., Mossakowski, D., Noordijk, J., Paarmann, W., Pizzolotto, R., Saska, P., Schwerk, A., Serrano, J., Szyszko, J., Taboada, A., Turin, H., Venn, S., Vermeulen, R., and Zetto, T. 2011. Forty years of carabid beetle research in Europe - from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. Zookeys, **100**: 55–148. doi: 10.3897/zookeys.100.1523.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture, Ecosystems & Environment, **74**: 187–228. https://doi.org/10.1016/S0167-8809(99)00037-7.

Kruse-Peeples, M. 2016, May 27. How to grow a Three Sisters garden. Available from https://www.nativeseeds.org/blogs/blog-news/how-to-grow-a-three-sisters-garden.

LaBerge, W.E. 1956. A revision of the bees of the genus *Melissodes* in North and Central America. Parts I., II (Hymenoptera, Apidae). The University of Kansas Science Bulletin, **37**: 911–1194. http://doi.org.uml.idm.oclc.org/10.5962/bhl.part.24549.

LaBerge, W.E. 1961. A revision of the bees of the genus *Melissodes* in North and Central America. Part III (Hymenoptera, Apidae). The University of Kansas Science Bulletin, **42**: 283–663. http://doi.org.uml.idm.oclc.org/10.5962/bhl.part.9821.

LaBerge, W.E. 1967. A revision of the bees of the genus *Andrena* of the Western Hemisphere. Part I. *Callandrena*. (Hymenoptera: Andrenidae). Bulletin of The University of Nebraska State Museum, **7**: 1–316.

LaBerge, W.E. 1973. A revision of the bees of the genus *Andrena* of the Western Hemisphere. Part VI. subgenus *Trachandrena*. Transactions of the American Entomological Society, **99**: 235–371.
LaBerge, W.E. 1980. A revision of the bees of the genus *Andrena* of the Western Hemisphere. Part X. subgenus *Andrena*. Transactions of the American Entomological Society, **106**: 395–525.
LaBerge, W.E. 1985. A revision of the bees of the genus *Andrena* of the Western Hemisphere. Part XI. minor subgenera and subgeneric key. Transactions of the American Entomological Society, **111**: 441–567.
LaBerge, W.E. 1987. A revision of the bees of the genus Andrena of the Western Hemisphere. Part XII. subgenera *Leucandrena, Ptilandrena, Scoliandrena* and *Melandrena*. Transactions of the American Entomological Society, **112**: 191–248. http://doi.org.uml.idm.oclc.org/10.1007/978-1-4020-6359-6_761.
Larochelle, A., and Larivière, M.-C. 2003. A natural history of the ground beetles (Coleoptera: Carabidae) of America north of Mexico. Pensoft Publishers, Bulgaria.
Laurance, W.F. 2010. Habitat destruction: death by a thousand cuts. *In* Conservation Biology for All. *Edited by* N.S. Sodhi and P.R. Ehrlich. Oxford University Press, pp. 73–87. doi: 10.1093/acprof:oso/9780199554232.001.0001.
Laverty, T.M. 1992. Plant interactions for pollinator visits: a test of the magnet species effect. Oecologia, **89**: 502–508. Available from https://www.jstor.org/stable/4219917.
Laverty, T.M., and Harder, L.D. 1988. The bumble bees of eastern Canada. The Canadian Entomologist, **120**: 965–967. doi: 10.4039/Ent120965-11.
LaZerte, S. 2021. GLMs and non-parametric stats. [PowerPoint slides]. Study Design and Quantitative Methods for Resource and Environmental. University of Manitoba.
LeBuhn, G., Droege, S., Connor, E., Gemmill-Herren, B., and Azzu, N. 2016. Protocol to detect and monitor pollinator communities. FAO. Available from https://openknowledge.fao.org/handle/20.500.14283/i5367e.
Lenth, R.V. 2024. emmeans: estimated marginal means, aka least-squares means. doi: 10.32614/CRAN.package.emmeans.
Lewandowski, S. 1987. Diohe'ko, the Three Sisters in Seneca life: implications for a native agriculture in the finger lakes region of New York State. Agriculture and Human Values, **4**: 76–93. doi: 10.1007/BF01530644.
Li, D. 2018. hillR: taxonomic, functional, and phylogenetic diversity and similarity through Hill Numbers. Journal of Open Source Software, **3**: 1041. doi: 10.21105/joss.01041.
Liao, H., Zhou, Z., Liu, Y., Luo, Y., Zhang, C., Feng, Y., Shu, Y., and Wang, J. 2024. 'The Three Sisters' (maize/bean/squash) polyculture promotes the direct and indirect defences of maize against herbivores. European Journal of Agronomy, **155**: 1–11. doi: 10.1016/j.eja.2024.127118.
Liebman, M., and Dyck, E. 1993. Crop rotation and intercropping strategies for weed management. Ecological Applications, **3**: 92–122. https://www.jstor.org/stable/1941795.
Lindroth, C.H. 1961–1969. The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska Parts 1 (1969), 2 (1961), 3 (1963), 4 (1966), 5 (1968), 6 (1969). Opuscula Entomologica Supplementum, 35, 20, 24, 29, 33, 34.
Lövei, G.L., and Sunderland, K.D. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology, **41**: 231–256. https://doi.org/10.1146/annurev.en.41.010196.001311.

Lüdecke, D., Ben-Shachar, M., Patil, I., Wiernik, B., Bacher, E., Thériault, R., and Makowski, D. 2022. easystats: an R framework for easy statistical modeling, visualization, and reporting. https://doi.org/10.32614/CRAN.package.easystats.

Malézieux, E., Crozat, Y., Dupraz, C., Laurans, M., Makowski, D., Ozier-Lafontaine, H., Rapidel, B., de Tourdonnet, S., and Valantin-Morison, M. 2009. Mixing plant species in cropping systems: concepts, tools and models. a review. Agronomy for Sustainable Development, **29**: 43–62. doi: 10.1051/agro:2007057.

Manitoba Agriculture. 2022. Seasonal summary crop report: 2022 provincial summary. Available from https://www.gov.mb.ca/agriculture/crops/seasonal-reports/crop-report-archive/pubs/seasonal-summary-11-01-2022.pdf.

McGillvray, B. 2023, June 29. Ottawa. Encyclopedia Britannica. Available from https://www.britannica.com/place/Ottawa.

McGinley, R.J. 1986. Studies of Halictinae (Apoidea: Halictidae), I: Revision of New World *Lasioglossum* Curtis. Smithsonian Contributions to Zoology, **429**: 1–294. http://doi.org.uml.idm.oclc.org/10.5479/si.00810282.429.

McLintock, P., Coats, K.S., and Bumsted, J.M. 2024, April 26. Manitoba. Encyclopedia Britannica. Available from https://www.britannica.com/place/Manitoba.

Michener, C.D. 2007. The bees of the world. *In* 2nd edition. The Johns Hopkins University Press, Baltimore.

Ministry of Agriculture Food and Agribusiness. 2022. Climate zones and planting dates for vegetables in Ontario. Available from https://www.ontario.ca/page/climate-zones-and-planting-dates-vegetables-ontario.

Mirshekari, B., and Valizadeh, N. 2010. Interference of redroot pigweed (*Amaranthus retroflexus* L.) in sunflower hybrids. Journal of Agriculture, Food, Environment and Animal Sciences, **8**: 810–814. Available from https://www.researchgate.net/publication/287351015.

Mitchell, T. 1960. Bees of the eastern United States, I. Technical Bulletin (North Carolina Agricultural Experiment Station), **141**: 1–538. http://doi.org.uml.idm.oclc.org/10.1007/978-1-4020-6359-6_761.

Mitchell, T. 1962. Bees of the eastern United States, II. Technical Bulletin (North Carolina Agricultural Experiment Station), **152**: 1–557.

Nerson, H. 2005. Effects of fruit shape and plant density on seed yield and quality of squash. Scientia Horticulturae, **105**: 293–304. doi: 10.1016/j.scienta.2004.11.008.

Ngapo, T.M., Bilodeau, P., Arcand, Y., Charles, M.T., Diederichsen, A., Germain, I., Liu, Q., Mackinnon, S., Messiga, A.J., Mondor, M., Villeneuve, S., Ziadi, N., and Gariépy, S. 2021. Historical Indigenous food preparation using produce of the Three Sisters intercropping system. Foods, **10**: 1–19. doi: 10.3390/foods10030524.

O'Connor, R.S., Kunin, W.E., Garratt, M.P.D., Potts, S.G., Roy, H.E., Andrews, C., Jones, C.M., Peyton, J.M., Savage, J., Harvey, M.C., Morris, R.K.A., Roberts, S.P.M., Wright, I., Vanbergen, A.J., and Carvell, C. 2019. Monitoring insect pollinators and flower visitation: the effectiveness and feasibility of different survey methods. Methods in Ecology and Evolution, **10**: 2129–2140. doi: 10.1111/2041-210X.13292.

OECD. 2015. Consensus document on the biology of common bean (*Phaseolus vulgaris* L.). Series on Harmonisation of Regulatory Oversight in Biotechnology. OECD Publishing, Paris, France.

Ollerton, J., Winfree, R., and Tarrant, S. 2011. How many flowering plants are pollinated by animals? Oikos, **120**: 321–326. doi: 10.1111/j.1600-0706.2010.18644.x.
Oluoch, M.O. 2012. Production practices of pumpkins for improved productivity. Scripta Horticulturae, **15**: 181–189. Available from https://www.researchgate.net/publication/316990877.
Ontario Biodiversity Council. 2024. State of Ontario's biodiversity. Peterborough, Ontario. Available from http://ontariobiodiversitycouncil.ca/sobr.
Ontario Ministry of Agriculture Food and Rural Affairs. 2022. Agronomy guide for field crops - publication 811. *Edited by* C. Brown, J. Follings, M. Moran, and B. Rosser. Ministry of Agriculture, Food and Rural Affairs, Toronto, Canada. Available from https://www.ontario.ca/files/2022-10/omafra-agronomy-guide-for-field-crops-en-2022-10-13.pdf.
Packer, L., Genaro, J.A., and Sheffield, C.S. 2007. The bee genera of eastern Canada. Canadian Journal of Arthropod Identification, **3**: 1–32. http://doi.org.uml.idm.oclc.org/10.3752/cjai.2007.03.
Paris, H.S., and Nerson, H. 2003. Seed dimensions in the subspecies and cultivar-groups of *Cucurbita pepo*. Genetic Resources and Crop Evolution, **50**: 615–625. https://doi.org/10.1023/A:1024464831595.
Pleasant, J.M. 2006. The science behind the Three Sisters mound system: an agronomic assessment of an Indigenous agricultural system in the northeast. *In* Histories of Maize: multidisciplinary approaches to the prehistory, linguistics, biogeography, domestication, and evolution of maize. *Edited by* J. Staller, R. Tykot, and B. Benz. Left Coast Press, New York. pp. 529–537. Available from https://www.ebsco.com/terms-of-use.
Pleasant, J.M. 2016. Food yields and nutrient analyses of the Three Sisters: a Haudenosaunee cropping system. Ethnobiology Letters, **7**: 87–98. doi: 10.14237/ebl.7.1.2016.721.
Pleasant, J.M., and Burt, R.F. 2010. Estimating productivity of traditional Iroquoian cropping systems from field experiments and historical literature. Journal of Ethnobiology, **30**: 52–79. https://doi.org/10.2993/0278-0771-30.1.52.
Portman, Z.M., Gardener, J., Lane, I., Gerjets, N., Petersen, J.D., Asher, J.S., Arduser, M., Evans, E.C., Boyd, C., Tomson, R., and Cariveau, D.P. 2023. A checklist of the bees (Hymenoptera: Apoidea) of Minnesota. Zootaxa, **5304**. doi: 10.4039/tce.2022.45.
Prendergast, K.S., Menz, M.H.M., Dixon, K.W., and Bateman, P.W. 2020. The relative performance of sampling methods for native bees: an empirical test and review of the literature. Ecosphere, **11**: 1–22. doi: 10.1002/ecs2.3076
R Core Team. 2024. R: a language and environment for statistical computing. Available from https://www.r-project.org/.
Ramirez, C., and Wright, A.J. 2025. Microclimate and growth advantages in the 'Three Sisters' planting food system in an urban garden. Plant Soil, **506**: 541–553. doi: 10.1007/s11104-023-06419-3.
Raven, P.H., and Wagner, D.L. 2021. Agricultural intensification and climate change are rapidly decreasing insect biodiversity. Proceedings of the National Academy of Science, **118**: 1–6. doi: 10.1073/PNAS.2002548117.
Reaume, T. 2009. 620 Wild plants of North America. University of Regina Press.
Rehan, S.M., and Sheffield, C.S. 2011. Morphological and molecular delineation of a new species in the *Ceratina dupla* species-group (Hymenoptera: Apidae: Xylocopinae) of eastern North America. Zootaxa, **2873**: 35–50.

Rényi, A. 1961. On measures of entropy and information. *In* 4th Berkeley symposium on mathematical statistics and probability. *Edited by* J. Neyman.

Riggi, L.G.A., Raderschall, C.A., Fijen, T.P.M., Scheper, J., Smith, H.G., Kleijn, D., Holzschuh, A., Aguilera, G., Badenhausser, I., Bänsch, S., Beyer, N., Blitzer, E.J., Bommarco, R., Danforth, B., González-Varo, J.P., Grab, H., Le Provost, G., Poveda, K., Potts, S.G., Rundlöf, M., Steffan-Dewenter, I., Tscharntke, T., Vilà, M., Westphal, C., Berggren, and Lundin, O. 2024. Early-season mass-flowering crop cover dilutes wild bee abundance and species richness in temperate regions: a quantitative synthesis. Journal of Applied Ecology, **61**: 452–464. doi: 10.1111/1365-2664.14566.

Rightmyer, M.G. 2008. A review of the cleptoparasitic bee genus *Triepeolus* (Hymenoptera: Apidae). - Part 1. Zootaxa, **1710**: 1–170. http://doi.org.uml.idm.oclc.org/10.11646/zootaxa.1710.1.1.

Roberts, R.B. 1973a. Bees of northwestern America: *Agapostemon* (Hymenoptera: Halictidae). Technical Bulletin (Oregon State University Agricultural Experiment Station), **125**: 1–23.

Roberts, R.B. 1973b. Bees of northwestern America: *Halictus* (Hymenoptera: Halictidae). Technical Bulletin (Oregon State University Agricultural Experiment Station), **126**: 1–23.

Robinson, S. 2023. A taxonomic revision of the subgenus *Protandrena* (*Pterosarus*) (Hymenoptera: Andrenidae) in Canada and the United States. [M.Sc.] thesis, University of Manitoba, Winnipeg, Manitoba.

Romankova, T. 2003. Bees of genus *Colletes* of Ontario (Hymenoptera, Apoidea, Colletidae). Journal of the Entomological Society of Ontario, **134**: 91–106.

Romankova, T.G. 2007. Bees of the genus *Hylaeus* of Ontario (Hymenoptera: Apoidea: Colletidea). Journal of the Entomological Society of Ontario, **138**: 137–154.

Ronald, M., and Charles, K. 2012. Weed suppression and component crops response in maize/pumpkin intercropping systems in Zimbabwe. Journal of Agricultural Science, **4**: 231–236. doi: 10.5539/jas.v4n7p231.

Roswell, M., Dushoff, J., and Winfree, R. 2021. A conceptual guide to measuring species diversity. Oikos, **130**: 321–338. doi: 10.1111/oik.07202.

Rühlemann, L., and Schmidtke, K. 2015. Evaluation of monocropped and intercropped grain legumes for cover cropping in no-tillage and reduced tillage organic agriculture. European Journal of Agronomy, **65**: 83–94. doi: 10.1016/j.eja.2015.01.006.

Scoggan, H.J. 1957. Flora of Manitoba. Department of Northern Affairs and Natural Resources, Ottawa.

Seddaiu, G., Iocola, I., Farina, R., Orsini, R., Iezzi, G., and Roggero, P.P. 2016. Long term effects of tillage practices and N fertilization in rainfed Mediterranean cropping systems: durum wheat, sunflower and maize grain yield. European Journal of Agronomy, **77**: 166–178. doi: 10.1016/j.eja.2016.02.008.

Sharma, I.P., Kanta, C., Dwivedi, T., and Rani, R. 2020. Indigenous agricultural practices: a supreme key to maintaining biodiversity. *In* Microbiological Advancements for Higher Altitude Agro-Ecosystems & Sustainability. Springer Singapore, Singapore. pp. 91–112. doi: 10.1007/978-981-15-1902-4_6.

Sheffield, C.S., Ratti, C., Packer, L., and Griswold, T. 2011. Leafcutter and mason bees of the genus *Megachile* Latreille (Hymenoptera: Megachilidae) in Canada and Alaska. Canadian Journal of Arthropod Identification, **18**: 1–107. doi: 10.3752/cjai.2011.18.

Shorthouse, D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. Available from https://www.simplemappr.net/ [accessed 13 October 2023].

Silva, N. 2012. Revision of the cleptoparasitic bee genus *Coelioxys* (Hymenoptera: Megachilidae) in Canada. [M.Sc.] thesis, York University, Toronto, Ontario.
Sircom, J., Arul Jothi, G., and Pinksen, J. 2018. Monitoring bee populations: are eusocial bees attracted to different colours of pan trap than other bees? Journal of Insect Conservation, **22**: 433–441. doi: 10.1007/s10841-018-0071-y.
Smith, R.E., Veldhuis, H., Mills, G.F., Eilers, R.G., Fraser, W.R., and Lelyk, G.W. 1998. Terrestrial ecozones, ecoregions, and ecodistricts of Manitoba an ecological stratification of Manitoba's natural landscapes. Technical bulletin 1998-9E (Brandon Research Centre). Agriculture and Agri-Food Canada, Brandon, Manitoba.
Soloneski, S., and Larramendy, M.L. (*Editors*). 2011. Herbicides, theory and applications. IntechOpen. http://dx.doi.org/10.5772/1430.
Statistics Canada. 2022a. Crop condition assessment program, April 11 to May 29, 2022. Available from https://www150.statcan.gc.ca/n1/daily-quotidien/220609/dq220609f-eng.htm
Statistics Canada. 2022b. Principal field crop areas, June 2022. Available from https://www150.statcan.gc.ca/n1/daily-quotidien/220705/dq220705b-eng.htm.
Staton, T., Walters, R.J., Breeze, T.D., Smith, J., and Girling, R.D. 2022. Niche complementarity drives increases in pollinator functional diversity in diversified agroforestry systems. Agriculture, Ecosystems & Environment, **336**: 1–10. doi: 10.1016/j.agee.2022.108035.
Stephen, W.P. 1954. A revision of the bee genus *Colletes* in America North of Mexico (Hymenoptera, Colletidae). The University of Kansas Science Bulletin, **36**: 149–527.
Swanton, C.J., Nkoa, R., and Blackshaw, R.E. 2015. Experimental methods for crop–weed competition studies. Weed Science, **63**: 2–11. doi: 10.1614/ws-d-13-00062.1.
Swanton, C.J., Weaver, S., Cowan, P., Van Acker, R., Deen, W., and Shreshta, A. 1999. Weed thresholds: theory and applicability. Journal of Crop Production, **2**: 9–29.
Terry, P.A., Pearson, D., and Holder, G. 2020. Sustainable agriculture: nutrition of Indigenous American 3 sisters garden compared to monoculture corn production and a cool old squash. Journal of Scientific Research and Reports, **26**: 99–108. doi: 10.9734/jsrr/2020/v26i830299.
Thiele, H.-U. 1977. Carabid beetles in their environments: a study on habitat selection by adaptations in physiology and behaviour. *Edited by* W.S. Hoar, B. Hoelldobler, H. Langer, and M. Lindauer. Springer, New York.
Tilman, D. 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences, **96**: 5995–6000. https://doi.org/10.1073/pnas.96.11.5995.
Township of Emo. 2025. About – township of Emo. Available from https://emo.ca/about/.
Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., and Thies, C. 2005. Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. Ecology Letters, **8**: 857–874. doi: 10.1111/j.1461-0248.2005.00782.x.
Uddin, Z., Suppakul, P., and Boonsupthip, W. 2016. Effect of air temperature and velocity on moisture diffusivity in relation to physical and sensory quality of dried pumpkin seeds. Drying Technology, **34**: 1423–1433. doi: 10.1080/07373937.2015.1119840.
Vincze, C., Leelőssy, Á., Zajácz, E., and Mészáros, R. 2025. A review of short-term weather impacts on honey production. International Journal of Biometeorology, **69**: 303–317. doi: 10.1007/s00484-024-02824-0.

Wagner, D.L. 2020. Insect declines in the Anthropocene. Annual Review of Entomology, **65**: 457–480. doi: 10.1146/annurev-ento-011019.
Wardhaugh, C.W. 2015. How many species of arthropods visit flowers? Arthropod-Plant Interactions, **9**: 547–565. doi: 10.1007/s11829-015-9398-4.
Warren, D.L., and Seifert, S.N. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecological Applications, **21**: 335–342. doi: 10.1890/10-1171.1.
Willey, R.W. 1979. Intercropping - its importance and research needs. part 1. competition and yield advantages. Field Crop Abstracts, **32**: 1–10.
Williams, P., Thorp, R., Richardson, L., and Colla, S. 2014. Bumble bees of North America: an identification guide. Princeton University Press, Princeton, NJ.
Wise, S.F., Horn, M., and Ewen, G. 2022, November 9. Ontario. Encyclopedia Britannica. Available from https://www.britannica.com/place/Ontario-province.
Yanovsky, E. 1936. Food plants of the North American Indians. US Department of Agriculture.
Zhang, C., Postma, J.A., York, L.M., and Lynch, J.P. 2014. Root foraging elicits niche complementarity-dependent yield advantage in the ancient “Three Sisters” (maize/bean/squash) polyculture. Annals of Botany, **114**: 1719–1733. doi: 10.1093/aob/mcu191.

Supplementary Tables and Figures

Table S 1. A visual outline of a traditional Three Sisters planting schedule compared to the adjusted and actual observed schedule followed for the 2022 field season. The traditional Three Sisters timeline was based on a combination of commonly referenced guidelines for growing the Haudenosaunee Three Sisters and select ethnobotanical literature due to lack of formal records. A solid-coloured bar represents the respective activity was performed directly in the field. A cross-hatched bar represents the respective activity was performed indoors. A single asterisk indicates that the associated activity was not completed for all sites. A double asterisk indicates that the associated activity was completed for all sites.

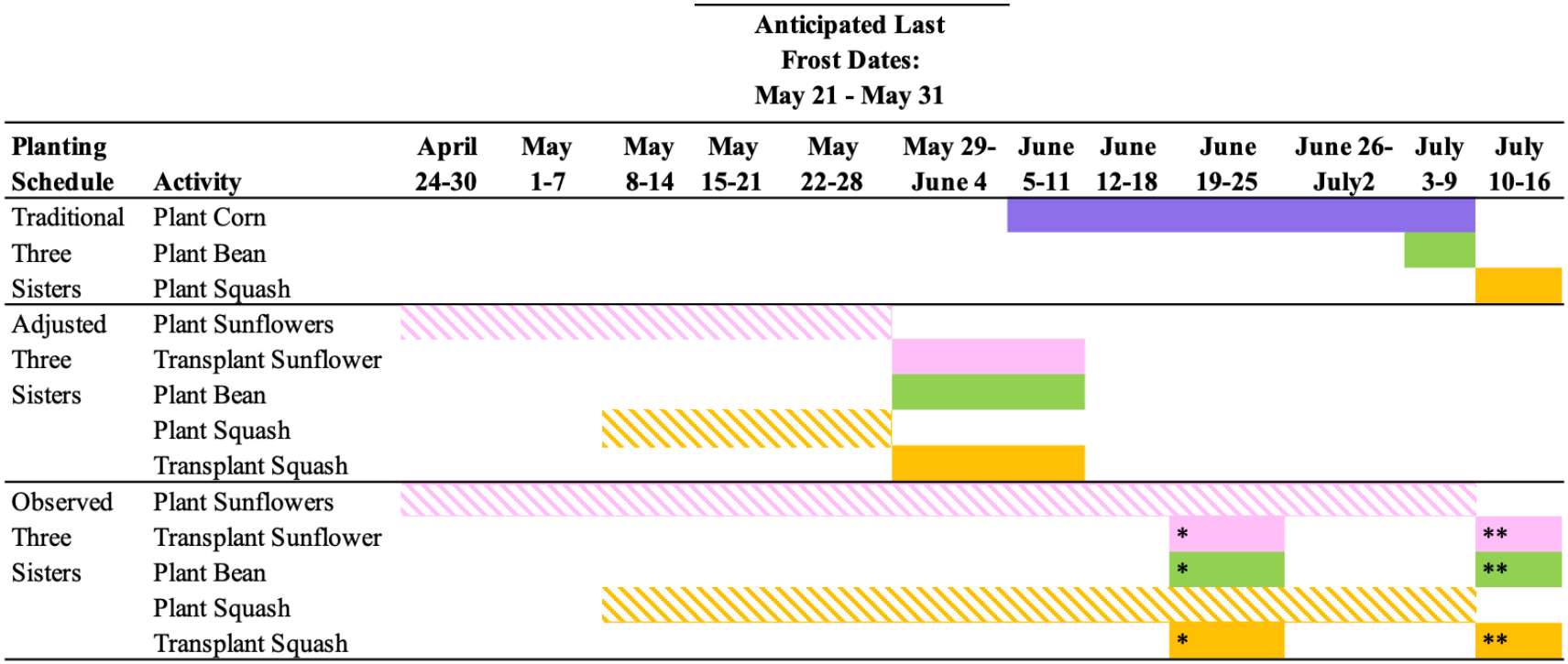

Table S 2. 2022 Field season study sites and corresponding dates of insect sampling procedures. Ontario sites (Emo and New Liskeard) were exclusively performed sampling for bees. Table does not include dates of sampling preformed outside the planned sampling window. Dashes (–) indicate no sampling was performed.

	Dates sampled			
Study Site	**July**	**August**	**September**	**Total Sampling Rounds**
Bee Bowls and Blue Vane Traps				
Brokenhead	20, 30	08, 11, 26	02, 09	7
Carman	21, 28	03, 19, 23, 31	07	7
Glenlea	22, 25	02, 09, 24	01, 11	7
The Point	15, 25	01, 08, 22, 30	06	7
Emo	–	09, 15, 25	–	3
New Liskeard	–	–	–	–
Targeted Aerial Netting				
Brokenhead	–	08, 11, 19, 26	02, 09	6
Carman	–	03, 10, 16, 23, 31	07	6
Glenlea	–	05, 09, 17, 24	01, 11	6
The Point	–	04, 11, 17, 22, 30	06	6
Emo	–	–	–	–
New Liskeard	–	–	–	–
Pitfall Traps				
Brokenhead	20-27	06-13, 19-26	02-09	4
Carman	21-28	03-10, 16-23	31(Aug)-07	4
Glenlea	15-22	02-09,17-24	01-08	4
The Point	14-21	01-08, 15-22	30(Aug)-06	4

Table S 3. Results of collinearity diagnostics for two models (treatment-focused and cropping-focused) of wild bee abundance and species richness. Variance inflation factor (VIF) values >5 are in bold. Dashes (–) indicate that the variable was not included in the model.

		Treatment-Focused Model	**Cropping-Focused Model**
Response Variable	**Predictor Variables**	**VIF**	**VIF**
Abundance	Treatment	**12.52**	–
	Cropping	–	**9.64**
	Plant Count	**11.83**	**9.67**
	Daily Average Temperature (°C)	**70.32**	**68.24**
	Daily Maximum Temperature (°C)	**55.11**	**53.39**
	Daily Minimum Temperature (°C)	4.98	4.94
	Daily Average Wind Speed (km/h)	**64.38**	**60.07**
	Daily Maximum Wind Speed (km/h)	**31.14**	**28.24**
	Daily Minimum Wind Speed (km/h)	**13.03**	**13.31**
Species Richness	Treatment	**14.19**	–
	Cropping	–	**9.56**
	Plant Count	**14.12**	**9.60**
	Daily Average Temperature (°C)	**73.32**	**73.22**
	Daily Maximum Temperature (°C)	**58.68**	**58.51**
	Daily Minimum Temperature (°C)	4.78	4.77
	Daily Average Wind Speed (km/h)	**60.47**	**57.15**
	Daily Maximum Wind Speed (km/h)	**28.86**	**26.75**
	Daily Minimum Wind Speed (km/h)	**12.37**	**12.39**

Table S 4. Results of collinearity diagnostics for three models (treatment-focused, cropping-focused, plant count-focused) of wild bee abundance and species richness. Variance inflation factor (VIF) values >5 are in bold. Dashes (–) indicate that the variable was not included in the model.

		Treatment-Focused Model	**Cropping-Focused Model**	**Plant Count-Focused Model**
Response Variable	**Predictor Variables**	**VIF**	**VIF**	**VIF**
Abundance	Treatment	1.06	–	–
	Cropping	–	1.00	–
	Plant Count	–	–	1.01
	Daily Average Temperature (°C)	**67.65**	**67.94**	**68.07**
	Daily Maximum Temperature (°C)	**53.19**	**53.32**	**53.36**
	Daily Minimum Temperature (°C)	4.89	4.83	4.88
	Daily Average Wind Speed (km/h)	**62.43**	**60.47**	**60.05**
	Daily Maximum Wind Speed (km/h)	**29.60**	**28.54**	**28.25**
	Daily Minimum Wind Speed (km/h)	**13.38**	**13.60**	**13.42**
Species Richness	Treatment	1.03	–	–
	Cropping	–	1.00	–
	Plant Count	–	–	1.01
	Daily Average Temperature (°C)	**67.48**	**67.65**	**67.55**
	Daily Maximum Temperature (°C)	**53.01**	**53.27**	**53.08**
	Daily Minimum Temperature (°C)	4.85	4.82	4.88
	Daily Average Wind Speed (km/h)	**62.39**	**59.98**	**59.09**
	Daily Maximum Wind Speed (km/h)	**28.97**	**27.86**	**27.41**
	Daily Minimum Wind Speed (km/h)	**13.41**	**13.43**	**13.14**

Table S 5. List of wild bee species caught during the 2022 field season. Sorted alphabetically by genus and species. Abundances presented by sampling method. List does not include excluded data. The following abbreviations are used for diet breadth (lecty): oligo., oligolectic; and poly., polylectic. No lecty information is provided for parasitic bee species. The following abbreviations are used for sampling method: BB., bee bowls; BVT., blue vane traps; and TAN., targeted aerial netting. Rare records for Manitoba are in bold, and for Canada include an asterisk.

Species	Lecty	Floral Host	Authority	BB	BVT	TAN	Total
Agapostemon (Agapostemon) texanus	poly.		Cresson 1872	8	13	0	21
Andrena (Callandrena s.l.) helianthi	oligo.	*Helianthus*	Robertson 1891	0	0	2	2
Andrena (Melandrena) lupinorum	oligo.	*Fabaceae*	Cockerell 1906	4	4	1	9
Andrena (Trachandrena) miranda	poly.		Smith 1879	1	5	0	6
Andrena (Andrena) thaspii	poly.		Graenicher 1903	5	3	0	8
Anthidium (Anthidium) tenuiflorae	poly.	*Phacelia*	Cockerell 1907	0	1	0	1
Anthophora (Clisodon) terminalis	poly.		Cresson 1869	2	12	0	14
Augochlorella aurata	poly.		(Smith 1853)	0	1	0	1
Bombus (Subterraneobombus) borealis	poly.		Kirby 1837	2	52	0	54
Bombus (Cullumanobombus) griseocollis	poly.		(De Geer 1773)	2	3	13	18
Bombus (Cullumanobombus) rufocinctus	poly.		Cresson 1863	3	20	0	23
Bombus (Pyrobombus) sandersoni	poly.		Franklin 1913	0	2	0	2
Bombus (Pyrobombus) ternarius	poly.		Say 1837	1	21	1	23
Bombus (Bombus) terricola	poly.		Kirby 1837	0	1	0	1
Bombus (Pyrobombus) vagans	poly.		Smith 1854	0	3	12	15
Ceratina (Zadontomerus) mikmaqi	poly.		Rehan and Sheffield 2011	0	6	0	6
Coelioxys (Boreocoelioxys) octodentatus	NA		Say 1824	1	0	1	2
Coelioxys (Boreocoelioxys) rufitarsis	NA		Smith 1854	1	0	1	2
Colletes hyalinus	poly.		Provancher 1888	2	0	0	2
Halictus (Seladonia) confusus	poly.		Smith 1853	7	1	3	11
Halictus (Protohalictus) rubicundus	poly.		(Christ 1791)	7	1	1	9

Heriades (Neotrypetes) carinata	poly.		Cresson 1864	4	17	4	25
Hoplitis (Alcidamea) pilosifrons	poly.		(Cresson 1864)	0	2	0	2
Hoplitis (Alcidamea) producta	poly.		(Cresson 1864)	1	0	0	1
Hylaeus (Prosopis) affinis	poly.		(Smith 1853)	2	3	0	5
Hylaeus (Prosopis) modestus	poly.		Say 1837	3	0	0	3
Lasioglossum (Dialictus) albipenne	poly.		(Robertson 1890)	1	7	1	9
Lasioglossum (Leuchalictus) leucozonium	poly.		(Schrank 1781)	3	0	0	3
Lasioglossum (Dialictus) lineatulum	poly.		(Crawford 1906)	0	3	0	3
Lasioglossum (Dialictus) novascotiae	poly.		(Mitchell 1960)	1	2	0	3
Lasioglossum (Dialictus) occidentale	poly.		(Crawford 1902)	17	4	0	21
Lasioglossum (Leuchalictus) paraforbesii	poly.		McGinley 1986	5	14	1	20
Lasioglossum (Dialictus) perpunctatum	poly.		(Ellis 1913)	15	12	0	27
Lasioglossum (Dialictus) semicaeruleum	poly.		(Cockerell 1895)	2	5	0	7
Lasioglossum (Dialictus) zephyrus	poly.		(Smith 1853)	1	0	0	1
Lasioglossum (Leuchalictus) zonulus	poly.		(Smith 1848)	0	2	1	3
Megachile (Litomegachile) brevis	poly.		Say 1837	1	1	0	2
Megachile (Megachile) inermis	poly.		Provancher 1888	0	1	11	12
Megachile (Xanthosarus) latimanus	poly.		Say 1823	0	2	4	6
Megachile (Xanthosarus) melanophaea	poly.		Smith 1853	0	1	0	1
Megachile (Megachile) montivaga	poly.		Cresson 1878	0	0	1	1
Megachile (Xanthosarus) perihirta	poly.		Cockerell 1898	0	5	1	6
Megachile (Sayapis) pugnata	oligo.	Compositae	Say 1837	0	0	1	1
Megachile (Megachile) relativa	poly.		Cresson 1878	2	0	0	2
Megachile (Eutricharaea) rotundata	poly.		(Fabricius 1787)	2	0	0	2
Melissodes (Eumelissodes) agilis	oligo.	*Helianthus*	Cresson 1878	6	117	19	142
Melissodes (Eumelissodes) bidentis*	oligo.	Compositae	Cockerell 1914	0	1	0	1
Melissodes (Melissodes) bimaculatus	poly.		(Lepeletier 1825)	0	2	0	2

Melissodes (Eumelissodes) confusus	oligo.	Compositae	Cresson 1878	18	54	0	72
Melissodes (Eumelissodes) druriellus	oligo.	*Solidago, Symphyotrichum*	(Kirby 1802)	0	4	1	5
Melissodes (Eumelissodes) trinodis	oligo.	*Helianthus* and related Compositae	Robertson 1901	1	68	6	75
Osmia (Melanosmia) simillima	poly.		Smith 1853	0	1	0	1
Protandrena (Pterosarus) albitarsis	oligo.	Compositae: Heliantheae	(Cresson 1872)	0	0	4	4
Protandrena (Pterosarus) parva	poly.		(Robertson1892)	0	2	0	2
Protandrena (Pterosarus) perlaevis	oligo.	*Helianthus*	(Cockerell 1896)	1	2	34	37
Triepeolus helianthi	NA		(Robertson 1897)	2	0	0	2
			Total Specimen	**134**	**481**	**124**	**739**
			Total Genera	**15**	**16**	**9**	**19**
			Total Species	**34**	**42**	**24**	**56**

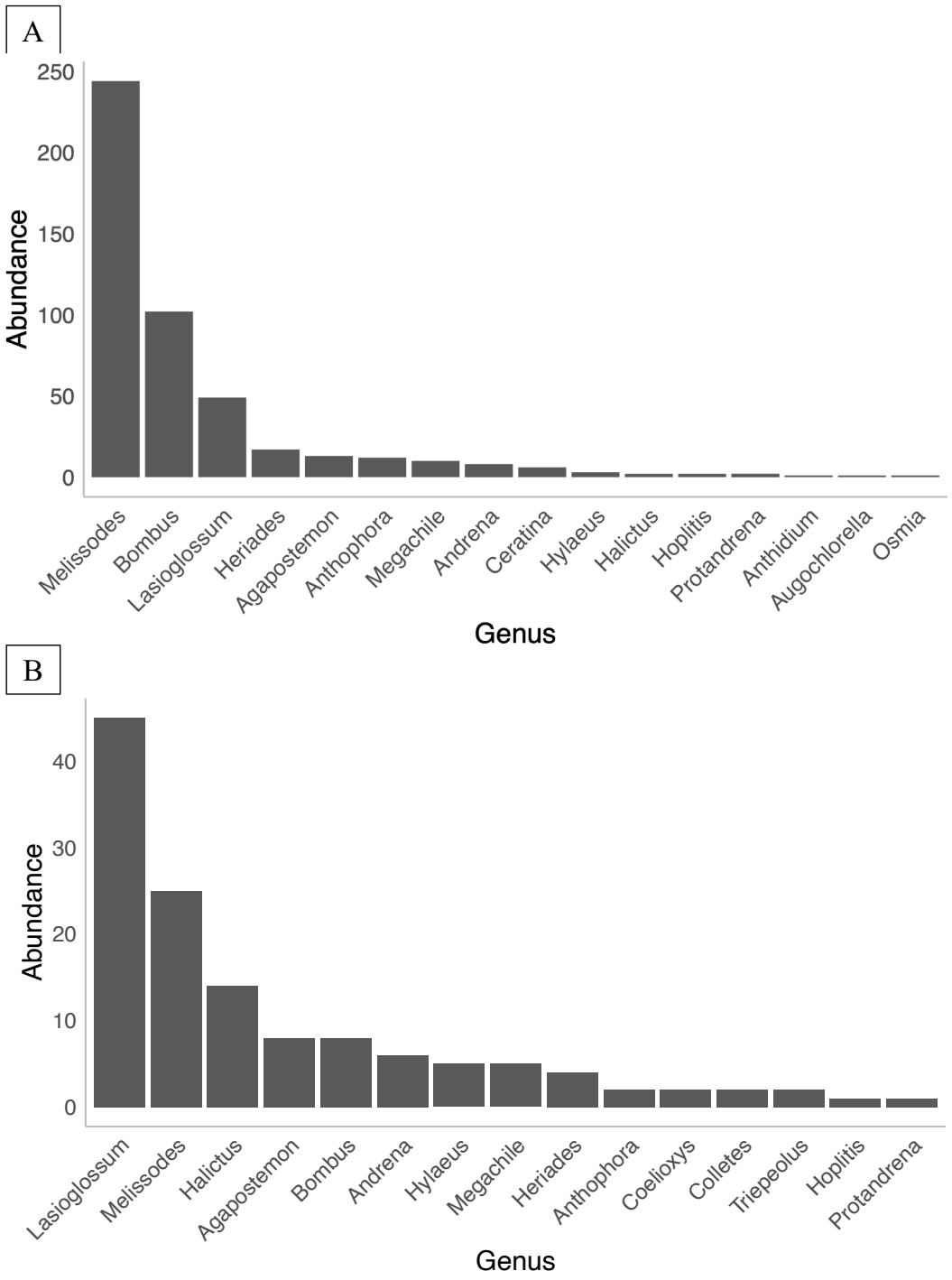

Figure S 1. Wild bee abundance by genus per passive sampling method across all sites and treatments. Graphs do not include excluded data. (A) Bee genera and abundances associated with BVT captures. *Anthidium* (1), *Augochlorella* (1), *Hoplitis* (2), and *Osmia* (1), each had ≤2 individuals. (B) Bee genera and abundances associated with bee bowl captures. *Anthophora* (2), *Coelioxys* (2), *Colletes* (2), *Hoplitis* (1), and *Protandrena* (1), and *Triepeolus* (2) had ≤2 individuals.

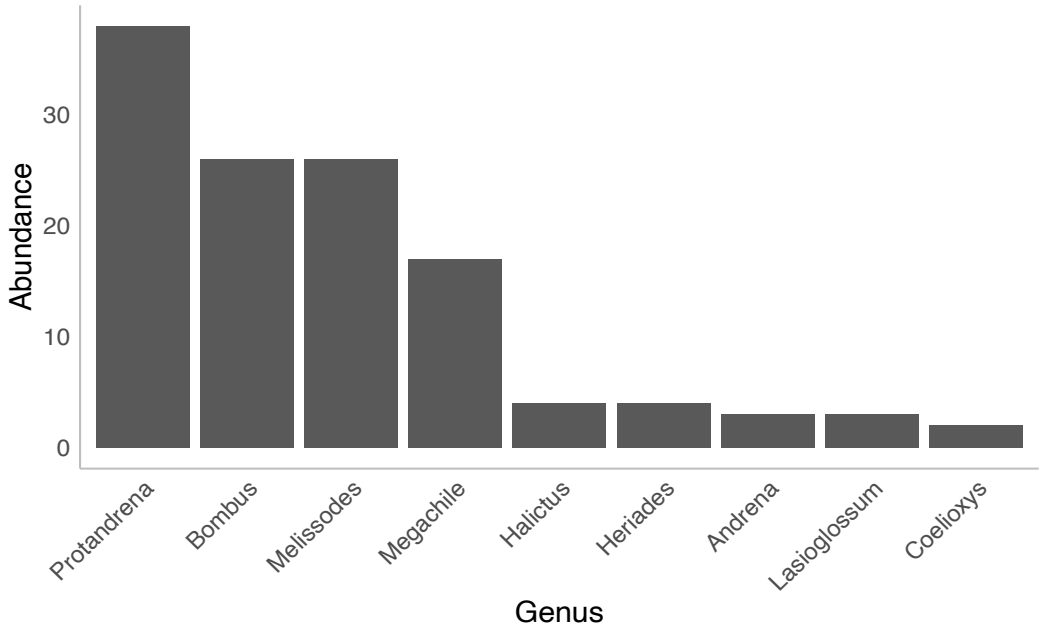

Figure S 2. Wild bee abundance by genus caught through targeted aerial netting across all sites and treatments. Graphs do not include excluded data. *Coelioxys* (2) was the only genera with ≤ 2 individuals.

Table S 6. List of wild bee species caught through targeted aerial netting during the 2022 field season. Sorted alphabetically by genus and species. Abundances presented per treatment. List does not include excluded data. The following abbreviations are used for diet breadth (lecty): oligo., oligolectic; and poly., polylectic. No lecty information is provided for parasitic bee species. The following abbreviations are used for treatment: 3S., Three Sisters; Be., Bean monocrop; Sq., Squash monocrop; Sun., Sunflower monocrop; SunBe., Sunflower Bean dicrop; SunSq., Sunflower Squash dicrop; and SqBe., Squash Bean dicrop. Rare records for Manitoba are in bold, and for Canada include an asterisk.

				Treatment						
Species	**Lecty**	**Floral Host**	**Authority**	**Sun**	**Be**	**Sq**	**SunBe**	**SunSq**	**SqBe**	**3S**
Andrena (Callandrena s.l.) helianthi	oligo.	*Helianthus*	Robertson 1891	1	0	0	0	0	0	1
Andrena (Melandrena) lupinorum	oligo.	*Fabaceae*	Cockerell 1906	1	0	0	0	0	0	0
Bombus (Cullumanobombus) griseocollis	poly.		(De Geer 1773)	2	0	0	0	6	3	2
Bombus (Pyrobombus) ternarius	poly.		Say 1837	1	0	0	0	0	0	0
Bombus (Pyrobombus) vagans	poly.		Smith 1854	4	2	0	0	4	0	2
Coelioxys (Boreocoelioxys) octodentatus	NA		Say 1824	1	0	0	0	0	0	0
Coelioxys (Boreocoelioxys) rufitarsis	NA		Smith 1854	0	0	0	0	0	0	1
Halictus (Seladonia) confusus	poly.		Smith 1853	1	0	0	1	1	0	0
Halictus (Protohalictus) rubicundus	poly.		(Christ 1791)	0	0	0	0	0	1	0
Heriades (Neotrypetes) carinata	poly.		Cresson 1864	3	0	0	0	0	0	1
Lasioglossum (Dialictus) albipenne	poly.		(Robertson 1890)	0	0	0	0	0	1	0
Lasioglossum (Leuchalictus) paraforbesii	poly.		McGinley 1986	0	0	0	1	0	0	0
Lasioglossum (Leuchalictus) zonulus	poly.		(Smith 1848)	0	0	0	0	0	1	0
Megachile (Megachile) inermis	poly.		Provancher 1888	5	0	0	3	2	0	1
Megachile (Xanthosarus)	poly.		Say 1823	1	0	0	0	0	0	3

latimanus										
Megachile (Megachile) montivaga	poly.		Cresson 1878	0	0	0	0	1	0	0
Megachile (Xanthosarus) perihirta	poly.		Cockerell 1898	1	0	0	0	0	0	0
Megachile (Sayapis) pugnata	oligo.	Compositae	Say 1837	0	0	0	1	0	0	0
Melissodes (Eumelissodes) agilis	oligo.	*Helianthus*	Cresson 1878	5	0	2	3	3	0	6
Melissodes (Eumelissodes) druriellus	oligo.	*Solidago, Symphyotrichum*	(Kirby 1802)	0	0	0	0	0	0	1
Melissodes (Eumelissodes) trinodis	oligo.	*Helianthus* and related Compositae	Robertson 1901	1	0	1	2	1	0	1
Protandrena (Pterosarus) albitarsis	oligo.	Compositae: Heliantheae	(Cresson 1872)	2	0	0	1	1	0	0
Protandrena (Pterosarus) perlaevis	oligo.	*Helianthus*	(Cresson 1872)	16	0	1	5	8	0	4
			Total Specimen	**45**	**2**	**4**	**17**	**27**	**6**	**23**
			Total Genera	**8**	**1**	**2**	**5**	**5**	**3**	**7**
			Total Species	**15**	**1**	**3**	**8**	**9**	**4**	**10**

Table S 7. List of carabid beetle species caught per treatment with pitfall traps during the 2022 field season. Sorted alphabetically by genus and species. Abundances presented per treatment. List does not include excluded data. The following abbreviations are used for treatment: 3S., Three Sisters; Be., Bean monocrop; Sq., Squash monocrop; Sun., Sunflower monocrop; SunBe., Sunflower Bean dicrop; SunSq., Sunflower Squash dicrop; and SqBe., Squash Bean dicrop.

		Treatment							
Species	**Authority**	**Sun**	**Be**	**Sq**	**SunBe**	**SunSq**	**SqBe**	**3S**	**Total**
Agonum (Olisares) corvus	(LeConte 1860)	0	1	1	0	0	0	0	**2**
Agonum (Olisares) cupreum	Dejean 1831	0	0	0	2	0	0	1	**3**
Agonum (Agonum) placidum	(Say 1823)	16	23	10	23	10	20	16	**118**
Amara (Curtonotus) carinata	LeConte 1848	85	90	45	125	69	65	44	**523**
Amara (Amara) confusa	LeConte 1848	1	2	1	1	0	1	2	**8**
Amara (Amarocelia) farcta	LeConte 1855	1	6	3	2	4	3	3	**22**
Amara (Amara) impuncticollis	(Say 1823)	13	4	2	13	2	8	10	**52**
Amara (Curtonotus) lacustris	LeConte 1855	4	1	4	1	1	1	1	**13**
Amara (Amara) littoralis	Mannerheim 1843	30	42	23	30	10	17	25	**177**
Amara (Percosia) obesa	(Say 1823)	109	77	66	100	92	105	78	**627**
Amara (Celia) pseudobrunnea	Lindroth 1968	6	1	2	10	5	6	5	**35**
Amara (Curtonotus) torrida	(Panzer 1797)	10	12	7	8	4	11	7	**59**
Anisodactylus (Gynandrotarsus) rusticus	(Say 1823)	0	0	0	1	0	0	0	**1**
Anisodactylus (Anadaptus) sanctaecrucis	(Fabricius 1798)	5	4	15	10	7	12	11	**64**
Bembidion (Trepanedoris) canadianum	Casey 1924	0	0	0	1	0	0	0	**1**
Bembidion (Furcacampa) mimus	Hayward 1897	1	2	2	2	2	1	1	**11**
Bembidion nudipenne	Lindroth 1963	3	1	0	1	0	0	1	**6**
Bembidion (Bembidion) quadrimaculatum	Say 1823	23	19	18	15	23	14	12	**124**
Bembidion (Furcacampa) timidum	(LeConte 1848)	1	0	0	0	0	0	0	**1**
Calosoma (Chrysostigma) calidum	(Fabricius 1775)	3	4	2	4	6	4	4	**27**
Carabus (Carabus) granulatus	Linné 1758	2	0	0	0	0	0	1	**3**
Chlaenius (Agostenus) alternatus	G.H. Horn 1871	3	2	4	1	1	0	1	**12**

Clivina (Clivina) fossor	(Linné 1758)	1	1	3	4	5	0	1	**15**
Cymindis (Tarulus) neglecta	Haldeman 1843	0	0	0	0	0	1	1	**2**
Diplocheila obtusa	(LeConte 1848)	0	0	0	0	1	0	0	**1**
Diplocheila undulata	Carr 1920	0	0	0	1	0	0	0	**1**
Dyschirius perversus	(Fall 1922)	1	1	0	0	0	0	0	**2**
Harpalus (Harpalus) affinis	(Schrank 1781)	2	4	0	3	2	1	2	**14**
Harpalus (Harpalus) amputatus	Say 1830	144	131	68	128	100	106	84	**761**
Harpalus (Plectralidus) erraticus	Say 1823	56	43	15	47	36	37	35	**269**
Harpalus (Pseudoophonus) erythropus	Dejean 1829	0	0	0	0	1	0	0	**1**
Harpalus (Euherpalops) funerarius	Csiki, 1932	6	5	5	5	6	12	8	**47**
Harpalus (Anamblystus) herbivagus	Say 1823	35	35	29	31	28	36	23	**217**
Harpalus lewisii	LeConte 1865	25	11	7	9	6	12	5	**75**
Harpalus ochropus	Kirby 1837	6	6	5	5	3	9	5	**39**
Harpalus (Bactroharpalus) opacipennis	(Haldeman 1843)	1	3	2	1	0	2	3	**12**
Harpalus (Pseudoophonus) pensylvanicus	(De Geer 1774)	559	739	324	629	446	534	401	**3632**
Harpalus plenalis	Casey 1914	3	4	0	1	1	1	0	**10**
Harpalus (Anamblystus) pleuriticus	Kirby 1837	15	17	15	12	7	10	9	**85**
Harpalus (Anamblystus) somnulentus	Dejean 1829	17	16	10	14	12	11	9	**89**
Harpalus (Platyharpalus) ventralis	LeConte 1848	0	1	1	2	0	0	2	**6**
Loricera pilicornis	(Fabricius 1775)	0	0	1	0	0	0	0	**1**
Microlestes linearis	(LeConte 1851)	5	3	0	3	3	2	5	**21**
Oxypselaphus pusillus	(LeConte 1854)	1	0	0	0	0	0	0	**1**
Platynus (Platynus) decentis	(Say 1823)	0	0	0	0	0	0	1	**1**
Poecilus corvus	(LeConte 1873)	18	19	15	28	17	19	22	**138**
Poecilus lucublandus	(Say 1823)	25	36	31	44	29	38	30	**233**
Poecilus scitulus	LeConte 1848	2	5	1	6	2	0	3	**19**
Pterostichus (Melanius) corvinus	(Dejean 1828)	0	0	0	1	0	0	0	**1**
Pterostichus (Phonias) femoralis	(Kirby 1837)	0	2	0	0	0	0	0	**2**

Pterostichus (Morphnosoma) melanarius	(Illiger 1798)	149	149	115	166	149	125	104	**957**
Sericoda quadripunctata	(De Geer 1774)	1	0	0	0	0	0	0	**1**
Synuchus impunctatus	(Say 1823)	15	0	0	0	0	0	0	**15**
	Grand Total	**1403**	**1522**	**852**	**1490**	**1090**	**1224**	**976**	**8557**
	Total Genera	**15**	**13**	**12**	**13**	**13**	**12**	**15**	**20**
	Total Species	**40**	**38**	**33**	**40**	**33**	**31**	**38**	**53**

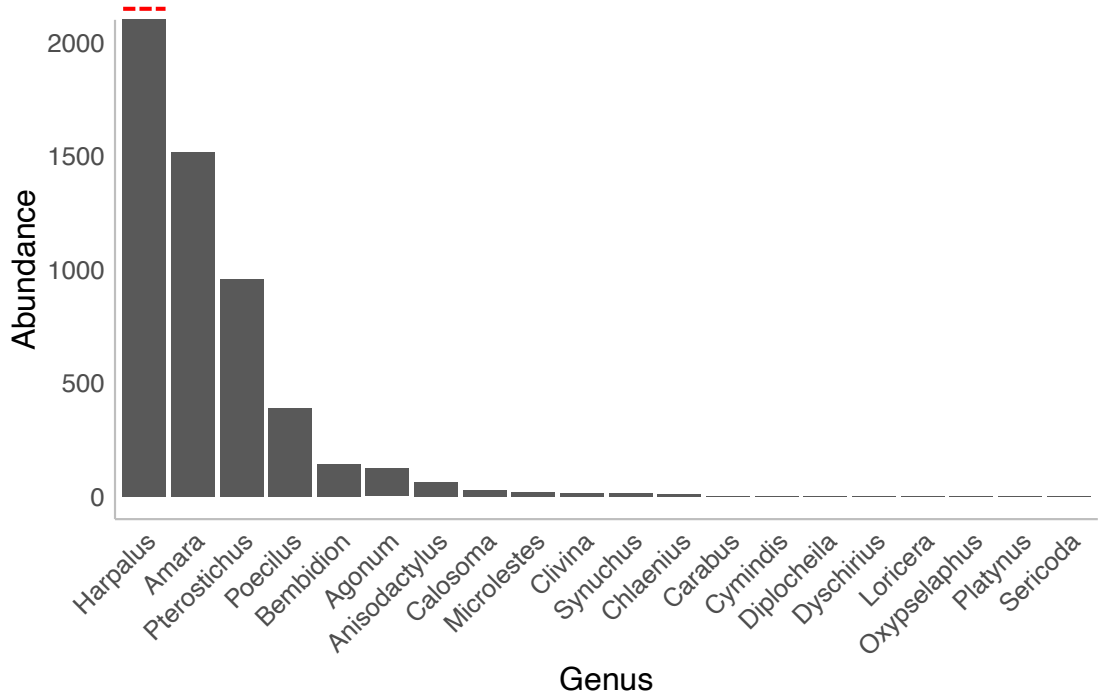

Figure S 3. Pitfall trap collected carabid beetle abundance per genus across all sites and treatments. Graphs do not include excluded data. Total *Harpalus* abundance was 5257 (greater than plot margins). *Cymindis* (2)*, Diplocheila* (2)*, Dyschirius* (2)**,** *Loricera* (1)*,* and *Oxypselaphus* (1), *Platynus* (1)*,* and *Sericoda* (1) had ≤2 individuals.

PREFACE TO CHAPTER 3

In Chapter two, I examined how the Three Sisters planting method and varying combinations of respective Indigenous crop plants influenced wild bee and ground beetle biodiversity metrics. I found that the Three Sisters treatment increased bee abundance and richness when compared to treatments that do not contain sunflowers, potentially supporting my expectation that increased plant diversity would drive greater bee captures. However, the absence of difference between the Three Sisters, sunflower monoculture, and sunflower-based dicrops suggests that sunflower presence alone may have been a primary driver of bee attraction, rather than the full intercrop composition. Limitations in sample size prevented robust comparisons for the remaining bee diversity and functional trait metrics (Hill-Shannon diversity and diet breadth). In contrast, carabid diversity and abundance showed no clear response to planting treatment, nor did harvest outcomes differ significantly across treatments. These patterns led me to speculate that local abiotic and biotic pressures may have obscured or superseded treatment effects.

Rather than continuing to compare cropping treatments, Chapter Three focuses on establishing a baseline of insect community composition within the Three Sisters system. In the context of accelerating global insect declines, collecting baseline data is an essential step for identifying conservation opportunities within agricultural landscapes. Given the immense diversity of insects in agroecosystems, wild bees and ground beetles were maintained as the insects of interest due to their critical roles in agriculture. To accomplish this, I altered the experiment performed in Chapter two to focus exclusively on the Three Sisters planting, both internally and in its relationship to the surrounding natural areas. Similar materials, methods, and analyses were used. By simplifying the experiment, I was able to build the first list of wild bee and ground beetle communities associated with the Three Sisters in southern Manitoba and northern Ontario. Understanding the insect-plant interactions of the Three Sisters can redefine landscape management and conservation practices while simultaneously increasing Indigenous-led research and knowledge.

CHAPTER 3: A COMPARISON OF THE THREE SISTERS CROP INFLUENCE ON WILD BEE AND GROUND BEETLE BIODIVERSITY AND THE OVERALL AGRICULTURAL PRODUCTIVITY OF THE THREE SISTERS SYSTEM

Abstract

Background. Modern large-scale agriculture has accelerated insect biodiversity loss worldwide, prompting renewed interest in intercropping systems that enhance ecosystem functions. The Indigenous Three Sisters method of growing corn, bean, squash, and sunflower together provides the temporal and morphological complementarity that supports crucial ecological processes and system productivity. In this study, I documented wild bee and ground beetle diversity and abundance associated with the Three Sisters and compared these communities to those in the surrounding natural area. Also, I explored the influence of edge effects of a Three Sisters garden area on insect biodiversity, the provisioning of potential ecosystem services, and harvest.

Methods. Five small sites were constructed in 2023, two located in southern Manitoba and three in northern Ontario, Canada. Sites included two sampling areas: an exclusively Three Sisters garden and an area that reflected the surrounding natural landscape. Bees were collected using bee bowls, blue vane traps, and targeted aerial netting, while carabid beetles were sampled through pitfall traps. I hypothesized that bee and beetle biodiversity metrics would be higher in the Three Sisters garden than in the natural area due to the complementary effects of the specific crop combination enhancing foraging and habitat resources. Additionally, I expected insects to be more abundant, diverse, and specialized within the interior of the Three Sisters garden compared to the edges, as the higher concentration of resources would support enhanced potential pollination and pest regulation services, ultimately resulting in greater yield and harvest quality.

Results. I identified 1,070 bees and 1,533 carabid beetles to species. Carabid beetle diversity was higher in the Three Sisters garden than in the surrounding natural area, suggesting that habitat structure rather than plant species richness may drive ground-dwelling insect communities. In contrast, neither bee nor beetle biodiversity metrics, even when abundances were utilized as proxies for pollination and pest control services, revealed any significant differences between the exterior and interior positions of the Three Sisters garden. Similarly, the yield and quality of the

harvest showed no significant differences across positions. Together, these results suggest that the uniform structure and resources within an exclusively Three Sisters garden likely minimized spatial differentiation, reducing the influence of edge effects on ecological interactions and agronomic outcomes. Further exploration revealed significant site-level effects on harvest metrics and the provisioning of potential ecosystem services, suggesting local environmental conditions strongly influence ecological and agronomic outcomes in a Three Sisters garden.

Conclusion. My study showed that an exclusively Three Sisters garden has the potential to enhance ground beetle diversity, likely through the creation of unique habitat structures as a by-product of complementary spatial arrangements of the crop plants. In contrast, the relatively homogeneous structure of the Three Sisters garden did not influence spatial patterns in bee community dynamics, potential ecosystem service provisioning, or final harvest yield and quality measures. The absence of influence of edge effects, combined with strong site-level variation in harvest metrics and the provision of potential pollination services, indicates that environmental context, management history, and crop traits may strongly influence Three Sisters functioning. Collectively, these findings suggest that the expression of complementarity in the Three Sisters system is context-dependent, with larger localized conditions overriding fine-scale spatial effects. Future studies should aim to collaborate with Indigenous communities to select for locally adapted heirloom crop varieties and integrate Traditional Ecological Knowledge (TEK) to foster sustainable agricultural innovation.

Introduction

Insects provide critical ecological functions in agroecosystems, including pollination, pest regulation, and nutrient cycling (Zhang et al. 2007). Among them, bees and ground beetles are particularly important (Wardhaugh 2015): bees maintain pollination services across both wild and cultivated plants (Klein et al. 2006), while carabids contribute to pest suppression and support the recycling of soil through their roles as predators, scavengers, and seed consumers (Lövei and Sunderland 1996, Holland 2002). In addition, both groups are highly sensitive to habitat changes in agricultural landscapes, making them useful bioindicators of ecosystem quality (Rainio and Niemela 2003, Zurbuchen et al. 2010, Schindler et al. 2013). Unfortunately, in recent decades, natural habitats have experienced rapid and extensive alterations due to

agricultural expansion, which has facilitated a global decline in insect populations (Fitzgerald-Moore and Parai 1996, Grevé et al. 2024)

Habitat loss through agriculture is the most detrimental event to occur in an ecosystem, as this foreshadows the intensification of agriculture, which simplifies the landscape to the point of requiring high-input chemicals (fertilizers, pesticides, fungicides, herbicides), large-scale irrigation infrastructure, and equipment (Fitzgerald-Moore and Parai 1996, Tilman 1999). These practices reduce ecosystem diversity, disrupt trophic interactions, and ultimately diminish the availability of ecological niches for plants and animals (Tscharntke et al. 2005, Laurance 2010, Bali and Kaleka 2022). Bees and beetles are particularly affected by the intensification of agriculture, exhibiting losses >70% globally, due to their foraging behaviours combined with central-place nesting in bees and habitat-structured distributions in ground beetles (Rainio and Niemela 2003, Zurbuchen et al. 2010, Schindler et al. 2013, Holliday et al. 2014, Hallmann et al. 2017, Lister and Garcia 2018).

Often accompanying habitat loss is fragmentation, defined as the break-up of land into smaller, disconnected patches, culminating in reduced habitat connectivity, altered resource distribution, and increased exposure to edge effects (Fahrig 2003, Bali and Kaleka 2022). The edge of an agricultural area is a transitional zone between the crop and surrounding vegetation, which often exhibits unique biotic and abiotic conditions compared to habitat interiors (Magura et al. 2017). These conditions create increased challenges for insects, including altered microclimate, uneven resource distribution, and shifts in species interactions, which can affect survival and reproduction (Ewers and Didham 2006, Nguyen and Nansen 2018).

In mitigating insect decline and reducing the effects of intensive agriculture, intercropping has increasingly been recognized as one of the most effective strategies for maintaining current crop production levels (Harwood 2024). By growing multiple species together in one field, intercropping systems create structurally and temporally diverse habitats that promote efficient resource use (Willey 1979, Bybee-Finley and Ryan 2018). This allows for the facilitation of beneficial interactions between crop plants and surrounding ecological communities, such as increased floral resources, enhanced pest suppression, improved soil nutrient cycling, and greater habitat provision for associated organisms (Willey 1979, Horwith 1985, Bybee-Finley and Ryan 2018). Although intercropping is often framed as a modern

agricultural advancement to enhance ecosystem function, it has historic roots in traditional Indigenous agriculture (Harwood 2024). In North America, the most well-known polyculture is the Three Sisters, which commonly consisted of maize (*Zea mays* L.*)*, bean (*Phaseolus* spp.), and squash (*Cucurbita* spp.) in a single mound with a floral complementary (e.g., sunflower) species planted to attract pollinators (Lewandowski 1987, Pleasant 2006, Pleasant and Burt 2010). In this system, maize or sunflower provided structural support for beans that replenish soil fertility through nitrogen fixation, and squash suppressed weeds while retaining soil moisture (Lewandowski 1987, Pleasant 2006). Beyond agronomic advantage, the Three Sisters represents a knowledge system that was developed through valuing cultural, spiritual, and ecological interactions above long-term resource maximization (Kimmerer 2012, Sharma et al. 2020).

Despite its historical and cultural significance, relatively little research has focused on the Three Sisters system. Most studies examine agronomic outcomes (Pleasant and Burt 2010, Zhang et al. 2014, Pleasant 2016, Terry et al. 2020, Kapayou et al. 2023, Cryan et al. 2024), while a small number have investigated ecological benefits, including insect community composition and plant-insect interactions (Gibson et al. 2024, Liao et al. 2024, Grof-Tisza et al. 2024). The findings from insect-focused studies are variable but generally indicate that the Three Sisters fosters higher insect abundances than monocultures (Gibson et al. 2024, Liao et al. 2024, Grof-Tisza et al. 2024). This variability highlights the inconsistencies in the understanding of ecological interactions within the Three Sisters system, emphasizing the importance of establishing baseline data to clarify its role in supporting biodiversity in modern agricultural landscapes.

In this chapter, I address the following objectives and associated predictions:

i. Document wild bee and ground beetle biodiversity within a Three Sisters garden and adjacent natural area in southern Manitoba and northern Ontario.
    - Prediction: Bee and beetle biodiversity metrics will be highest in the interior of a Three Sisters garden, lower at the garden edge, and lowest in the adjacent natural habitat.
ii. Assess functional dimensions of bee communities by quantifying dietary specialization across positions within a Three Sisters garden

- Prediction: The proportion of specialist bees will be greatest in the interior of the Three Sisters garden when compared to the edge.

iii. Evaluate end-of-season yield and quality of individual crop plants within the Three Sisters garden relative to the edge and associated ecological function related to the Indigenous planting method.

- Prediction: Interior Three Sisters plantings will produce greater yield and higher-quality harvest when compared to those located at the edge, consistent with potential benefits of increased pollinator activity and pest control.

The goal of this study is to establish a baseline of wild bees and beetle assemblages associated with the Three Sisters. More broadly, this research seeks to contribute to the creation of space for Indigenous communities to engage in research grounded in TEK, to foster community cohesion, to facilitate intergenerational knowledge transmission, and to highlight that meaningful reconciliation requires more than rhetorical acknowledgment; it necessitates practical, hands-on action that yields tangible, community-driven outcomes.

Materials and Methods

Terminology

The term “traditional Three Sisters”, as used throughout this study, follows the definition and contextual framing established in Chapter two. Haudenosaunee agricultural practices are most frequently cited in this chapter because they are both most commonly documented and regionally relevant for this study.

Study Sites

This study was conducted in five field sites during the 2023 field season (May–September) (Figure 16). Two sites were located in southern Manitoba, Canada and three sites were located in northern Ontario (western and eastern), Canada; Brokenhead Ojibway First Nations Reserve, Scanterbury, Manitoba (50.337151 N, 96.584218 W), Glenlea Research Station, Glenlea, Manitoba (49.64548 N, 97.130381 W), The Ontario Crops Research Centre, Emo, Chapple, Ontario (48.38130N, -93.51507 W), The Ontario Crops Research Centre, New Liskeard, New Liskeard, Ontario (47.521857N, 79.669402 W), and a private residence located

near the Collège Boréal, Greater Sudbury, Ontario (46.617295 N, -81.280569 W). Hereafter, the sites above will be referred to as their general location: Brokenhead, Glenlea, Emo, New Liskeard, and Sudbury, respectively.

For climate and ecozone characteristics of the southern Manitoba and select northern Ontario sites (Emo and New Liskeard), see Chapter two. The added Sudbury site exhibited climate conditions broadly comparable to the other Ontario sites, but differed in plant hardiness, falling between zones 3b and 4a (Government of Canada 2020).

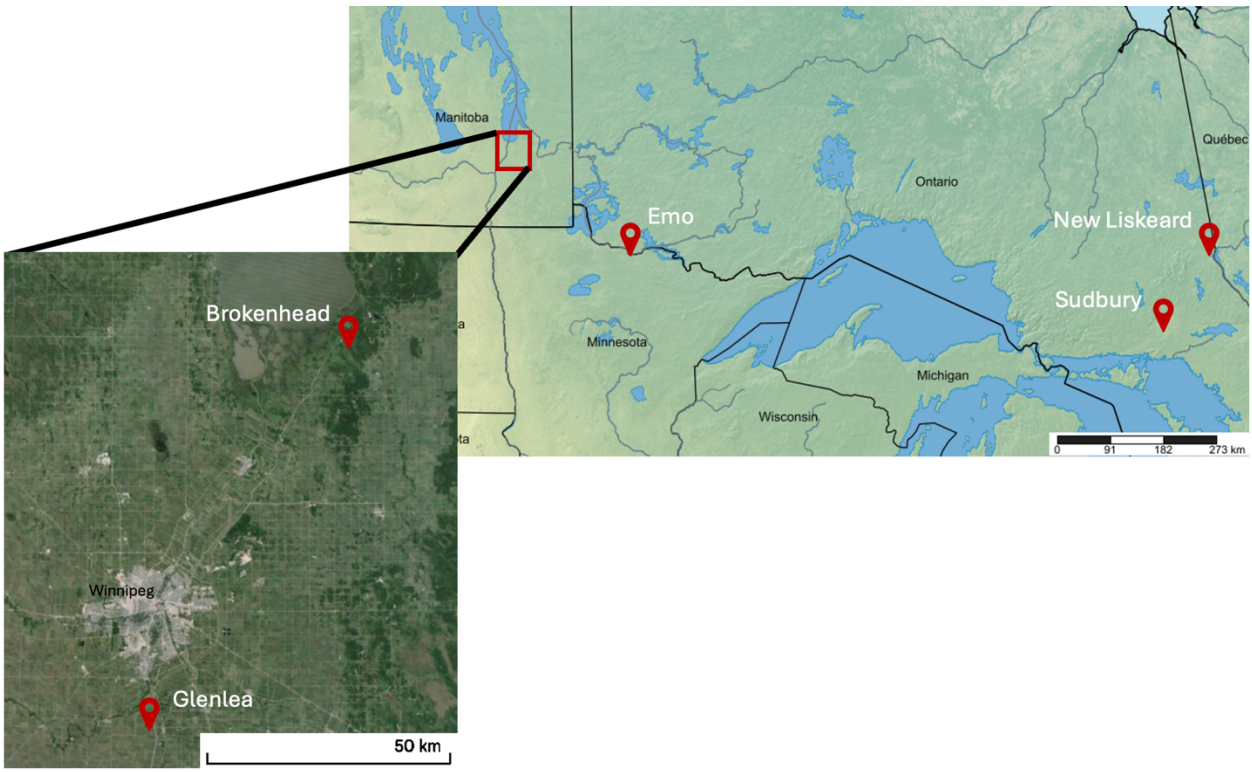

Figure 16. Locations of the study sites across southern Manitoba and northern Ontario. Red points indicate the respective study sites specific locations. White text refers to the study sites abbreviated designation. Maps created with SimpleMappr (Shorthouse 2010) and Google Earth (Google Earth 10.55.0.1 2023).

Study Site Selection

Site selection parameters remained consistent with those outlined in Chapter two and were followed in the decision to add the Sudbury site, located in a small-scale garden previously

planted with a variety of native plants and seasonal vegetables. Two southern Manitoba sites previously used in Chapter two (Carman and The Point) were removed from this study as higher priority was placed on sites with easier access and upkeep, distance from the University of Manitoba Campus (Fort Gary), and relevance to the 2023 field season goals. For details regarding land-use history of the remaining selected 2023 study sites (Brokenhead, Glenlea, Emo, and New Liskeard), refer to Chapter two.

Study Areas

Each study site included two study areas, an exclusively all Three Sisters garden and natural area representative of the general study site's natural landscape (e.g., long grass prairie, forest, etc.) that would not be disturbed by human intervention (e.g., lawn maintenance, selective gardening, etc.) for the entirety of the field season. Both areas were located within a 250 m radius. The Three Sisters garden area was a 25 m x 25 m square array (23 m x 23 m without optional exclusionary fence) (Figure 17A). Similar to Chapter two, the small size of the study areas was maintained to both follow guidance from members of the Brokenhead community and traditional small-scale Haudenosaunee farming methods, as well as to take into account limited access to maintenance equipment and ease of replication. Ideally, the shape and size of the natural area mirrored that of the Three Sisters garden area; however, due to limited space, the majority of the Ontario sites placed the natural area along a fence line. In study sites with high wildlife presence, the Three Sisters garden area was surrounded by a two-layered electric fence (100 m total, Peavey Mart, Winnipeg, Manitoba) to deter wildlife (Government of Manitoba n.d.). The natural area was left free of restrictive fencing, with the only alterations including the implementation of certain insect sampling methods.

Within the Three Sisters garden area there was 49 individual Three Sisters plantings, as described in the Plant Selection and Three Sisters Planting Layout section. Each planting was placed within a 1 m x 1 m space, arranged in 7 x 7 rows, spaced 2 m apart (Figure 17A–B). Three Sisters plantings may have included a 1 m diameter dirt mound (Figure 17C), but the addition of this feature was decided upon on a site-by-site basis. To orient and standardize the placement of a Three Sisters planting, a 1 m x 1 m quadrate (metal) was used.

Both areas were also divided into spatial positions corresponding to either specific Three Sisters plantings or to distance-based zones relative to the edges of an area (Figure 17D–E).

Depending on the data collection procedure, a maximum of three positions (exterior, middle, and interior) were assigned within an area (Table 12). Two distinct positional frameworks were used rather than applying three positions across all sampling methods in order to maintain a balanced sampling effort.

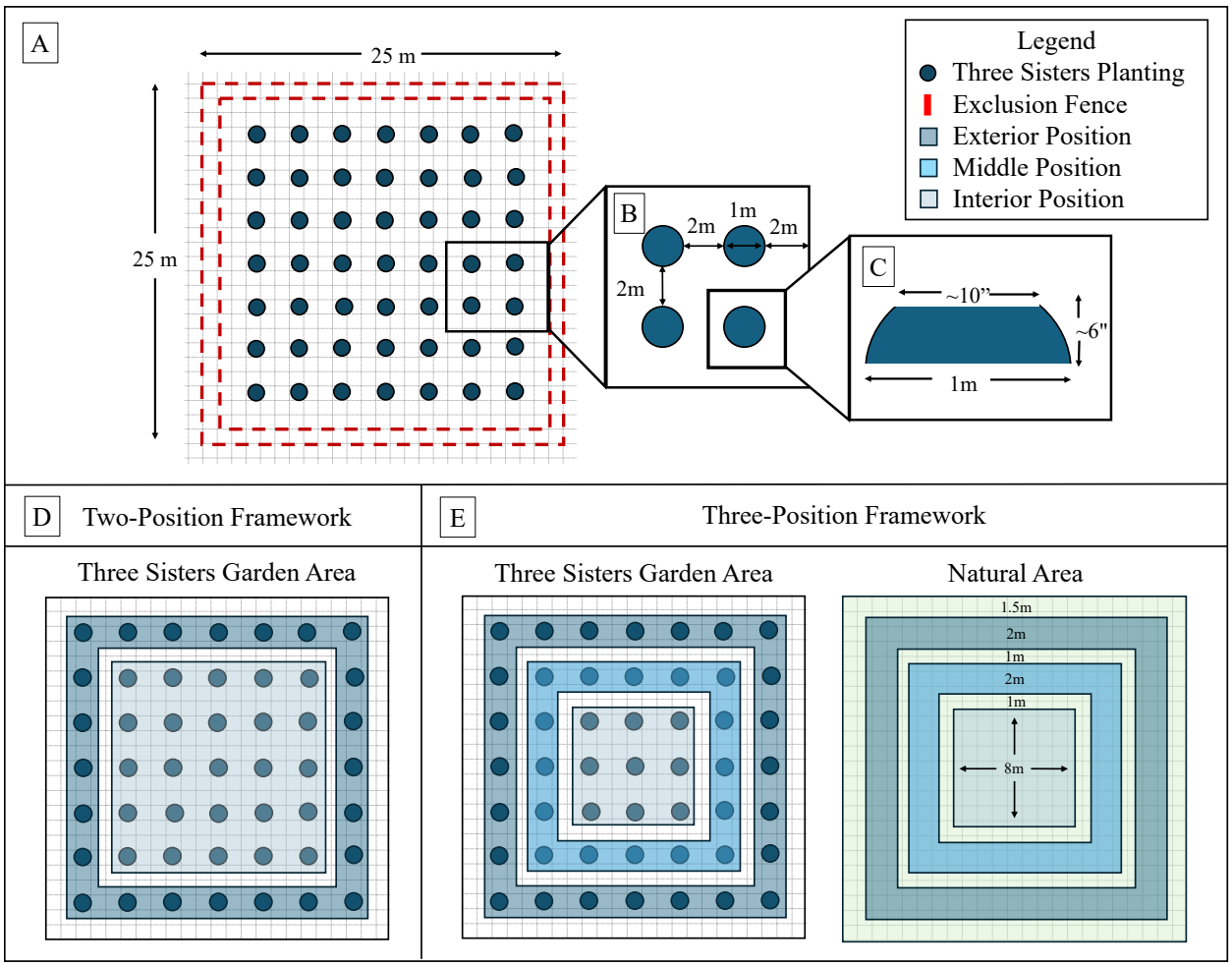

Figure 17. Visual representation of a Three Sisters garden area layout including two- and three-position frameworks. Each grid square represents 1 m^2. (A) Three Sisters planting layout within the Three Sisters garden area including the optional exclusionary fence. The natural area mirrored the same measurements, either 25 m x 25 m or 23 m x 23 m, depending on study site requirements. (B) Measurements between Three Sisters planting and edge of the study area. (C) Measurements of a single mound (use was optional). (D) Diagram depicting the layout of a two-position framework (exterior and interior) within the Three Sisters garden area, used in targeted aerial netting wild bee sampling and harvest procedures. A depiction of the two-position framework was not included as respective sampling procedures were restricted to the Three Sisters garden area. (E) Diagrams depicting placement of the three-position framework (exterior, middle, and interior) within the Three Sisters garden area and natural area, used in pitfall trap sampling procedures. The spacing from the edge of the natural area to the exterior position are based off a 25 m x 25 m study site.

Table 12. Sampling area and spatial position variables associated with respective data collection procedures.

Collection Procedure	Variable	Category	Description
	Area	Three Sisters Garden Area, Natural Area	In reference to either the Three Sisters garden area or natural area within a study site
Targeted Aerial Netting, Harvest	Position - Three Sisters Garden Area	Exterior	The outermost row of Three Sisters plantings closest to the perimeter of the study area, includes 24 Three Sisters plantings
		Interior	The centre of the study area, includes 25 Three Sisters plantings
Pitfall Traps	Position - Three Sisters Garden Area	Exterior	The outermost row of the Three Sisters planting closest to the perimeter of the study area, includes 24 Three Sisters plantings and 4 pitfall traps.
		Middle	The intermediate rows between the exterior and interior positions of the study area, includes 16 Three Sisters plantings and 4 pitfall traps.
		Interior	The centre of the study area includes 9 Three Sisters plantings and 4 pitfall traps
	Position - Natural Area	Exterior	The zone starting approximately 1–1.5 m inward from the edge of the natural area perimeter, covering approximately 2 m along each side of the area, includes 4 pitfall traps
		Middle	The zone starting approximately 1 m inward from the edge of the exterior position, covering approximately 2 m along each side of the sampling area, includes 4 pitfall traps
		Interior	The zone starting approximately 1 m inward from the edge of the middle position, covering approximately 64 m^2, includes 4 pitfall traps

Plant Selection and Three Sisters Planting Layout

Traditionally, the Three Sisters system consisted of corn (*Zea mays* L.), bean (*Phaseolus* spp.), and squash (*Cucurbita* spp.) planted within a raised soil mound, with a pollinator-attractive crop such as sunflower (*Helianthus annuus* L.) occasionally incorporated (Lewandowski 1987, Pleasant 2006, Pleasant and Burt 2010). In Chapter two, the Three Sisters was defined as sunflower, bean, and squash planted within a constructed mound (Figure 18B). However, for this study, to increase functional diversity, all four crop plants were incorporated into the Three

Sisters system, resulting in a standardized planting of eight individual plants: two corn, two beans, two squash, and two sunflowers (Figure 18C). Furthermore, the mound in which the Three Sisters and other treatments in Chapter two were planted was optional due to the time constraints. Therefore, throughout this chapter, the term "Three Sisters planting" referred to the outlined eight plant assemblage in a 1 m^2 area, regardless of whether a soil mound was constructed. The Three Sisters plantings served as the primary unit of replication used for this study.

The seed layout within the Three Sisters planting followed that of a traditional Haudenosaunee Three Sisters (Pleasant and Burt 2010, Kruse-Peeples 2016), modified to accommodate four varieties of plants (Figure 18A). The seed varieties and distributor (Ontario Seed Co. Ltd, Kitchener, Ontario) for the sunflower ('Lemon Queen'), common pole bean (*Phaseolus vulgaris* L. 'Blue Lake'), and pumpkin (*Cucurbita pepo* L. 'Jack-Be-Little') were maintained from Chapter two. Selection of the corn cultivar followed the multi-step process described in Chapter two, resulting in the inclusion of a flint corn variety (*Z. mays* convar. *mays* 'Assiniboine flint', Heritage Harvest Seeds, Fisher Branch, Manitoba). The number of seeds purchased included the total seeds required per site, plus an additional 50% to account for failed seed emergence.

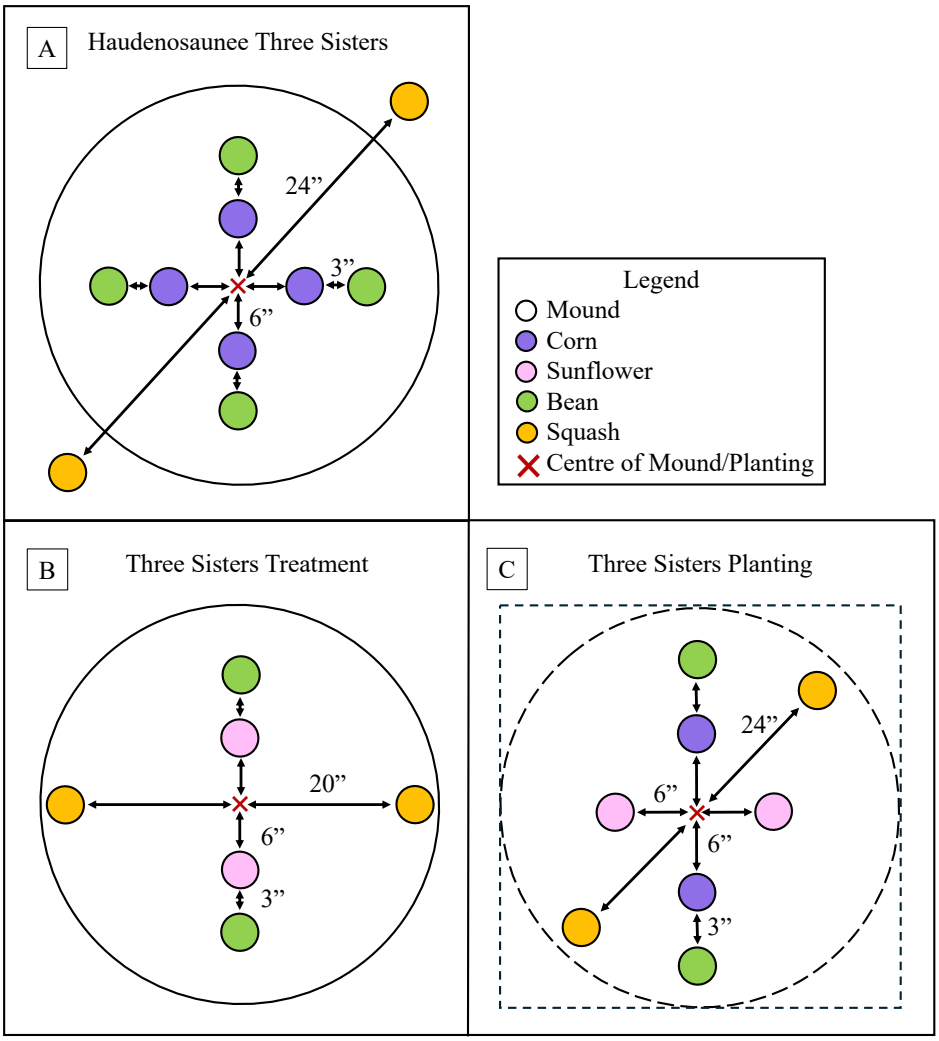

Figure 18. Comparison of a traditional Three Sisters planting layout within a mound to the 2022 field season Three Sisters treatment mound and the 2023 field season Three Sisters planting within a mound or 1 m^2 space. Diagrams include planting orientation of respective crop plant species and measurements relative to the centre of mound (1 m diameter) or 1 m^2 space. (A) Traditional Haudenosaunee Three Sisters mound. (B) Three Sisters treatment mound used in the 2022 field season study. Included a mound with reduced spacing between plants and used sunflower as a replacement for corn. (C) Three Sisters planting used in the 2023 field season containing four plant species. The dashed square represents the standardized 1 m^2 planting space, while the dashed circle indicates the optional inclusion of a 1 m diameter soil mound.

Planting Schedule and Maintenance

For the 2023 field season, an adjusted Three Sisters planting schedule (Table S 8) was designed based the 2022 observed planting schedule (Table S 1) and traditional Haudenosaunee Three Sisters practices (Hurt 1987, Pleasant and Burt 2010, Kruse-Peeples 2016), with

modifications to accommodate four crop species and direct field sowing. Challenges experienced in the 2022 field season related to plant health, transplanting, and wildlife disturbance informed these adjustments. All crops except squash were directly sown into the field, and hand weeding was conducted for the entirety of a study site. The only deviation from the adjusted 2023 planting schedule was that all sites were planted 2–3 weeks early due to the last frost date occurring in early May (Table S 8).

Corn was planted first, sown directly into the field once soil temperatures were consistently above 10ºC (Government of Manitoba n.d., Ontario Ministry of Agriculture Food and Rural Affairs 2022). One week later, sunflowers were planted directly into the field while squash was germinated indoors under controlled conditions. Manitoba sites utilized an incubation chamber (Percival Model I-36LLVL, Perry, Iowa) at the University of Manitoba, and Ontario sites used comparable facilities. Chamber conditions were identical to those outlined in Chapter two. Squash seeds were planted in plastic plugs (42 mm, University of Manitoba, Plant Science Department) containing Promix Growing Medium (University of Manitoba, Plant Science Department). To induce squash seed cracking, plugs were covered with a damp towel or plastic wrap for 48–72 hours after watering to maintain high moisture levels. After emergence, squash plants were periodically hardened outdoors before transplanting. Beans were directly planted into the field approximately 4–5 weeks after initial corn planting date, once the corn had reached at least 6″ tall. Within the 3–5 week transplanting window, approximately one week after the beans started to germinate, squash plants were transplanted into the field (NeSmith 1993).

Seed density and planting depths followed regional agronomic recommendations (Government of Manitoba 2007, n.d., n.d., Ontario Ministry of Agriculture Food and Rural Affairs 2022). Corn was sown two kernels per hole (2″ depth), sunflowers at three seeds per hole (1.5″–2″ depth), squash at one seed per hole/plug (1" depth), and beans at two seeds per hole (1″ depth). Marked sticks were used to ensure consistent planting depths. Prior to insect sampling, all plants sown directly into the field were thinned to a single plant in its respective spot to maintain consistent plant numbering within each Three Sisters planting.

Insect Surveying Procedures

To provide data regarding wild bee and ground beetle communities I performed the same four insect surveying methods, maintained the similar insect preservation practices, and utilized identical weather collection techniques, thresholds, and variables outlined in Chapter two (Table 1).

Wild Bees

Targeted Aerial Netting

To document wild bee biodiversity within the Three Sisters garden area and assess for potential edge effects, targeted aerial netting was conducted once a week between August 8, 2023–September 6, 2023, for a total of 3–5 sampling occurrences depending on the site (Table S 9). Sampling was performed per Three Sisters planting following the protocol described in Chapter two. For this sampling method, the Three Sisters garden area was divided into two positions to ensure balanced sampling effort across the study area (Table 12; Figure 17D).

Bee Bowls and Blue Vane Traps (BVTs)

To determine the general bee community differences between the Three Sisters garden area and the adjacent natural area, the bee bowl and BVT sampling procedures outlined in Chapter two were followed. However, the number of traps used for sampling was reduced by half (Figure 19). Frequency of sampling was once a month for a total of three sampling events (Table S 9).

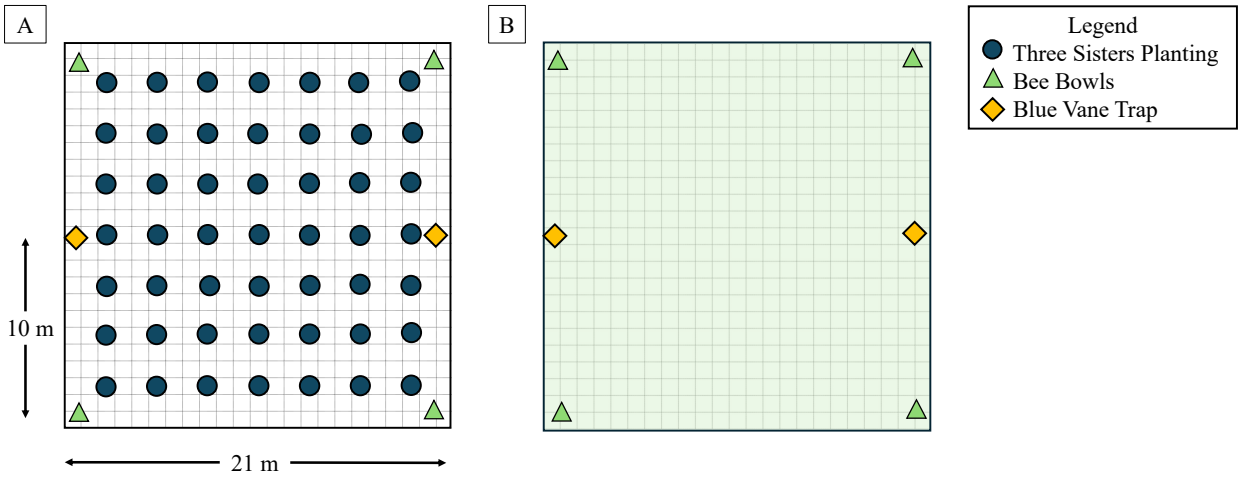

Figure 19. Visual representation of bee bowl and blue vane trap placement within the respective study area. (A) Location of bee bowls and blue vane traps in the Three Sisters garden area. Each grid square represents 1 m^2. (B) Location of bee bowls and blue vane traps in the natural area.

Carabid Beetles

Pitfall Traps

To evaluate ground beetle biodiversity metrics within the Three Sisters garden area and the surrounding natural area, as well as potential edge effects, the pitfall trap sampling method (Figure 6) outlined in Chapter two was conducted on a biweekly basis for a total of 4–6 sampling events, depending on the site (Table S 9). However, the number of pitfall traps within the Three Sisters garden area was reduced from every mound to a total of 12 pitfall traps (Figure 20A). The pattern in which the pitfall traps were placed in the Three Sisters garden area was mirrored in the natural area (Figure 20B), resulting in 24 pitfall traps per study site. The choice to decrease the number of traps was based on lessening the potentially negative ecological impact of sampling on beetle and other ground-dwelling animal communities, reducing the chance of oversampling, and accommodating assigned spatial positions used to assess edge effects. The Three Sisters garden and natural areas were divided into three positions for beetle sampling to maintain equal sampling effort (Table 12; Figure 20C-D).

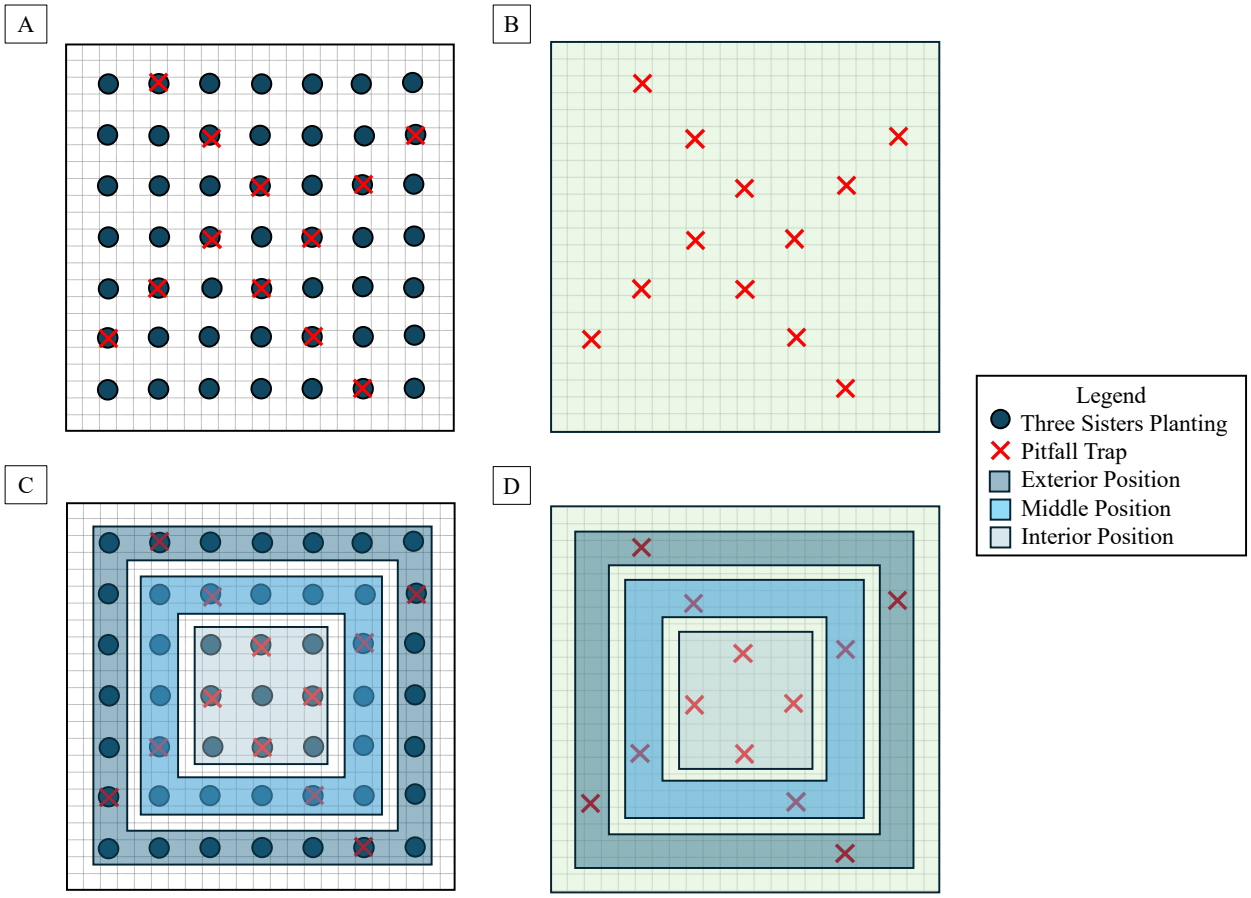

Figure 20. Visual representation of the placement of pitfall traps within the respective study area and orientation within the three-position framework. Each grid square represents 1 m^2. (A) Location of pitfall traps in the Three Sisters garden area. (B) Location of pitfall traps in the natural area. (C) Orientation of pitfall traps within the corresponding spatial positions in the Three Sisters garden area. (D) Orientation of pitfall traps within the corresponding spatial positions in the natural area.

Processing Insects

I used the same procedure for processing and identifying bees and ground beetles to species as outlined in Chapter two, Sections Processing Bees and Processing Ground Beetles.

Insect Biodiversity Metrics

I measured the same insect biodiversity metrics and classified bees by identical diet breadth as explained in Chapter two (Table 13), but the units in this study were defined by insect sampling method and sampling area. In addition, I further explored the abundance metric as an

indicator of the likelihood of ecosystem service provisioning. Specifically, wild bee abundance was used as a proxy for potential pollination delivery, and ground beetle abundance was used as a proxy for potential pest control distribution (Table 13). This approach is commonly employed in agroecological studies where direct measurement of ecosystem service rates was not feasible (Noriega et al. 2018). The application of these proxies was for harvest-focused analyses and is described in the Modelling Harvest Metrics section.

Table 13. Insect biodiversity metrics and functional trait variables. Metrics calculated based on sampling area and insect surveying procedures sampling unit (Three Sisters garden area: Three Sisters planting – targeted aerial netting, pitfall traps; Trap – bee bowls, and BVTs; Natural area: Trap – pitfall traps, bee bowls, and BVTs) per sampling event at each study site.

Scale	Variable	Description
Community-Level Metrics	Abundance	The total number of individuals
	Species Richness	Total number of unique species
	Hill-Shannon Diversity	A measure of community diversity that accounts for both species richness and relative abundance, expressed in units of "effective number of species"
Community-Level Metrics (Proxies)	Pollination	Wild bee abundance values averaged across sampling round per Three Sisters planting at each study site, used as a proxy for potential pollination provision
	Pest Control	Ground beetle abundance values averaged across sampling round per Three Sisters planting at each study site, used as a proxy for potential pest control provision
Species-Level Trait - Diet Breadth	Oligolectic	Plant specialist, narrow diet range
	Polylectic	Plant generalist, broad diet range

Plant Surveying Procedures

Crop Plant Survey

The crop plant survey methods were identical to those in Chapter two. The variables used were similar; however, the "cropping" variable was not applicable to this study, as all Three Sisters plantings had an equal number of plant species, and the "plant count" variables were used exclusively for pre-analysis data screening (Table 3).

Non-Crop Plant Survey

To account for the effects of local land cover on insect captures, non-crop plant (floral and non-floral weeds) diversity and ground cover characteristics were recorded through vegetation surveys. The percentage of bare ground, grass cover, and floral cover was estimated within a 1 m x 1 m quadrat, using scale diagrams provided by Anderson (1986) as a guide. A total of ten samples spaced 5 m apart along two 25 m line transects (Figure 21), placed perpendicular to an edge of the respective Three Sisters garden area, were recorded monthly. If possible, sampling transects prioritized the inclusion of wildflowers but avoided roads/ditches and agricultural cropland. Within each 5 m segment, quadrat placement was randomized to reduce sampling bias. A measure of floral richness was derived from these percent estimates similar to Chapter two, however, the count reflected the species found within the study site as a whole (Table 14). The procedure to identify plants was identical to the steps outlined in Chapter two.

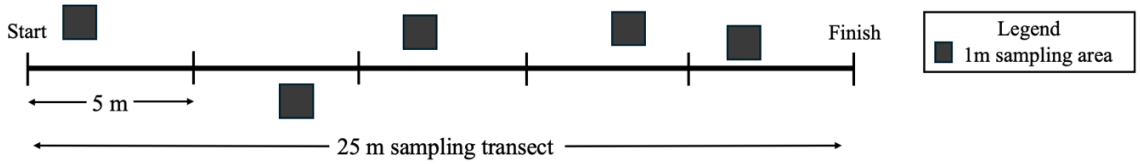

Figure 21. Visual representation of transect sampling.

Table 14. Non-crop plant variables representing local land cover. Calculated per ten 1 m^2 quadrats per study site.

Variable	Description
Floral Richness	Total number of individual plant species (excluding grasses)
Bare Ground (%)	Average bare ground
Grass Cover (%)	Average area covered by grass

Harvest Procedures

To assess edge effects and potential insect ecosystem services influence on Three Sisters harvest metrics, mature crops were collected by hand at the end of the growing season prior to

each sites anticipated first frost date of September 18, 2023–September 26, 2023 (Government of Manitoba n.d., Ministry of Agriculture 2024). For this method, the Three Sisters garden area was divided using the two-position framework (Table 12; Figure 17D). Initial counts for each plants total harvest per Three Sisters planting was recorded and prepared for drying by stripping harvest of unneeded plant material (flower petals, bean hulls, leaves, peduncles, and stems). Unlike Chapter two, sunflower seeds were left on the heads and corn husks were left on to aid in the drying process. All harvested material was initially placed on large wire racks to dry using ambient room temperature (~20–25ºC) and constant airflow provided by an oscillating fan (FAO 2001, 2013, Uddin et al. 2016, Gunathilake et al. 2018).

Within a week of harvest, select plant material was transferred to a dedicated drying facility, with temperatures ranging from 15–21Cº (FAO 2013). For the Manitoba sites, initial drying occurred in the Bobiwash lab (University of Manitoba, Winnipeg) and the drying facility was located at the Plant Science Field Station (University of Manitoba, Fort Garry Campus, Winnipeg). Ontario sites used comparable on-site facilities for both procedures. The order in which harvest was transferred was based on moisture content and availability of space, with sunflower moved first, followed by corn, then squash.; beans remained on the wire racks. Sunflower heads were placed in large breathable bags (e.g., cheesecloth or paper bags) on drying tables with airflow from above and below (FAO 2001, Barrozo et al. 2014). Approximately two weeks after collection date, corn husks, silk, and kernels were separated from the cob and placed in containers on racks with ceiling fans providing airflow. Due to a plentiful harvest, four per Three Sisters planting were randomly selected for processing. Whole squash weights (peduncle-less) were recorded (g), seeds were removed, placed in small mesh or brown paper bags, and placed onto the drying tables. Samples were checked daily for spoilage and periodically rotated to avoid mould (Gunathilake et al. 2018). All plant material/seeds were dried for one to two weeks or until crispy/brittle to the touch.

All samples were then assessed for debris and large excess plant material (e.g., petals, involucre bracts, squash pulp etc.) was removed by hand. This step included the removal of sunflower seeds from the sunflower head. Fine debris was removed using a seed blower (Hoffman Manufacturing Inc. HMC67L. 115 V/60 Hz). The airflow was standardized by always starting with the air intake gate set to 15, increasing to 25 for 30 to 45 second intervals

depending on the amount of debris and seed weight of each sample. In the event that seeds and fine debris were of similar weights, seeds were then sifted by hand using seed-cleaning screens to avoid loss of samples in the blower. A range of cleaning screen sizes were used, including round hole and oblong slot screens (Dominion Seed, Winnipeg), with either the 7/64" round hole screen and/or 1/8" oblong slot screen being the most used for this task. The seeds were then counted per Three Sisters planting with a seed counter (Seed counter Model U, International Marketing Design Corp. San Antonio, TX USA). Finally, all seeds had their dry weights recorded in grams to two decimal places. The Ontario sites either sent their respective harvest to the Bobiwash lab to be processed or followed the above outlines as closely as possible with the drying facilities and equipment on site. All excess plant materials were composted at each respective site except for the excess squash collected in Manitoba, which was donated to Little Red Barn Animal Sanctuary (Winnipeg, Manitoba).

Harvest Metrics

I measured the same harvest metrics (yield and quality) using the "dry" weights (g) and seed counts for each crop species as outlined in Chapter two, but the units in this study were defined by Three Sisters planting (Table 15). For squash, only four randomly selected squash per planting were processed due to the large harvest, and seed samples were lost before weighing; therefore, squash quality was estimated using the average weight of a single whole squash as a proxy for seed weight (Paris and Nerson 2003, Nerson 2005).

Table 15. Harvest metrics. Calculated per Three Sisters planting and single unit of harvest (corn ear, sunflower head, bean pod, or squash) at each study site.

Variable	Description
Corn Yield	Average number of kernels per single ear of corn
Corn Quality (g)	Average weight of a single dry corn kernel per single ear of corn
Sunflower Yield	Average number of seeds per single sunflower head
Sunflower Quality (g)	Average weight of a single seed per single sunflower head
Bean Yield	Average number of beans per single bean pod
Bean Quality (g)	Average weight of a single bean per single bean pod
Squash Yield	Average number of squash seeds per single squash
Squash Quality (g)	Average whole weight of a single squash, used as a proxy for single seed weight; n=4 squash per Three Sisters planting

Statistical Analysis

The following assessments and analyses were performed in R (R Core Team 2024).

Pre-Analysis Data Screening

Exploratory statistics were conducted to assess for inconsistencies and inaccuracies in the collected data, with parameters for exclusion mirroring those outlined in Chapter two. This pre-analysis screen resulted in all bee bowl and BTV samples being excluded from analysis, with additional omissions including New Liskeard pitfall trap samples and data collected by Sudbury across all sampling methods.

Modelling Insect Biodiversity Metrics

To assess the effect the Three Sisters garden area had on insect biodiversity metrics and wild bee diet breadth (lecty) (Table 13), I ran independent generalized linear mixed models (GLMMs) using glmmTMB package (Brooks et al. 2017). Wild bee and ground beetle data were analyzed in separate model sets. For wild bee analyses, models included "position" (exterior vs. interior; Table 12) as my focal predictor to determine whether biodiversity metrics or the proportion of specialists relative to generalists differed within the Three Sisters garden area (Table 13). For ground beetle analyses, I explored the biodiversity differences between the Three Sisters garden area and the natural area, as well as the potential additive or interactive effect of position (exterior vs. middle vs. interior) within study areas (Table 12). To accomplish this, ground beetle models initially included two focal predictors ("area" and "position") as an interaction term. All models included the nested random variables for site and mound, as well as a crossed random factor for sampling round to account for changes in insect communities over time.

Global models were created for each biodiversity metric, including the focal predictor(s) and relevant local climate (Table 1) and landcover (Table 14) covariates. The following were the global model structures used for wild bee (6) and ground beetle biodiversity metric analysis (7):

$$Y \sim position + daily\ average\ temperature + daily\ maximum\ temperature + daily\ minimum\ temperature + daily\ average\ wind\ speed + daily\ maximum\ wind\ speed + daily\ minimum\ wind\ speed + (1|site/mound) + floral\ richness + (1|sampling\ round) \quad (6)$$

$$Y \sim area * position + weekly\ average\ temperature + weekly\ maximum\ temperature + weekly\ minimum\ temperature + bare\ ground + grass\ cover + (1|site/mound) + (1|sampling\ round) \quad (7)$$

Diet breadth was analyzed as the proportion of wild bee species classified by lecty (specialist vs. generalist) using a subset of the wild bee model structure (6) that excluded weather-related variables, as this response reflected a functional trait rather than an abundance metric influenced by sampling conditions.

Global models were initially evaluated for convergence issues and overall goodness-of-fit. If a global model failed to converge or could not be fit using an appropriate distribution, the principle of parsimony was followed, defined as the simplest model that could adequately explain the data; in this context, models would retain only the focal predictor(s) and appropriate random effects (Warren and Seifert 2011). For models that successfully converged or met assumptions, multicollinearity among additional environmental and landscape variables was assessed using the 'easystats' package (Lüdecke et al. 2022). Variables that indicated moderate or high correlation (e.g., VIF score of >5) were removed from further consideration (Craney and Surles 2002). For ground beetle models, the significance of the interaction term was then evaluated, and if the interaction was found not to be statistically significant ($p<0.05$), it was removed. Both variables were retained as primary predictors to assess their individual contributions, re-tested for multicollinearity, and run accordingly.

Abundance and species richness models were first fit with either a Poisson or a negative binomial distribution, depending on overdispersion. Hill-Shannon diversities were created using the 'hillR' package (Li 2018) and were initially fit using a Gaussian distribution. However, if goodness-of-fit was not achieved, Hill-Shannon diversity values were aggregated across Three

Sisters plantings by averaging within each site, area (beetle model only), position, and sampling round. Models were refit with site and sampling round as crossed random effects and run accordingly. Diet proportion models, limited to wild bee analysis, were run using a binomial distribution. Model diagnostics were assessed using histograms and quantile-quantile plots from the 'DHARMa' package (Hartig 2024). When more than one distribution provided an adequate fit, models were compared using analysis of deviance (LaZerte 2021). If no significant differences were detected, the model with the lowest Akaike's Information Criterion (AIC) was selected, as it indicated the most parsimonious and optimal balance between model fit and complexity (Akaike 1998, Warren and Seifert 2011). In the final chosen models, if significance was found ($p<0.05$) a pairwise post-hoc test was performed using the 'emmeans' package (Lenth 2024), with p-values adjusted using the False Discovery Rate (FDR) method to control for multiple testing.

Modelling Harvest Metrics and Potential Ecosystem Service Provisioning

To assess differences in yield and quality of Indigenous significant crop plants (Table 15) among the positions (exterior vs. interior; Table 12) within a Three Sisters garden area, and to test whether the position of Three Sisters plantings potentially provisioned ecological services relevant to crop performance (Table 13), I ran non-parametric Kruskal-Wallis tests (Kruskal and Wallis 1952). Non-parametric testing was selected due to limited replication of study sites after pre-analysis data screening and sampling events (n=1). The Kruskal-Wallis test was consistently used for all response variables rather than alternating among other tests (e.g., Wilcoxon Signed-Rank test) because it accommodates predictor variables with two or more levels, is robust to non-normality or unequal group sizes, and allows for consistent comparisons of harvest metric and ecosystem service response variables. Regarding the ecological services models, site-level mean abundances were calculated from wild bees caught through targeted aerial netting and ground beetles captured through pitfall traps. Mean wild bee abundance was considered a proxy for pollination services and mean ground beetle abundance was considered a proxy for pest control services (Table 13). Proxies were calculated by averaging abundance across all sampling rounds and Three Sisters plantings within each site to mirror the single sampling event of the harvest procedure. The focal predictor "area" was not included in either ecosystem service models as captures were restricted to within the Three Sisters garden area to mirror harvest metric models

to allow for appropriate comparison. The "position" variable for the pollination model reflected a two-position framework (exterior and interior), while the pest control model used the three-position framework (exterior, middle, and interior) (Figure 17D–E). Where significance was found ($p<0.05$), a Dunn's test with a Benjamini-Hochberg correction was conducted using the 'FSA' package (Dinno 2024, Ogle et al. 2025).

Results

Insect Biodiversity Metric Analysis

Wild bee and ground beetle global models included all focal predictors and were initially run individually for each insect biodiversity response variable. For wild bee abundance, ground beetle abundance, and ground beetle species richness, the global models initially met convergence and goodness-of-fit criteria (Table S 10). However, the contribution of environmental and landscape variables was inconsistent across models; therefore, to facilitate comparison between the effect of "position" and/or "area" on response variables, these global models were simplified following the principle of parsimony. The above models retained only focal predictors and random effects. The remaining insect response global models all failed goodness-of-fit, and therefore, the principle of parsimony was applied. The following were the final model structures for both wild bee (8) and ground beetle (9) count and diet breadth response models:

$$Y \sim position + (1|site/mound) + (1|sampling\ round) \quad (8)$$

$$Y \sim area * position + (1|site/mound) + (1|sampling\ round) \quad (9)$$

Concerning Hill-Shannon diversity models for both wild bees and ground beetles, data was required to be aggregated per Three Sisters planting. The following was the altered model structure for wild bee (10) and ground beetle (11) Hill-Shannon diversity analysis:

$$Y \sim position + (1|site) + (1|sampling\ round) \quad (10)$$

$$Y \sim area * position + (1|site) + (1|sampling\ round) \quad (11)$$

Wild Bee

Captures

I collected 1,070 bees through the three described collection methods, identifying 39 species from 12 genera. Similar to Chapter two, misaligned and uncertain data was excluded from statistical analysis, culminating in 853 specimens from 8 genera and 28 species for potential analysis (Table S 11). Furthermore, bee bowl and BVT data was deemed unusable for analysis, resulting in only the 701 wild bees captured through targeted aerial netting to be appropriate for analysis. Bee bowl and blue vane trap data are presented only in the results section and are not interpreted further than presenting total counts and relative abundances (expressed as a percentage of total individuals) of select genera and species (Table S 11; Table S 12; Figure S 4).

Bee Bowls and Blue Vane Traps (BVTs)

Collectively, the bee bowls and BVT traps captured 152 wild bees from both the Three Sisters garden area and natural area, with the majority being polylectic (Table S 12). Since BVTs caught 93.4% of the 152 bees, this method also saw the highest number of genera and species. Out of the five genera captured through BVT, *Bombus* (98 specimens, 69%) and *Melissodes* (30 specimens, 21.1%) dominated samples, with the two genera, *Agapostemon* and *Anthophora,* being exclusive to this method (Figure S 4A). *Bombus (Subterraneobombus) borealis* Kirby was the most abundant species with 48 specimens, followed by *Bombus (Pyrobombus) vagans* Smith and *Melissodes (Eumelissodes) agilis* Cresson*,* both with 20 specimens each (Table S 12). Bee bowl captures were from three genera and seven species, totalling 10 specimens, none of which had an abundance exceeding three observations (Figure S 4B). When comparing the two sampling methods between the two study areas, wild bee abundances and species richness did not differ greatly, but leaned in favour of the Three Sisters garden area (Table S 12). The opposite was observed at the genus level, with higher abundance and richness seen in the natural area, with differences of only one or two individuals or species.

Targeted Aerial Netting

Targeted aerial netting caught 701 wild bees, representing 20 species in 5 genera (Table S 13). Dominant genera are presented with total count and relative abundance (expressed as a percentage of total individuals). The majority of wild bees were within the *Bombus* genus (592 specimens, 84.5%), followed by *Melissodes* (92 specimens, 13.1%) (Figure S 5). The remaining genera (*Megachile*, *Protandrena*, and *Triepeolus*) accounted for <2% of collected specimens. When comparing the exterior position of the Three Sisters garden area to the interior, I found that in general slightly more bees were caught within (375 specimens, 53.5%), opposed to the exterior (326 specimens, 46.5%). Notably, one bee species, *Bombus (Thoracobombus) pensylvanicus* (De Geer), was captured, representing the second recorded occurrence in Manitoba since 1948.

Effect of Position

The subsequent biodiversity analyses were conducted using wild bee captures via targeted aerial netting as this method provided a direct link between wild bees and plants they visit. Targeted aerial netting was restricted to the Three Sisters garden area, as the natural areas did not produce consistent floral resources to allow for targeted sampling.

Abundance

The model examining wild bee abundance was fit using a negative binomial distribution. Results indicated no significant effect of positions within the Three Sisters garden area on bee abundance (GLMM, χ^2= 1.0187, df= 1, p= 0.3128) (Figure 22).

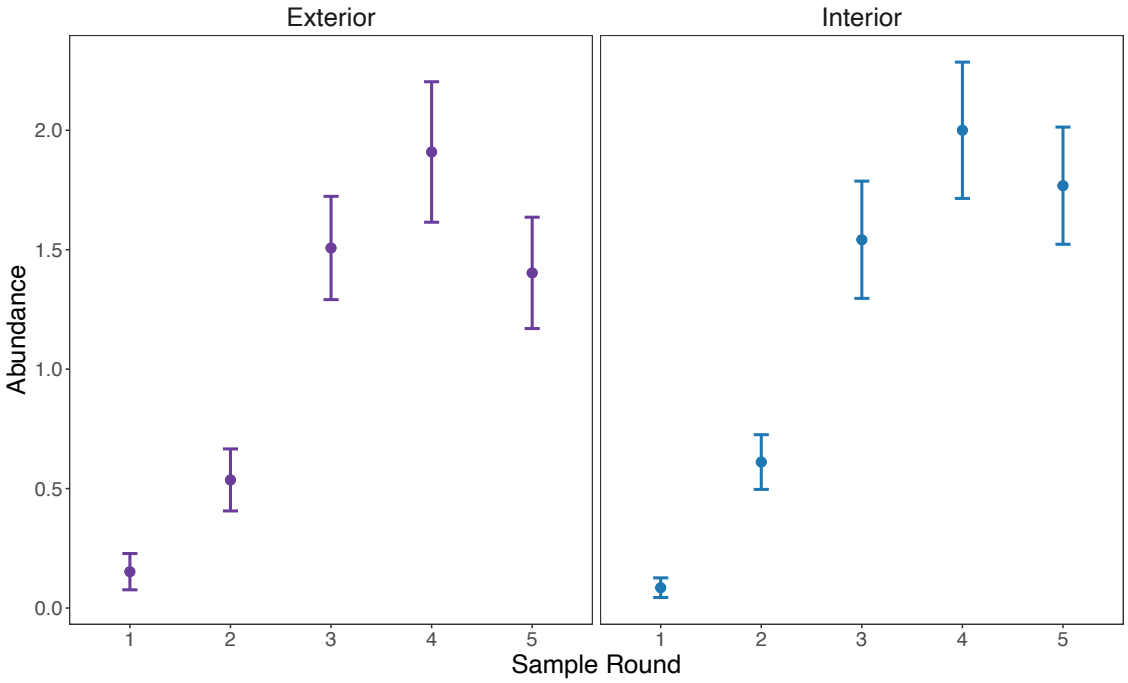

Figure 22. Effect of position within the Three Sisters garden area on wild bee abundance. Points represent the average number of wild bees captured through targeted aerial netting per sampling event. Error bars represent the 95% confidence intervals of the mean.

Species Richness

Species richness was analyzed using a Poisson distribution. The position of a Three Sisters planting within a garden plot was found to not influence bee species richness (GLMM, χ^2= 0.5020, df= 1, p= 0.4786) (Figure 23).

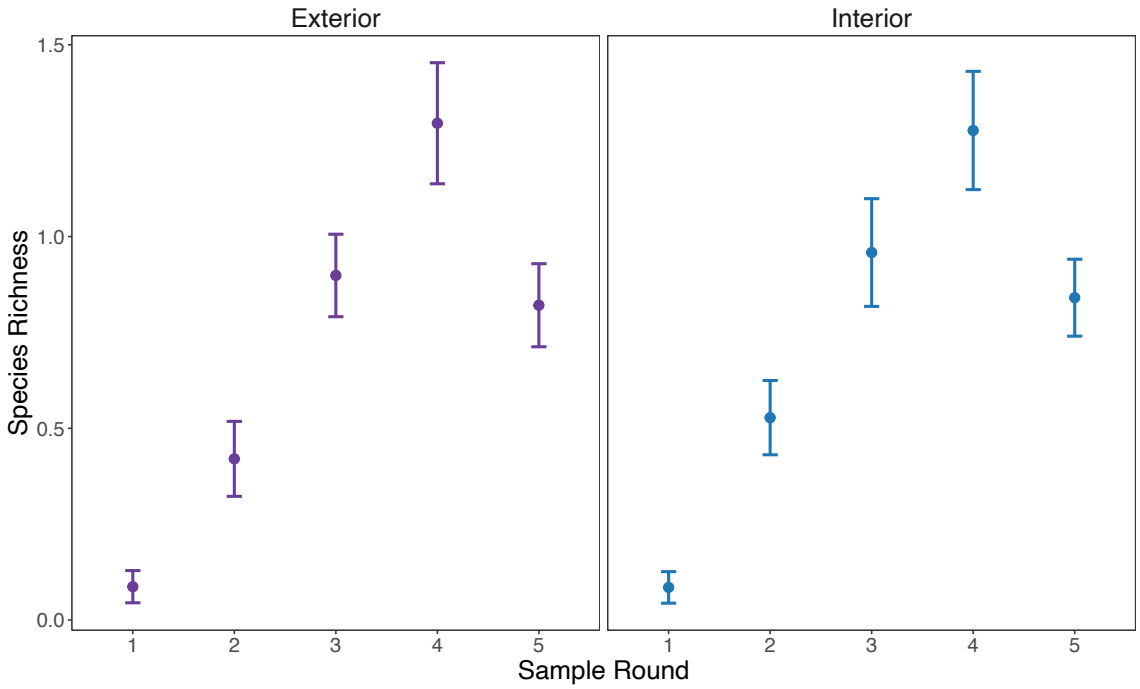

Figure 23. Effect of position within the Three Sisters garden area on wild bee species richness. Points represent the average number of wild bee species captured through targeted aerial netting per sampling event. Error bars represent the 95% confidence intervals of the mean.

Hill-Shannon Diversity

The Hill-Shannon diversity model was fit with a Gaussian distribution after simplifying the data (averaging by Three Sisters planting). Results revealed that position of a Three Sisters planting did not have a significant effect on bee Hill-Shannon diversity (GLMM, χ^2= 0.062, df= 1, p= 0.8033) (Figure 24).

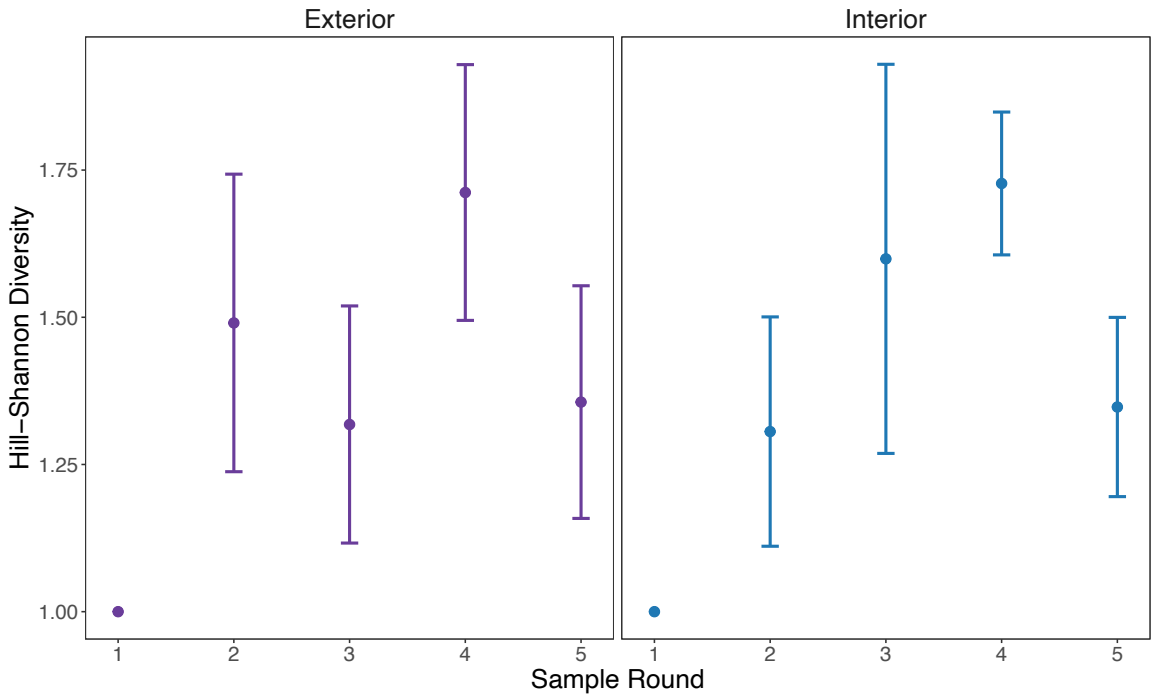

Figure 24. Effect of position within the Three Sisters garden area on wild bee Hill-Shannon diversity. Points represent the average Hill-Shannon diversity of wild bees captured through targeted aerial netting per sampling event. Error bars represent the 95% confidence intervals of the mean.

Diet Proportion

The wild bee diet proportion model was fit using the binomial distribution. No significant effect of position was found on the proportion of oligolectic versus polylectic wild bee individuals (GLMM, χ^2= 0.1741, df= 1, p= 0.6765) (Figure 25).

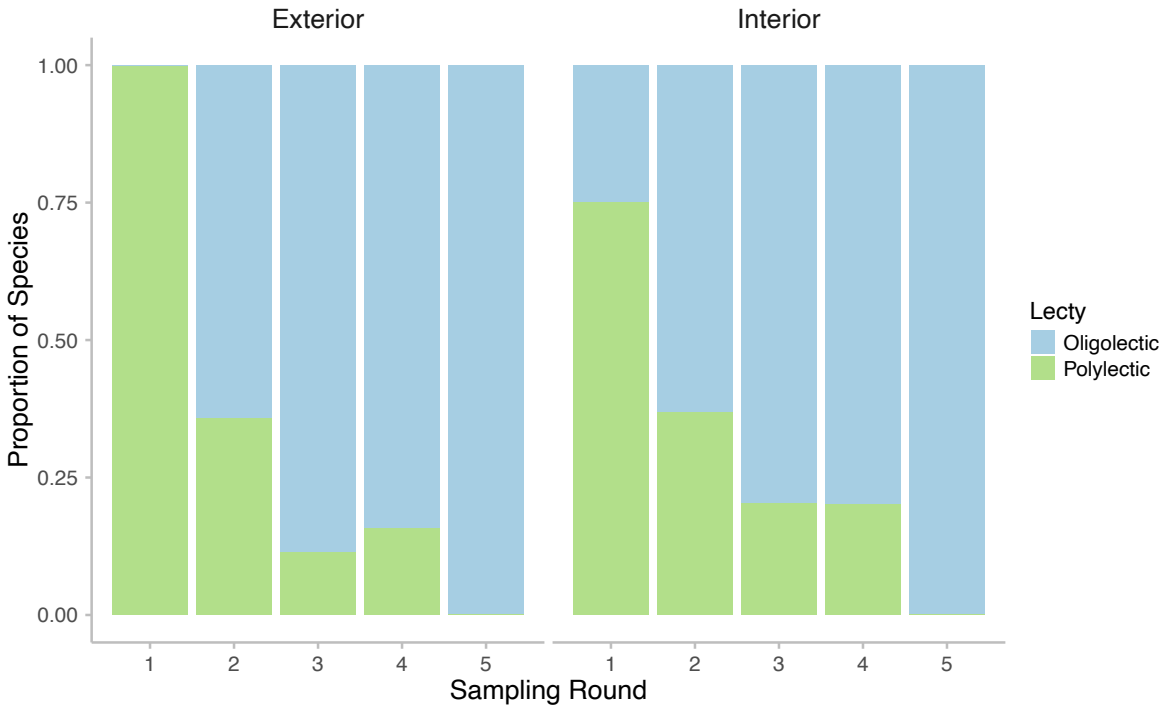

Figure 25. Effect of position within the Three Sisters garden area on the relative composition of specialist and generalist wild bee species captured through targeted aerial netting. Bars represent the proportion of oligolectic and polylectic species per sampling event. Graph does not include parasitic bee species.

Carabid Beetles

Captures

I collected 1,538 carabid beetles, identifying 1,533 specimens to 61 species within 24 genera, with the remaining five unidentifiable beyond the carabid family. After the exclusion of non-reflective data, a total of 770 carabid beetles in 21 genera across 52 species were used for statistical analysis (Table S 14; Figure S 6). Pitfall trap captures were dominated by *Pterostichus* in terms of abundance (31.1% of all individuals), with the most abundant species being *Pterostichus (Morphnosoma*) *melanarius* (Illiger) (236 specimen, 30.6% of all individuals) (Figure S 6). Dominant genera are presented with total count and relative abundance (expressed as a percentage of total individuals). The *Amara, Bembidion,* and *Harpalus* genera were also abundant and accounted for 16.4%, 12.5% and 10.9% of all individuals collected, respectively. With the majority of carabid specimens being rare (>25 individuals), six species stood out as

relatively abundant (>100 individuals). They were, in decreasing order (relative abundance in parentheses, rounded up to the first decimal place), *Bembidion (Furcacampa) mimus* Hayward (7.5%)*, Poecilus lucublandus* (Say) (6.5%)*, Synuchus impunctatus* (Say) (4.5%)*, Harpalus (Pseudoophonus) pensylvanicus* (De Geer) (3.9%), *and Clivina (Clivina) fossor* (Linné) (3.7%).

Effect of Area and Position

In all ground beetle models, the interaction between the variables "area" and "position" was found to not have significance; therefore, it was removed. The following ground beetle biodiversity metric models were performed with the variables "area" and "position" as individual fixed effects.

Abundance

Carabid beetle abundance was fit using a negative binomial distribution. I found that area had a highly significant effect on ground beetle abundance (GLMM, χ^2=13.1969, df= 1, p=0.0002804). In contrast, position within the Three Sisters garden area showed no significant interaction (GLMM, χ^2=1.0744, df= 2, p=0.5843779). I performed a post-hoc analysis to see how the Three Sisters garden area and natural area differed from each other (Table 16). Pairwise comparisons revealed that across all positions within the Three Sisters garden area consistently supported 58.1% more beetles compared to the same positions in the natural area (Figure 26). In addition to these consistent effects, the Three Sisters exterior captured 63% more beetles than the natural interior and 84.1% more than the natural area's middle. These results indicate that carabid abundance was consistently and significantly higher in the Three Sisters planting than in the natural area, regardless of spatial position.

Table 16. Results (response scale) of ground beetle abundance post-hoc pairwise comparison. The ratio represents an odds ratio comparing the two respective areas to the three positions within those areas and the p-value reflects whether or not that ratio is different from 1. The following abbreviations are used for area: 3S., Three Sisters garden area; and Nat., Natural area. The following abbreviations are used for position: Ext., Exterior; Mid., Middle; and Int., Interior. Significant p-values are shown in bold (α=0.05).

Area and Position Pairs	Ratio	Std. Error	z	p
3S Ext - Nat Ext	1.5810	0.1990	3.633	**0.0014**
3S Ext - 3S Int	1.0310	0.1540	0.205	0.8373
3S Ext - Nat Int	1.6300	0.3150	2.524	**0.0290**
3S Ext - 3S Mid	1.1650	0.1800	0.988	0.4403
3S Ext - Nat Mid	1.8410	0.3630	3.095	**0.0074**
Nat Ext - S3 Int	0.6520	0.1290	-2.166	0.0649
Nat Ext - Nat Int	1.0310	0.1540	0.205	0.8373
Nat Ext - 3S Mid	0.7370	0.1480	-1.517	0.2155
Nat Ext - Nat Mid	1.1650	0.1800	0.988	0.4403
3S Int - Nat Int	1.5810	0.1990	3.633	**0.0014**
3S Int - 3S Mid	1.1300	0.1740	0.790	0.4957
3S Int - Nat Mid	1.7850	0.3550	2.913	**0.0107**
Nat Int - 3S Mid	0.7150	0.1430	-1.684	0.1727
Nat Int - Nat Mid	1.1300	0.1740	0.790	0.4957
3S Mid - Nat Mid	1.5810	0.1990	3.633	**0.0014**

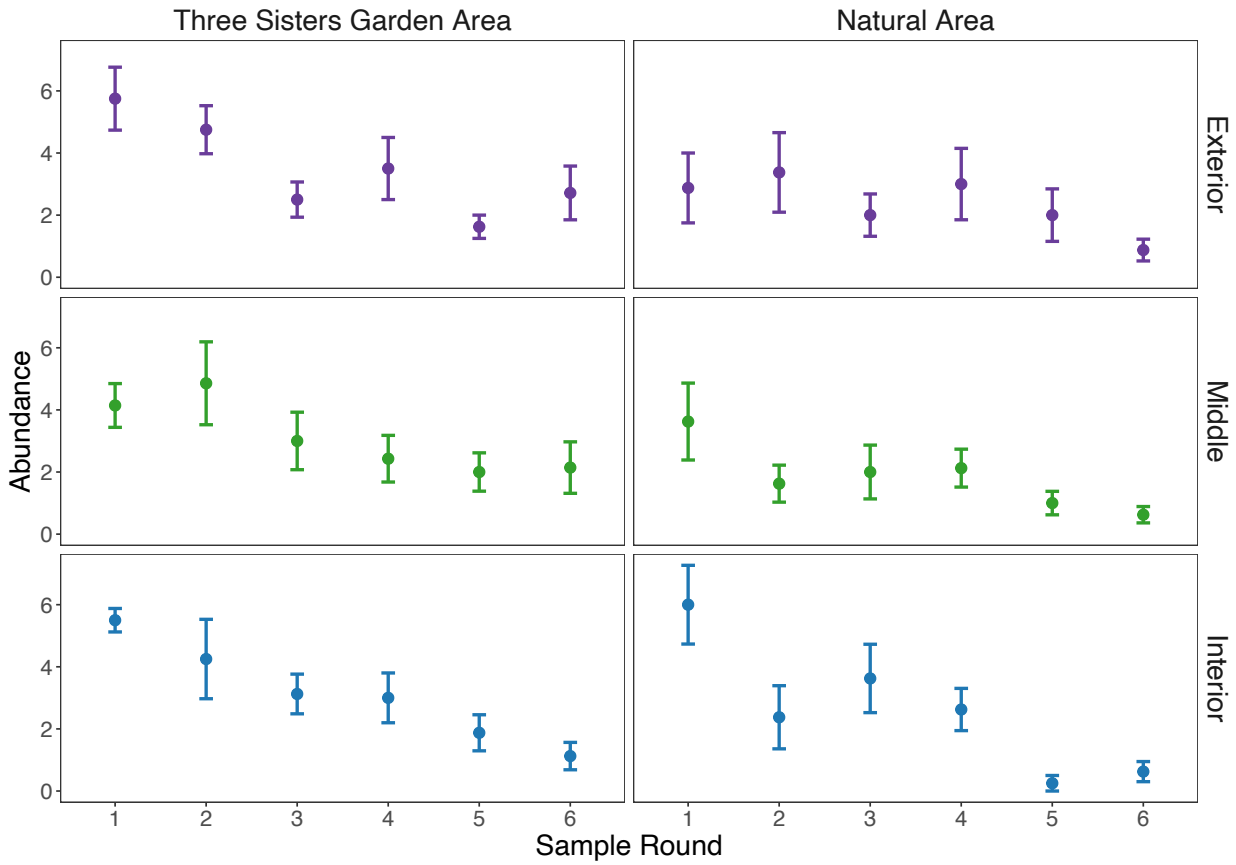

Figure 26. Effect of area and position on ground beetle abundance. Panels are arranged by area (columns: Three Sisters Garden Area and Natural Area) and position (rows: Exterior, Middle, and Interior). Points represent the average number of ground beetles captured per sampling event. Error bars represent the 95% confidence intervals of the mean.

Species Richness

The species richness model was fit using a Poisson distribution. Area was found to have a highly significant effect on ground beetle species richness (GLMM, χ^2=25.0266, df= 1, p=5.655e^{-07}), whereas position did not (GLMM, χ^2=0.0389, df= 2, p=0.980759). I conducted a post-hoc analysis to evaluate how the areas differed from one another (Table 17). Each Three Sisters garden area position hosted significantly more carabid species than its counterpart in the natural area, with all equivalent positions showing a 68.7% increase in richness (Figure 27). Beyond these matched comparisons, all other cross-position contrasts between areas supported this trend. The Three Sisters garden area exterior compared to the natural area interior and

middle closely aligned with the increases observed between equivalent position comparisons, with 65.1% and 68.6% more species, respectively. While the Three Sisters garden area interior contained 72.2% more species than the natural areas' middle position. Conversely, the natural area exterior caught 42% fewer species than the Three Sisters garden areas interior and 40.8% fewer species than the Three Sisters garden areas middle. Similarly, the natural area interior supported 40% fewer species than the Three Sisters garden areas' middle position. Together, these results highlight a consistent and substantial reduction in species richness within the natural area.

Table 17. Results (response scale) of ground beetle species richness post-hoc pairwise comparison. The ratio represents an odds ratio comparing the two respective areas to the three positions within those areas and the p-value reflects whether or not that ratio is different from 1. The following abbreviations are used for area: 3S., Three Sisters garden area; and Nat., Natural area. The following abbreviations are used for position: Ext., Exterior; Mid., Middle; and Int., Interior. Significant p-values are shown in bold (α=0.05).

Area and Position Pairs	Ratio	Std. Error	z	p
3S Ext - Nat Ext	1.6870	0.1760	5.003	**<0.0001**
3S Ext - 3S Int	0.9790	0.1210	-0.174	0.9957
3S Ext - Nat Int	1.6510	0.2680	3.090	**0.0038**
3S Ext - 3S Mid	0.9990	0.1270	-0.005	0.9957
3S Ext - Nat Mid	1.6860	0.2730	3.219	**0.0032**
Nat Ext - S3 Int	0.5800	0.0939	-3.363	**0.0023**
Nat Ext - Nat Int	0.9790	0.1210	-0.174	0.9957
Nat Ext - 3S Mid	0.5920	0.0989	-3.136	**0.0037**
Nat Ext - Nat Mid	0.9990	0.1270	-0.005	0.9957
3S Int - Nat Int	1.6870	0.1760	5.003	**<0.0001**
3S Int - 3S Mid	1.0210	0.1290	0.165	0.9957
3S Int - Nat Mid	1.7220	0.2780	3.365	**0.0023**
Nat Int - 3S Mid	0.6050	0.1010	-3.012	**0.0043**
Nat Int - Nat Mid	1.0210	0.1290	0.165	0.9957
3S Mid - Nat Mid	1.6870	0.1760	5.003	**<0.0001**

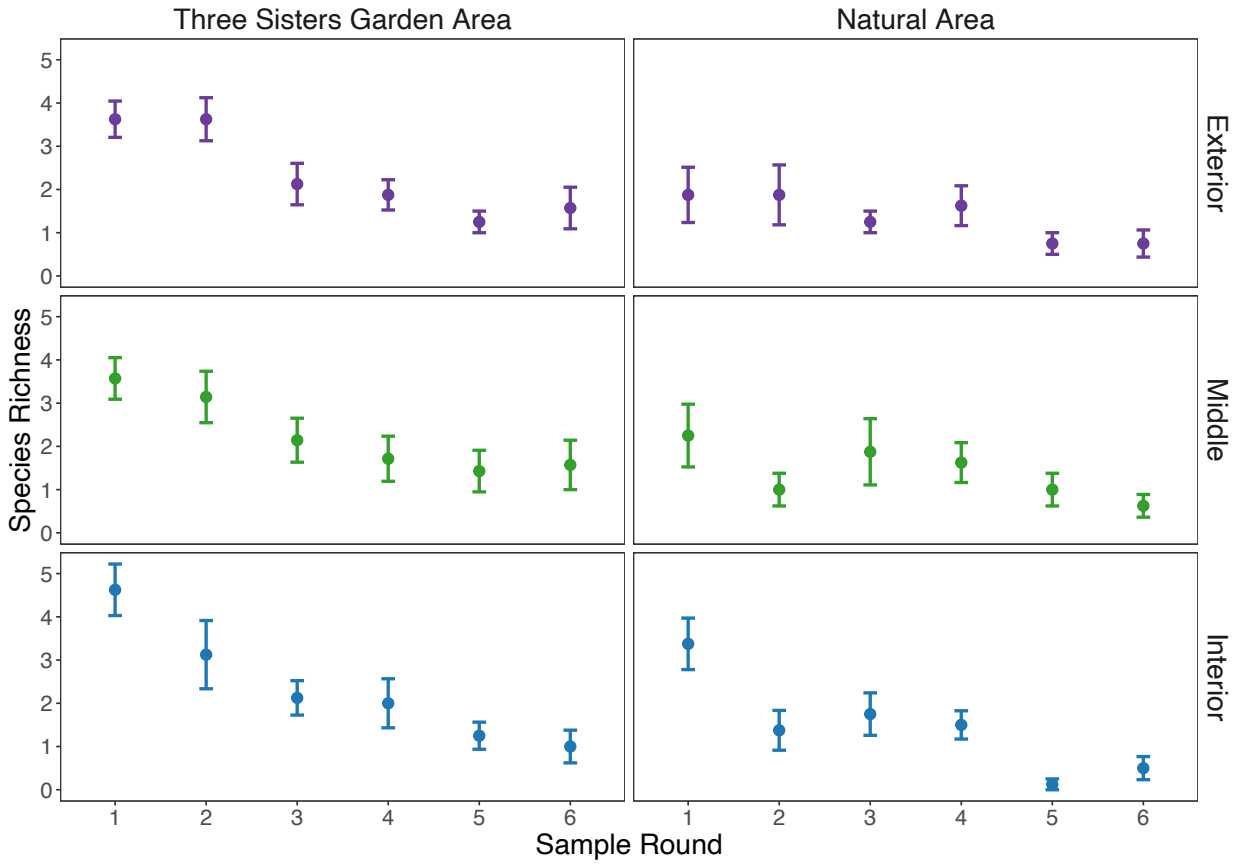

Figure 27. Effect of area and position on ground beetle species richness. Panels are arranged by area (columns: Three Sisters Garden Area and Natural Area) and position (rows: Exterior, Middle, and Interior). Points represent the average number of ground beetle species captured per sampling event. Error bars represent the 95% confidence intervals of the mean.

Hill-Shannon Diversity

The Hill-Shannon diversity model fit using a Gaussian distribution after that data was simplified, I found that area had a highly significant effect on ground beetle Hill-Shannon diversity (GLMM, χ^2=30.3240, df= 1, p=3.656e^{-08}); however, position within the Three Sisters garden area showed no significant effect (GLMM, χ^2=1.3453, df= 2, p=0.5103). I performed a post-hoc analysis to understand how these areas differed from each other (Table 18).

In contrast to the above models, the Hill-Shannon diversity Gaussian distribution resulted in post-hoc comparisons being reported as estimated differences in average diversity between groups rather than ratios. Despite these differences in interpretation, the pattern of significance was consistent with observations from the ground beetle abundance and richness model (Figure

28). The Three Sisters garden area exhibited significantly higher mean Hill-Shannon diversity than the natural area. The natural area exterior had significantly lower diversity than the interior and middle position of the Three Sisters garden area, while the natural area interior also showed lower diversity compared to the Three Sisters garden area middle. The Hill-Shannon diversity incorporates both richness and evenness, providing a comprehensive measure of community structure. These results indicate that the Three Sisters garden area supported not only greater species richness but also more evenly distributed beetle communities.

Table 18. Results (response scale) of ground beetle Hill-Shannon diversity post-hoc pairwise comparison. The estimate represents the difference in mean Hill-Shannon diversity values between the two respective areas to the three positions within those areas and the p-value reflects whether the estimate differs from 0. The following abbreviations are used for area: 3S., Three Sisters garden area; and Nat., Natural area. The following abbreviations are used for position: Ext., Exterior; Mid., Middle; and Int., Interior. Significant p-values are shown in bold (α=0.05).

Area and Position Pairs	Estimate	Std. Error	z	p
3S Ext - Nat Ext	0.7528	0.1370	5.507	**<0.0001**
3S Ext - 3S Int	-0.1509	0.1680	-0.898	0.4300
3S Ext - Nat Int	0.6019	0.2190	2.752	**0.0144**
3S Ext - 3S Mid	-0.1797	0.1660	-1.082	0.3863
3S Ext - Nat Mid	0.5731	0.2150	2.664	**0.0163**
Nat Ext - S3 Int	-0.9037	0.2150	-4.211	**0.0002**
Nat Ext - Nat Int	-0.1509	0.1680	-0.898	0.4300
Nat Ext - 3S Mid	-0.9325	0.2150	-4.335	**0.0002**
Nat Ext - Nat Mid	-0.1797	0.1660	-1.082	0.3863
3S Int - Nat Int	0.7528	0.1370	5.507	**<0.0001**
3S Int - 3S Mid	-0.0288	0.1680	-0.171	0.8645
3S Int - Nat Mid	0.7240	0.2150	3.374	**0.0027**
Nat Int - 3S Mid	-0.7816	0.2190	-3.574	**0.0017**
Nat Int - Nat Mid	-0.0288	0.1680	-0.171	0.8645
3S Mid - Nat Mid	0.7528	0.1370	5.507	**<0.0001**

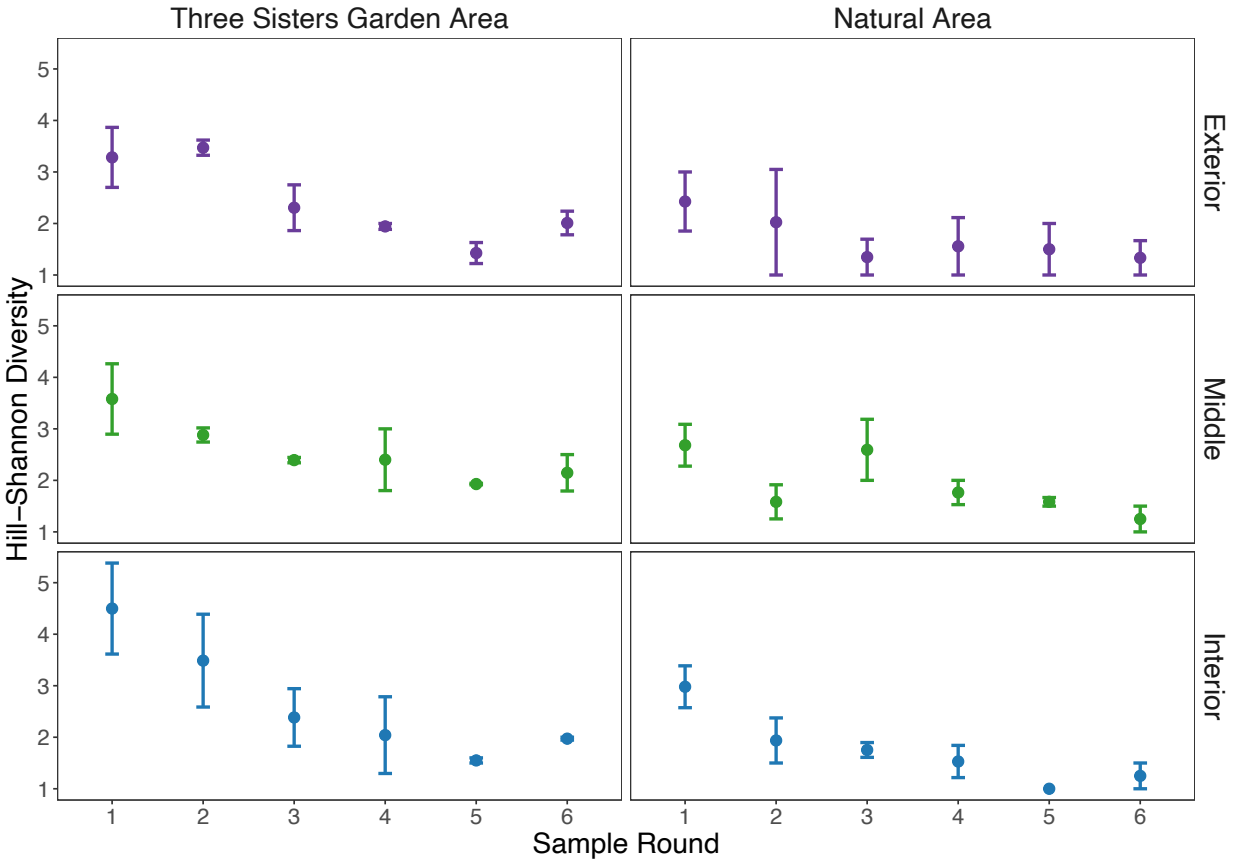

Figure 28. Effect of area and position on ground beetle Hill-Shannon diversity. Panels are arranged by area (columns: Three Sisters Garden Area and Natural Area) and position (rows: Exterior, Middle, and Interior). Points represent the average Hill-Shannon diversity of ground beetles captured per sampling event. Error bars represent the 95% confidence intervals of the mean.

Harvest Metric and Ecosystem Services Analysis

In the 2023 growing season, approximately 4,000 seeds were sown, totalling 1,960 plants across five sites (392 plants/site). Due to harvest loss from spoilage at certain sites, only three sites were used in analysis. Resulting in the final harvest being collected from 268 corn stalks (420 ears), 232 sunflower plants (5,320 heads), 275 bean stalks (995 bean pods), and 267 squash plants (1998 individual squash). The survival rate of each plant species was exceptionally high in descending order: bean (93.5%), corn (91.1%), squash (90.8%), and sunflower (78.9%).

Effect of Position

Yield

Kruskal-Wallis tests indicated no significant difference in the average corn yield (Kruskal-Wallis, χ^2=1.2449, df= 1, p=0.2645), sunflower yield (Kruskal-Wallis, χ^2=1.2656, df= 1, p=0.2606), bean yield (Kruskal-Wallis, χ^2=1.8818, df= 1, p=0.1701), or squash yield (Kruskal-Wallis, χ^2=2.3415, df= 1, p=0.1260) between the position in which the Three Sisters plantings were placed within a Three Sisters garden area (Figure 29).

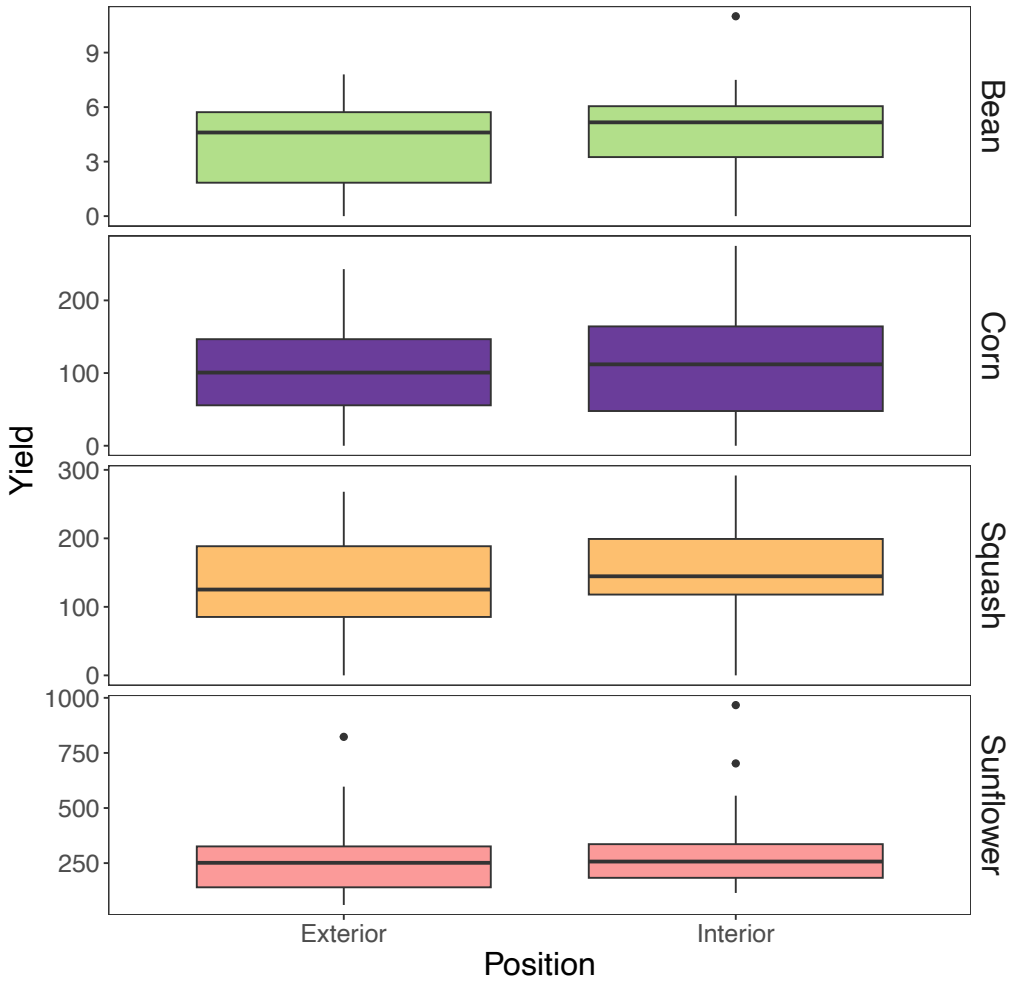

Figure 29. Effect of position on crop plant yield. Boxes represent the distribution of mean seed yield (average number of seeds per individual harvestable unit: corn ear, sunflower head, bean pod, or squash). The central line indicates the median, boxes show the interquartile range, and whiskers extend to 1.5x the interquartile range from the box, with points beyond representing outliers.

Quality

The non-parametric Kruskal-Wallis tests detected no significant differences in the average corn quality (Kruskal-Wallis, χ^2=0.8292, df= 1, p=0.3625), sunflower quality (Kruskal-Wallis, χ^2=1.0691, df= 1, p=0.3011), bean quality (Kruskal-Wallis, χ^2=0.1441, df= 1, p=0.7043), or squash quality (Kruskal-Wallis, χ^2=3.2599, df= 1, p=0.0710) among the exterior and interior positions of Three Sisters plantings within the Three Sisters garden area (Figure 30).

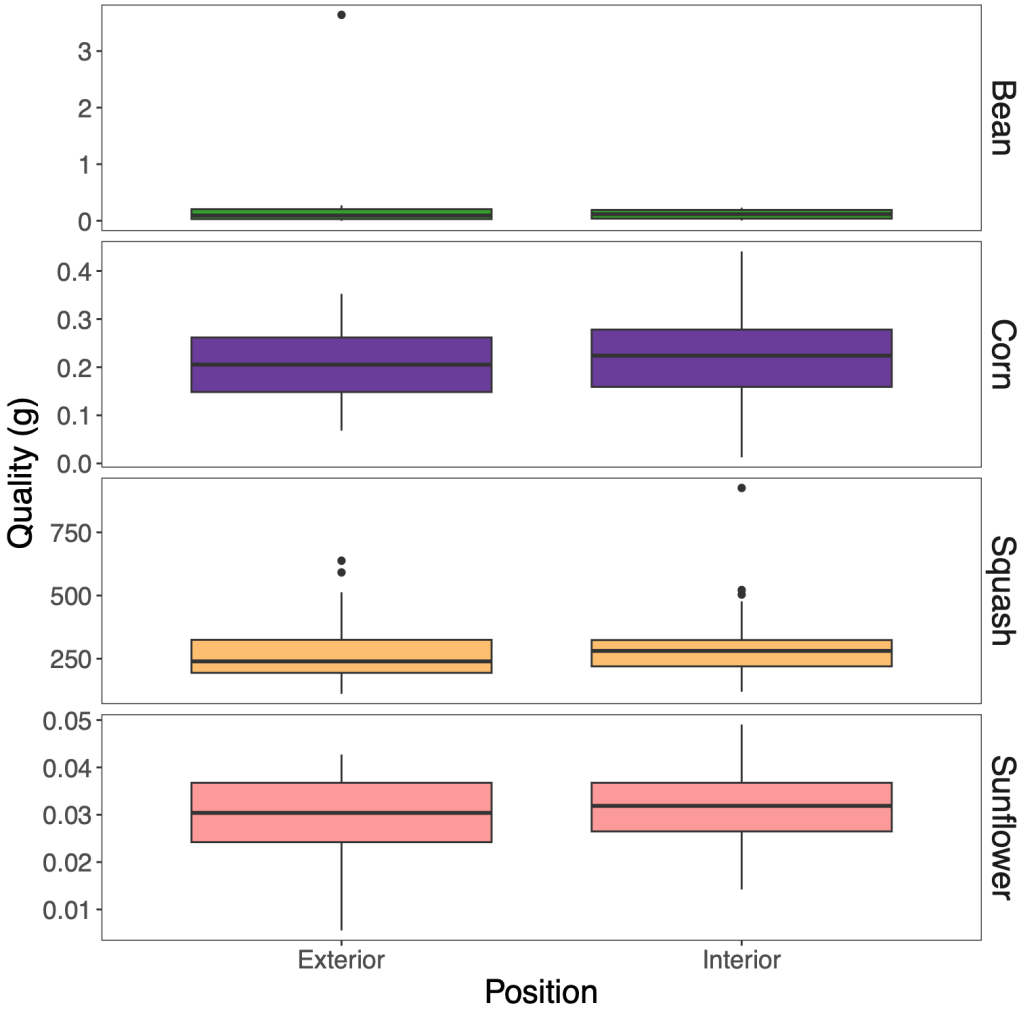

Figure 30. Effect of position on crop plant quality (g). Boxes represent the distribution of mean seed weight (g) (average weight (g) of a single seed per individual harvestable unit: corn ear, sunflower head, bean pod, squash). Squash seed quality is represented by a proxy measure based on the average weight (g) of four whole fruit. The central line indicates the median, boxes show the interquartile range, and whiskers extend to 1.5x the interquartile range from the box, with points beyond representing outliers.

Pollination Potential

The Kruskal-Wallis test found no differences in the averaged wild bee abundances (proxy for potential pollination services) among positions (Kruskal-Wallis, χ^2=1.1987, df= 1, p=0.2736) in the Three Sisters garden area (Figure 31).

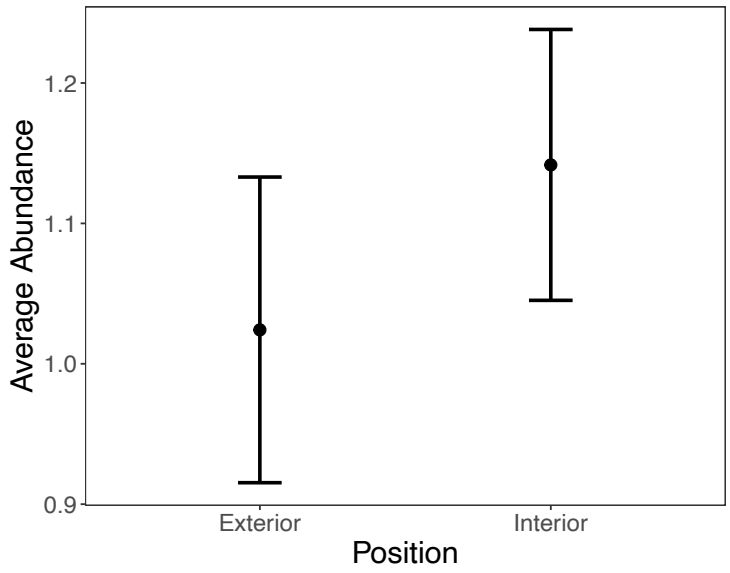

Figure 31. Effect of position on pollination potential within the Three Sisters garden area. Points represent the average abundance of wild bees captured through targeted aerial netting during the 2023 field season per position, used as a proxy for pollination. Error bars represent the 95% confidence intervals of the mean.

Pest Control Potential

Using site-level mean ground beetle abundance as a proxy for potential pest control, the Kruskal-Wallis test indicated no significant differences between positions (Kruskal-Wallis, χ^2=1.009, df= 2, p=0.6038) within the Three Sisters garden area (Figure 32).

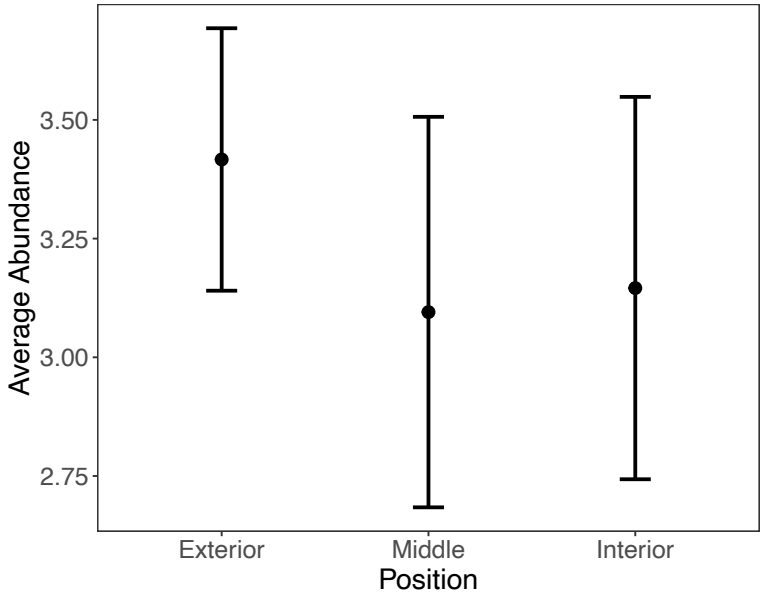

Figure 32. Effect of position on pest control potential within the Three Sisters garden area. Points represent the average abundance of ground beetles captured per position during the 2023 field season, used as a proxy for pest control. Error bars represent the 95% confidence intervals of the mean.

Effect of Study Site

As a result of both the harvest and ecological service metrics revealing no differences between spatial positions within a Three Sisters garden area, I decided to further explore whether the sites chosen were a factor influencing these metrics.

Yield

The Kruskal-Wallis tests indicated that the average corn yield (Kruskal-Wallis, χ^2=23.843, df= 2, p=6.646e^{-06}), sunflower yield (Kruskal-Wallis, χ^2=84.907, df= 2, p=2.2e^{-16}), bean yield (Kruskal-Wallis, χ^2=48.2860, df= 2, p=3.272e^{-11}), and squash yield (Kruskal-Wallis, χ^2=62.057, df= 2, p=3.346e^{-14}) strongly differ by the location in which the Three Sisters garden area was implemented. I performed a Dunn's test to determine how the study sites differed from one another (Table 19).

Yields per plant species differed strongly among study sites (Figure 33). Corn yield differed significantly among select sites, with Glenlea producing considerably more kernels than

both Brokenhead and New Liskeard, which did not differ from each other. Regarding sunflower yield, Brokenhead yielded a significantly greater number of seeds per head when compared to Glenlea and New Liskeard. Both differed from each other, with Glenlea having significantly higher yields than New Liskeard. Bean yield varied significantly across sites, driven by Glenlea's significantly lower yield relative to both Brokenhead and New Liskeard, both of which showed no difference between each other's yields. Finally, for squash yield, New Liskeard produced the highest yield followed by Glenlea and Brokenhead.

Table 19. Results of crop plant yield post-hoc pairwise comparison. The z-statistic represents the standardized difference in ranks between the two respective study sites listed and the p-value reflects whether or not that difference is significantly different from 0. Significant p-values are shown in bold (α=0.05).

	Corn Yield		**Sunflower Yield**		**Bean Yield**		**Squash Yield**	
Study Site Pairs	**z**	**p**	**z**	**p**	**z**	**p**	**z**	**p**
Brokenhead – Glenlea	-4.096	**0.0001**	2.754	**0.0059**	5.759	**<0.0001**	-4.968	**<0.0001**
Brokenhead – New Liskeard	0.137	0.8909	9.005	**<0.0001**	-0.510	0.6099	-7.778	**<0.0001**
Glenlea – New Liskeard	4.334	**<0.0001**	6.086	**<0.0001**	-6.294	**<0.0001**	-2.742	**0.0061**

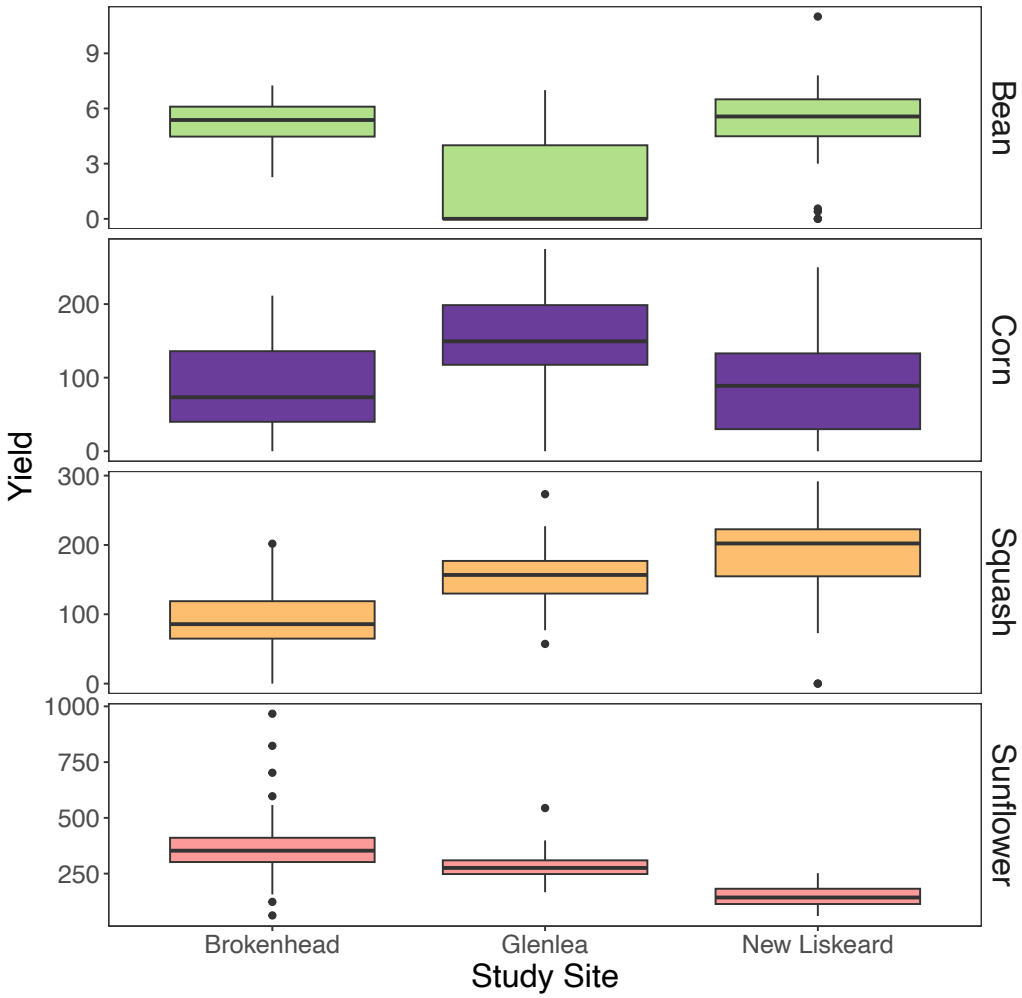

Figure 33. Effect of study site on crop plant yield. Boxes represent the distribution of mean seed yield (average number of seeds per individual harvestable unit: corn ear, sunflower head, bean pod, or squash). The central line indicates the median, boxes show the interquartile range, and whiskers extend to 1.5x the interquartile range from the box, with points beyond representing outliers.

Quality

The non-parametric Kruskal-Wallis tests detected no significant differences in the average corn quality (Kruskal-Wallis, χ^2=0.8292, df= 1, p=0.3625), sunflower quality (Kruskal-Wallis, χ^2=1.0691, df= 1, p=0.3011), bean quality (Kruskal-Wallis, χ^2=0.1441, df= 1, p=0.7043), or squash quality (Kruskal-Wallis, χ^2=3.2599, df= 1, p=0.0710) among the exterior and interior positions of Three Sisters plantings within the Three Sisters garden area.

The Kruskal-Wallis test results found that the average corn quality (Kruskal-Wallis, χ^2= 65.494, df= 2, p= $6e^{-15}$), sunflower quality (Kruskal-Wallis, χ^2= 73.501, df= 2, p= $2.2e^{-16}$), bean quality (Kruskal-Wallis, χ^2= 71.723, df= 2, p= $2.663e^{-16}$), and squash quality (Kruskal-Wallis, χ^2= 51.177, df= 2, p= $7.711e^{-12}$) strongly varied by study site location. I performed a Dunn's test to determine how the study sites differed from one another (Table 20).

The quality of seeds per plant species differed significantly among most study sites (Figure 34). In terms of kernel quality, all locations differed significantly: Glenlea produced the heaviest kernels, Brokenhead the lightest, and New Liskeard intermediate. Concerning sunflower quality, New Liskeard produced significantly heavier seeds than Glenlea and Brokenhead. The latter two being significantly different from one another in the described respective order. Regarding bean quality, New Liskeard produced significantly heavier seeds when compared to both Brokenhead and Glenlea, between which, no significant difference in quality was detected. Finally, squash quality (whole fruit mass) showed a familiar pattern where New Liskeard produced the heaviest squash, followed by Glenlea, then Brokenhead.

Table 20. Results of crop plant quality post-hoc pairwise comparison. The z-statistic represents the standardized difference in ranks between the two respective study sites listed and the p-value reflects whether or not that difference is significantly different from 0. Significant p-values are shown in bold (α=0.05).

	Corn Quality (g)		**Sunflower Quality (g)**		**Bean Quality (g)**		**Squash Quality (g)**	
Study Site Pairs	**z**	**p**	**z**	**p**	**z**	**p**	**z**	**p**
Brokenhead – Glenlea	-7.899	**<0.0001**	-3.063	**0.0022**	1.173	0.2409	-2.833	**0.0046**
Brokenhead – New Liskeard	-5.619	**<0.0001**	-8.471	**<0.0001**	-7.312	**<0.0001**	-7.088	**<0.0001**
Glenlea – New Liskeard	5.619	**0.0144**	-5.298	**<0.0001**	-6.691	**<0.0001**	-4.329	**<0.0001**

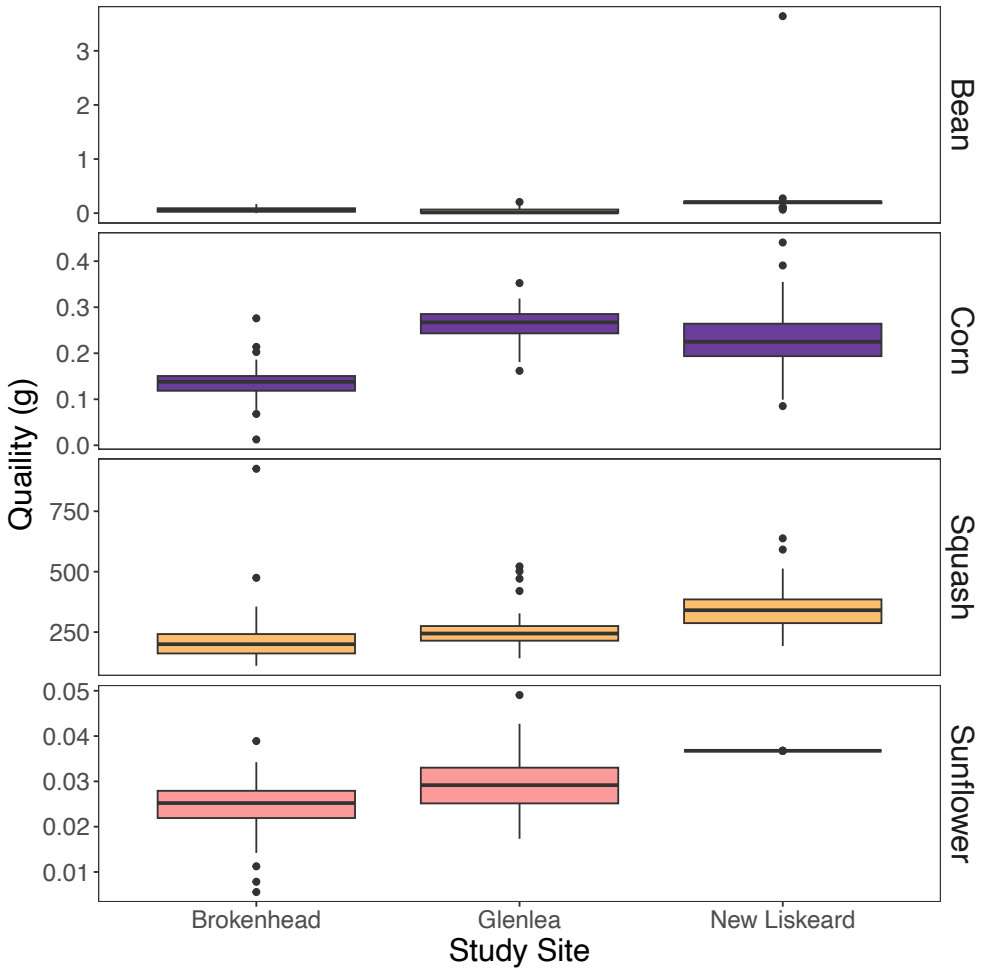

Figure 34. Effect of study site on Three Sisters crop quality (g). Boxes represent the distribution of mean seed weight (g) (average weight (g) of a single seed per individual harvestable unit: corn ear, sunflower head, bean pod, squash). Squash seed quality is represented by a proxy measure based on the average weight (g) of four whole fruit. The central line indicates the median, boxes show the interquartile range, and whiskers extend to 1.5x the interquartile range from the box, with points beyond representing outliers.

Pollination Potential

Utilizing wild bee abundance as a proxy for pollination, the Kruskal-Wallis test indicated that pollination differed by location (Kruskal-Wallis, χ^2=30.228, df= 2, p=2.729e^{-07}). I performed a Dunn's test to determine how the study sites differed from one another (Table 21). All study sites were significantly different from one another, with potential pollination (mean bee abundance) being highest in Brokenhead, followed by Glenlea, and lowest in New Liskeard (Figure 35).

Table 21. Results of pollination potential post-hoc pairwise comparison. The z-statistic represents the standardized difference in ranks between the two respective study sites listed and the p-value reflects whether or not that difference is significantly different from 0. Significant p-values are shown in bold (α=0.05).

Study Site Pairs	z	p
Brokenhead - Glenlea	2.797	**0.0077**
Brokenhead - New Liskeard	5.498	**<0.0001**
Glenlea - New Liskeard	2.701	**0.0069**

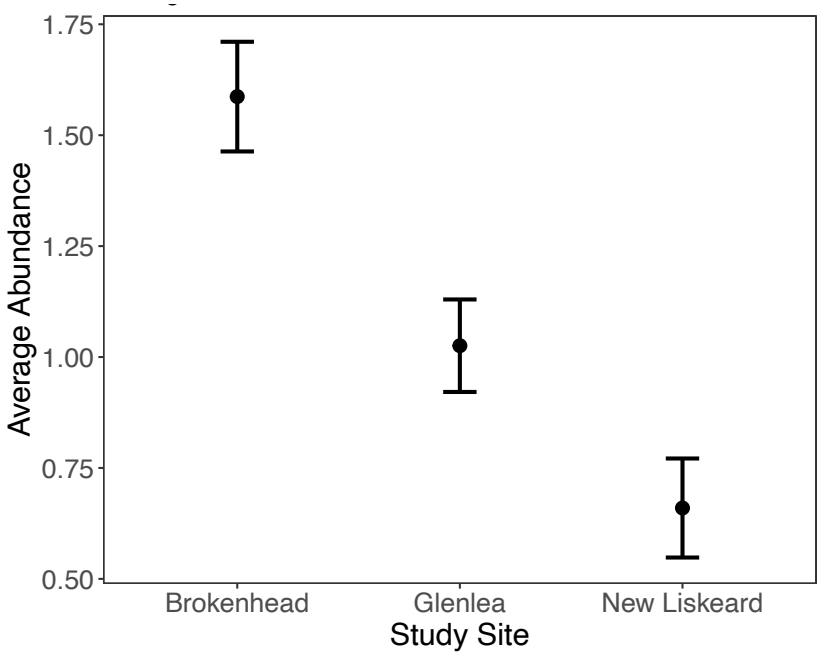

Figure 35. Effect of study site on pollination potential within the Three Sisters garden area. Points represent the average abundance of wild bees captured through targeted aerial netting during the 2023 field season per study site, used as a proxy for pollination. Error bars represent the 95% confidence intervals of the mean.

Pest Control Potential

Using the estimated number of beetles as a proxy for pest control within a Three Sisters garden, the Kruskal-Wallis test showed potential pest control services (mean beetle abundance) (Kruskal-Wallis, χ^2=1.3022, df= 1, p=0.2538) did not strongly vary between study site locations (Figure 36).

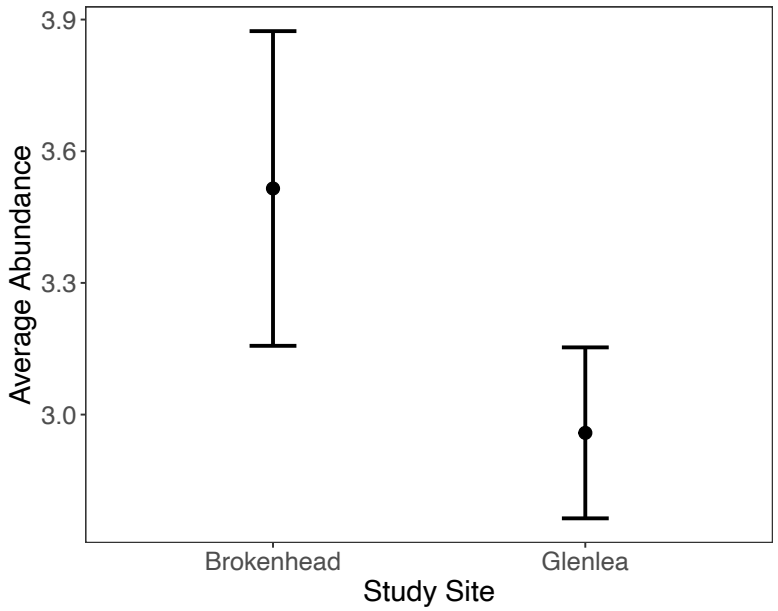

Figure 36. Effect of study site on pest control potential within the Three Sisters garden area. Points represent the average abundance of ground beetles captured per study site during the 2023 field season, used as a proxy for pest control. Error bars represent the 95% confidence intervals of the mean.

Discussion

Wild Bees

I hypothesized that the interior of a Three Sisters garden area would support greater wild bee abundance, species richness, Hill-Shannon diversity, and a higher proportion of specialist bees relative to Three Sisters plantings positioned at the edge. Contrary to this expectation, position within the Three Sisters garden did not significantly influence any of the biodiversity metrics examined. Edge effects typically emerge when there is a change in resources or structure (e.g., shade, wind exposure, floral density) between two areas; however, these results indicate that at the spatial scale of this study, the Three Sisters garden area did not function as a heterogeneous habitat with ecologically distinct edges from the surrounding landscape.

In agricultural landscapes, wild bees typically detect edge effects over distances ranging from 50–500 m (Olynyk et al. 2021), whereas the maximum distance between edge and interior

Three Sisters plantings in my study was approximately 12 m. Empirical studies consistently show that even small-bodied bees (e.g., *Halictus* or *Lasioglossum* spp.) that travel shorter distances routinely forage beyond this range, while larger taxa such as *Bombus* spp. may travel several kilometres (Gathmann and Tscharntke 2002, Westphal et al. 2006, Greenleaf et al. 2007, Osborne et al. 2008, Zurbuchen et al. 2010). An absence of edge effects may indicate nesting activity occurring directly within the Three Sisters garden area, as untilled areas in agricultural systems support nesting (Arthur et al. 2010), and traditional hand-weeding was exclusively conducted. However, even some oligolectic bees that strictly nest within sunflower fields may adjust their range to track their preferred floral hosts, such as sunflower specialist *M. agilis* with a foraging range of 740± 250 m (Gathmann and Tscharntke 2002, Greenleaf et al. 2007, Sardiñas et al. 2016). These patterns suggest that bees captured within the plot were unlikely to restrict their activity to the exterior or interior Three Sisters plantings, limiting the potential for spatial structuring of abundance, diversity, or functional composition (Westphal et al. 2006, Jha and Kremen 2013).

In addition, the relatively uniform and dense distribution of crop plants and high-rewarding floral resources across the Three Sisters garden area and individual Three Sisters plantings likely reduced fine-scale heterogeneity in foraging opportunities (Amaya-Márquez 2009, Nicholls and Hempel de Ibarra 2017). In particular, the vigorous growth of the sunflowers may have visually and nutritionally dominated the Three Sisters garden area, such that bees perceived the plot as a high-reward pseudo-monoculture within a larger landscape mosaic of distinct microhabitats rather than a four-species intercrop (Barley et al. 2022, Morán et al. 2023), masking any spatially partitioned community-level responses (Olynyk et al. 2021). Furthermore, the absence of strong edge effects may be exacerbated by the tendency for bees to forage broadly across a landscape, even in the presence of resource-rich patches (Jha and Kremen 2013, Sardiñas et al. 2016). Regarding the relationship between plant density and pollinator visitation, mixed results have been reported, with local spatial scale remaining understudied for the majority of bee taxa (Lázaro and Totland 2010, Barley et al. 2022). In a recent study, the density of conspecific plants did not affect pollinator abundance; however, *Bombus* spp. were observed to visit denser flower patches, whereas smaller-bodied bees, such as *Ceratina* spp., were found in less dense areas (Barley et al. 2022). These differences may be attributed to resource competition or direct interference, with larger bees displacing smaller species at denser floral aggregations

(Westphal et al. 2006). My observations are consistent with these findings as, *Bombus* spp. were the most numerous bees captured within the Three Sisters garden areas. Overall, these patterns reflect the complexity of foraging decisions, with plant density exerting species-specific effects and visitation rates often driven by other factors such as floral traits or ecological interactions (Barley et al. 2022).

The structure of the surrounding landscape may have also influenced the uniformity of bee activity. Study sites were located in areas bordering other agricultural experimental plots, semi-natural habitats, or a combination of both. Such landscapes appear heterogeneous, but habitat-specific conditions or characteristics can promote either more or less permeable edges within a given habitat (Duelli et al. 1990). Vegetation of a similar height is a common structural characteristic which creates a "soft edge" between natural and cultivated areas and facilitates an almost continuous transition of insect communities, especially for flying insect taxa (Duelli et al. 1990, Bailey et al. 2014, Purvis et al. 2020). The structural similarity between the Three Sisters plantings and the surrounding tall native grasses (Brokenhead) or organic agricultural plots (Glenlea), specifically in the Manitoba sites, may have been more complementary to the chosen Indigenous crop plants than expected. Therefore, creating either a "soft" or non-existent edge to the Three Sisters garden area, allowing for spillover of pollinator activity and further diluting spatial differentiation within the Three Sisters garden area (Purvis et al. 2020).

Compounding these conditions, 2023 was Canada's most destructive year for wildfires, with smoke plumes leading to sustained poor air quality across North America (Coallier et al. 2025). While the known effect of wildfire smoke on insects is limited, select species (e.g., *Apis* spp.) responses suggest impaired navigation, reduced foraging activity, and interference with olfactory cues (Liu et al. 2022, Sanderfoot et al. 2022, Kaminskaia et al. 2025, Coallier et al. 2025). If similar responses occurred in unmanaged bees, coupled with the potential of smoke altering flower phenology, we can expect a disruption in the plant-pollinator relationship, ultimately limiting the ability to detect subtle spatial patterns within a Three Sisters garden area, especially specialist pollinators (Kaminskaia et al. 2025).

Carabid Beetles

Overall, the Three Sisters garden area supported significantly higher ground carabid beetle abundance, species richness, and Hill-Shannon diversity than the adjacent natural area,

consistent with my prediction. Despite the natural area being botanically diverse, beetle communities were consistently less abundant and less diverse, suggesting that plant diversity alone does not necessarily translate to suitable habitat for ground-dwelling beetles. The dense and spatially uniform cover provided by squash likely created favourable microhabitat conditions through increased shade, moisture retention, and thermal buffering, all of which are known to enhance carabid activity and persistence (Lövei and Sunderland 1996, Holland 2002, Pleasant 2006). Early-season hand-weeding and consistently high temperatures minimize spontaneous vegetation across sites (Liebman and Mohler 2009), reducing confounding effects of weed-mediated habitat complexity, and allowing crop architecture itself to drive observed patterns. While cucurbit-derived allelochemicals may influence insect communities, their role in shaping carabid assemblages remains speculative in this context and is likely a secondary driver to physical habitat structure (Bruno et al. 2023).

Contrary to expectations, no spatial variation in ground beetle biodiversity was detected between interior, middle, and exterior positions within either plot area. This likely reflects the relatively small spatial scale of this study, at which carabid communities may have perceived both the Three Sisters garden area and natural area as structurally homogeneous habitats. Several previous studies have shown that strong edge effects on beetle assemblages often emerge in only larger areas or where sharp structural contrasts occur between habitats (Ewers and Didham 2008, Soga et al. 2013, Nguyen and Nansen 2018). Ewers and Didham (2008) found that edge influences on beetle communities persisted at distances ≥250 m for 20% of common beetle species, far exceeding the spatial dimensions of my study areas.

Moreover, insect community level responses to habitat edges could also be influenced by land-use history. Generally, boundaries between habitats have modestly dissimilar structure and environmental conditions, but the magnitude and influence of these differences depend on previous management (Magura et al. 2017, Nguyen and Nansen 2018, Kemmerling et al. 2022). Edges maintained by natural processes often support higher species richness than the interiors of a habitat, whereas agriculturally maintained edges show little difference, and responses can vary among taxa, diversity metrics, and locations (Magura et al. 2017, Kemmerling et al. 2022). Although my study sites differed in land-use history (small-scale organic agricultural field (Glenlea) vs. natural prairie habitat shaped by prior bison grazing (Brokenhead)), both were

managed with continuous low-intensity maintenance practices during the field season. This likely reduced habitat contrast at sampling area boundaries, therefore dampening edge effects and minimizing differences among interior, middle, and exterior positions.

Harvest

Contrary to my expectations, no significant differences in yield or quality were detected between the exterior and interior positions within the Three Sisters garden area. The limited scale of my study area, although designed to reflect a traditional Three Sisters garden, may have been too small to generate strong environmental gradients, particularly for above-ground interactions. Yield advantage of the Three Sisters has been successfully documented at small-scale sites in connection with below-ground root structure; however, it required greater replication of study sites (Zhang et al. 2014). The absence of spatial differentiation in harvest metrics was mirrored in the provisioning of potential pollination and biological control services. While these variables represented proxies rather than direct measures, the lack of differences implies that all positions experienced comparable access to key regulating ecosystem services. This apparent homogenization is likely a consequence of the limited spatial extent of the study area, as pollinators typically forage over distances that exceed those separating the interior and exterior positions, and the effects of edges on ground beetles often only manifest at larger spatial scales (Ewers and Didham 2008, Olynyk et al. 2021). Collectively, these results suggest that the Three Sisters garden area was spatially homogeneous, enforced by the lack of provisioning of ecosystem services, limiting the emergence of detectable differences in crop performance, and the number of study sites reduced power to detect subtle effects.

Although the original hypothesis focused on spatial position within the Three Sisters fields, the strongest patterns observed in this study with respect to harvest metrics were instead related to site-level differences. All four crops exhibited significant variation in performance among the three fields, though the magnitude and direction of these effects varied by crop and metric. Glenlea and New Liskeard, both located on long-term research stations of varying intensities, exhibited a positively coupled yield and quality responses for corn and squash, respectively, consistently outperforming Brokenhead. Although Brokenhead was located on abandoned farmland, which could have provided an overall advantage (Rey Benayas and Bullock 2012), consistent lower yield and quality may reflect site-specific environmental factors

(e.g., soil moisture accumulating from river proximity, microclimate conditions, or localized nutrient limitations) overriding expected benefits from combined conservation and intercropping approaches. The legacy effects of past crop rotations, nutrient inputs, or organic matter accumulation can enhance both biomass allocation and reproductive output (Cuddington 2011). Maize kernel abundance is commonly considered highly correlated with environmental and management factors, whereas kernel weight is a highly heritable trait; however, increased nitrogen availability has shown to override genotype effects (Ruiz et al. 2022). In this context, as corn was planted first, it is likely that the organic farming methods used in Glenlea left soils with higher levels of residual nitrogen, providing corn with an early developmental advantage over other sites (Shen et al. 2017). Squash grows best in soil with high organic matter but is highly sensitive to waterlogging due to its shallow root system (Ertek et al. 2004). Ertek et al. (2004) and Okasha et al. (2020) document a positive correlation of fruit abundance and whole fruit weight in response to specific irrigation frequencies, noting five- or seven-day intervals for optimal paralleled crop growth. The watering plan used in my study mirrored this ideal schedule. Together, these factors likely lead to consistently higher yield and quality metrics for corn and squash at these specific sites.

In contrast, sunflower exhibited site-specific divergence, with Brokenhead yielding the most but producing the lightest seeds, whereas New Liskeard yielded the least but produced the highest quality seeds. Multiple studies report sunflower seed mass as highly variable not only across multiple sites in a single year but over several years at a single location, tying differences to environmental factors (Susko and Lovett-Doust 2000, Mrdja et al. 2012). This suggests that site-level environmental conditions favouring initial reproduction at Brokenhead did not necessarily support seed filling (Mrdja et al. 2012). In addition, the decoupled harvest metrics could also be explained by wildlife presence, as the highest abundance of birds was observed at the most natural site (Brokenhead), where birds likely consumed the larger seeds prior to harvest, potentially biasing the results.

Unlike the other three crops, *P. vulgaris* yield and quality are frequently negatively associated (White and González 1990). I found similar results for the Brokenhead site, the opposite for New Liskeard (coupled increased metrics), and no differences in yield between Brokenhead and New Liskeard or quality between Brokenhead and Glenlea. There are a few

broad hypotheses to explain this outcome, such as source/sink imbalances or genetics (White and González 1990). However, it is likely that the chosen Three Sisters planting layout placed in the southern Manitoba and northern Ontario study sites might not be the most beneficial for all plants within the system (Munson-Scullin and Scullin 2005).

Examining the patterns of potential ecological service provisioning between study sites provided additional context for the observed variation in harvest metrics. Mean wild bee abundance, used as a proxy for pollination, varied significantly among sites, with Brokenhead exhibiting the greatest potential pollination benefit, followed by Glenlea and New Liskeard. Interestingly, this order follows a gradient from the least to most intensively managed sites, suggesting possible influence from more natural environmental conditions. However, this pattern was only true for sunflower yield; for the remaining crops, the relationship between pollination and yield or quality was inconsistent. In contrast, mean ground beetle abundance, used as a proxy for pest control, did not differ between sites, indicating comparable potential for biological control services. Although intercropping systems are often assumed to consistently enhance pollination and pest suppression, these results align with empirical evidence demonstrating that these services are more commonly variable (Landis et al. 2000, Norris et al. 2018). Overall, my findings regarding the effect of position and site on these metrics suggest that the Three Sisters gardens area has the potential to provision key ecosystem services, but their capacity to positively influence crop performance depends on crop-specific and site-specific environmental and management factors (Munson-Scullin and Scullin 2005).

Conclusion

My study suggests that a Three Sisters garden area is a potential strategy for integrating land sharing and sparing approaches into modern agricultural systems, as it can function as a small-scale, ecologically diverse patch while simultaneously acting as multiple traditional biodiversity-enhancing techniques (e.g., floral strips, cover crops, hedgerows). Wild bee abundance and diversity were uniform across the Three Sisters garden area, with no detectible edge effects, likely reflecting the high density and even distribution of floral resources, and the mobility of pollinators. This indicates that the garden provided a continuous foraging habitat, analogous to a bee pasture or floral strip rather than discrete spatial niches. Ground beetle communities benefited from the complementary structure of the intercrop, with dense squash

cover creating favourable microhabitats and potentially enhancing recruitment or survival of other beneficial arthropods through companion effects, seen in hedgerows and cover crops. Site-level differences in yield and harvest quality further underscored the context-dependent nature of the Three Sisters, influenced by environmental conditions, management history, and crop-specific traits. Together, these results demonstrate that Three Sisters can simultaneously provide multiple ecological and agronomic functions, with synergistic effects from interactions among crop traits, the distribution of floral and structural resources, and spatial arrangement. Future research should prioritize exploring the Three Sisters garden at a larger spatial scale, across a wide range of environmental gradients with differing plant densities, management practices, and varying surrounding landscape complexity over multiple years.

Moreover, expanding beyond observational metrics to include functional traits such as nesting guild, body size, and phenology could reveal more nuanced patterns in bee and beetle community responses to the Three Sisters structure. Such work would help determine whether edge effects and spatial structuring of bee assemblages emerge under conditions with stronger habitat contrast or greater distances between interior edges. Comparative studies across regions, soil types, and management histories could also clarify the consistency of biodiversity response and harvest outcomes, providing a broader ecological context for understanding the multi-functional roles of this traditional intercrop. Finally, collaborative work with Indigenous communities could ensure future research reflects both ecological processes and traditional knowledge, offering a more holistic understanding of how the Three Sisters system can support pollinators, beneficial arthropods, and sustainable food production.

References

Akaike, H. 1998. Information theory and an extension of the maximum likelihood principle. *In* Selected Papers of Hirotugu Akaike. *Edited by* E. Parzen, K. Tanabe, and G. Kitagawa. Springer, New York, NY. pp. 199–213. https://doi.org/10.1007/978-1-4612-1694-0_15.

Amaya-Márquez, M. 2009. Floral constancy in bees: a revision of theories and a comparison with other pollinators. Revista Colombiana de Entomología, **35**: 206–216. Available from http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120-04882009000200017&lng=en&nrm=iso.

Anderson, W.E. 1986. A guide for estimating cover. Rangelands, **8**: 236–238. Available from http://www.jstor.org/stable/3901027.

Arthur, A.D., Li, J., Henry, S., and Cunningham, S.A. 2010. Influence of woody vegetation on pollinator densities in oilseed Brassica fields in an Australian temperate landscape. Basic and Applied Ecology, **11**: 406–414. doi: 10.1016/j.baae.2010.05.001.

Bailey, S., Requier, F., Nusillard, B., Roberts, S.P.M., Potts, S.G., and Bouget, C. 2014. Distance from forest edge affects bee pollinators in oilseed rape fields. Ecology and Evolution, **4**: 370–380. doi: 10.1002/ece3.924.

Bali, G.P.K., and Kaleka, A.S. 2022. Potential reasons for insect decline. *In* Global Decline of Insects. *Edited by* H.A. Farag El-Shafie. IntechOpen, London, United Kingdom. pp. 25–42. http://dx.doi.org/10.5772/intechopen.94711.

Barley, T.A., Martinez Algarin, M.G., and Bauer, J.T. 2022. The effects of flower patch density on pollinator visitation. Environmental Entomology, **51**: 482–491. doi: 10.1093/ee/nvab143.

Barrozo, M.A.S., Mujumdar, A., and Freire, J.T. 2014. Air-drying of seeds: a review. Drying Technology, **32**: 1127–1141. doi: 10.1080/07373937.2014.915220.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Mächler, M., and Bolker, B.M. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal, **9**: 378–400. doi: 10.32614/RJ-2017-066.

Bruno, P., Arce, C.C.M., Machado, R.A.R., Besomi, G., Spescha, A., Glauser, G., Jaccard, C., Benrey, B., and Turlings, T.C.J. 2023. Sequestration of cucurbitacins from cucumber plants by *Diabrotica balteata* larvae provides little protection against biological control agents. Journal of Pest Science, **96**: 1061–1075. doi: 10.1007/s10340-022-01568-3.

Bybee-Finley, K.A., and Ryan, M.R. 2018. Advancing intercropping research and practices in industrialized agricultural landscapes. Agriculture, **8**: 1–24. doi: 10.3390/agriculture8060080.

Coallier, N., Perez, L., Franco, M.F., Cuellar, Y., and Vadnais, J. 2025. Poor air quality raises mortality in honey bees, a concern for all pollinators. Communications Earth & Environment, **6**: 1–9. doi: 10.1038/s43247-025-02082-x.

Craney, T.A., and Surles, J.G. 2002. Model-dependent variance inflation factor cutoff values. Quality Engineering, **14**: 391–403. doi: 10.1081/QEN-120001878.

Cryan, T., Musselman, O., Baumgardner, A.W., Osborn, S., Beuscher, C.J., Stehn, C., Burt, A., Chaaban, R., Lopez, A., Lewis, G., Mata, G., Rothenberg, M., Plascencia-Marquez, A., Greer, R., Stremlau, R., Murphy, H., Newman, L.R., Merrill, A., and Wadgymar, S.M. 2024. Yield, growth, and labor demands of growing maize, beans, and squash in monoculture versus the Three Sisters. Plants People Planet, **7**: 204–214. doi: 10.1002/ppp3.10576.

Cuddington, K. 2011. Legacy effects: the persistent impact of ecological interactions. Biological Theory, **6**: 203–210. doi: 10.1007/s13752-012-0027-5.

Dinno, A. 2024. dunn.test: dunn's test of multiple comparisons using rank sums. doi: 10.32614/CRAN.package.dunn.test.

Duelli, P., Studer, M., Marchand, I., and Jakob, S. 1990. Population movements of arthropods between natural and cultivated areas. Biological Conservation, **54**: 193–207. https://doi.org/10.1016/0006-3207(90)90051-P.

Ertek, A., Şensoy, S., Küçükyumuk, C., and Gedik, I. 2004. Irrigation frequency and amount affect yield components of summer squash (*Cucurbita pepo* L.). Agricultural Water Management, **67**: 63–76. doi: 10.1016/j.agwat.2003.12.004.

Ewers, R.M., and Didham, R.K. 2006. Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews, **81**: 117–142. doi: 10.1017/S1464793105006949.

Ewers, R.M., and Didham, R.K. 2008. Pervasive impact of large-scale edge effects on a beetle community. Ecology, **105**: 5426–5429. doi: 10.1073/pnas.0800460105.
Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics, **34**: 487–515. doi: 10.1146/annurev.ecolsys.34.011802.132419.
FAO. 2001. Grain crop drying, handling and storage. *In* Rural structures in the tropics. Design and development. Rome, Italy. pp. 363–411.
FAO. 2013. Genebank standards for plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy.
Fitzgerald-Moore, P., and Parai, B.J. 1996. The Green Revolution.
Gathmann, A., and Tscharntke, T. 2002. Foraging ranges of solitary bees. Journal of Animal Ecology, **71**: 757–764. doi: 10.1046/j.1365-2656.2002.00641.x.
Gibson, S.D., Onuferko, T.M., Myers, L., and Colla, S.R. 2024. Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes region. PeerJ, **12**: 1–24. doi: 10.7717/peerj.17401.
Google Earth 10.55.0.1. 2023. Manitoba, Canada. Available from https://earth.google.com/web/ [accessed 13 October 2023].
Government of Canada. 2020, December 18. Canada's plant hardiness site. Available from https://planthardiness.gc.ca/?lang=en&m=24&speciesid=1000000&s=p&phz=phz1991-2020&bc=1.
Government of Manitoba. 2007. Gardening basics. Available from https://www.gov.mb.ca/inr/pdf/pubs/mafri-gbasic.pdf.
Government of Manitoba. (n.d.). Agricultural climate of Manitoba. Available from https://www.gov.mb.ca/agriculture/weather/agricultural-climate-of-mb.html.
Government of Manitoba. (n.d.). Planting corn in a cool spring. Available from https://www.gov.mb.ca/agriculture/crops/crop-management/planting-corn-early-cool-spring.html.
Government of Manitoba. (n.d.). Sunflower production and management. Available from https://www.gov.mb.ca/agriculture/crops/crop-management/sunflowers.html.
Government of Manitoba. (n.d.). Wildlife deterrents. Available from https://www.gov.mb.ca/nrnd/fish-wildlife/wildlife/wildlife_human/wd.html.
Greenleaf, S.S., Williams, N.M., Winfree, R., and Kremen, C. 2007. Bee foraging ranges and their relationship to body size. Oecologia, **153**: 589–596. doi: 10.1007/s00442-007-0752-9.
Grevé, M.E., Marx, M.T., Eilmus, S., Ernst, M., Herrmann, J.D., Baden, C.U., and Maus, C. 2024. Insect decline—evaluation of potential drivers of a complex phenomenon. Insects, **15**: 1–37. doi: 10.3390/insects15121021.
Grof-Tisza, P., Muller, M.H., Gónzalez-Salas, R., Bustos-Segura, C., and Benrey, B. 2024. The Mesoamerican milpa agroecosystem fosters greater arthropod diversity compared to monocultures. Agriculture, Ecosystems & Environment, **372**: 1–10. doi: 10.1016/j.agee.2024.109074.
Gunathilake, D.M.C.C., Senanayaka, D.P., Adiletta, G., and Senadeera, W. 2018. Drying of agricultural crops. *In* Advances in Agricultural Machinery and Technologies. *Edited by* G. Chen. CRC Press, pp. 331–365. https://doi-org.uml.idm.oclc.org/10.1201/9781351132398.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., and De Kroon, H. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One, **12**: 1–21. doi: 10.1371/journal.pone.0185809.

Hartig, F. 2024. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. Available from https://github.com/florianhartig/dharma.

Harwood, J. 2024. The forgotten history of intercropping. Plants People Planet, **6**: 1121–1128. doi: 10.1002/ppp3.10502.

Holland, J.M. 2002. Carabid beetles: their ecology, survival and use in agroecosystems. *In* The Agroecology of Carabid Beetles. *Edited by* J.M. Holland. Intercept, pp. 1–66. Available from https://www.researchgate.net/publication/262827188.

Holliday, N.J., Floate, K.D., Cárcamo, H., Pollock, D.A., Stjernberg, A., and Roughley, R.E. 2014. Ground beetles (Coleoptera: Carabidae) of the prairie grasslands of Canada. *In* Arthropods of Canadian Grasslands: Biodiversity and Systematics Part 2. *Edited by* D.J. Giberson and Cárcamo H.A. Biological Survey of Canada, pp. 1–85. http://dx.doi.org/10.3752/9780968932179.ch1.

Horwith, B. 1985. A role for intercropping in modern agriculture. Bioscience, **35**: 286–291. Available from http://links.jstor.org/sici?sici=0006-3568%28198505%2935%3A5%3C286%3AARFIIM%3E2.0.CO%3B2-H.

Hurt, D.R. 1987. Indian agriculture in America: prehistory to the present. University Press of Kansas.

Jha, S., and Kremen, C. 2013. Resource diversity and landscape-level homogeneity drive native bee foraging. Proceedings of the National Academy of Sciences, **110**: 555–558. doi: 10.1073/pnas.1208682110.

Kaminskaia, E., Stuligross, C., and Rafferty, N.E. 2025. Pollination in a fire-prone world: Reduced solar radiation and warming alter plant–pollinator interactions. Functional Ecology, **00**: 1–12. doi: 10.1111/1365-2435.70082.

Kapayou, D.G., Herrighty, E.M., Hill, C.G., Camacho, V.C., Nair, A., Winham, D.M., and McDaniel, M.D. 2023. Reuniting the Three Sisters: collaborative science with Native growers to improve soil and community health. Agriculture and Human Values, **40**: 65–82. doi: 10.1007/s10460-022-10336-z.

Kemmerling, L.R., Rutkoski, C.E., Evans, S.E., Helms, J.A., Cordova-Ortiz, E.S., Smith, J.D., Vázquez Custodio, J.A., Vizza, C., and Haddad, N.M. 2022. Prairie strips and lower land use intensity increase biodiversity and ecosystem services. Frontiers in Ecology and Evolution, **10**: 1–18. doi: 10.3389/fevo.2022.833170.

Kimmerer, R.W. 2012. Searching for synergy: integrating traditional and scientific ecological knowledge in environmental science education. Journal of Environmental Studies and Sciences, **2**: 317–323. doi: 10.1007/s13412-012-0091-y.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. 2006. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences, **274**: 303–313. doi: 10.1098/rspb.2006.3721.

Kruse-Peeples, M. 2016, May 27. How to grow a Three Sisters garden. Available from https://www.nativeseeds.org/blogs/blog-news/how-to-grow-a-three-sisters-garden.

Kruskal, W.H., and Wallis, W.A. 1952. Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association, **47**: 583–621. https://doi.org/10.2307/2280779.

Landis, D.A., Wratten, S.D., and Gurr, G.M. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology, **45**: 175–201. https://doi.org/10.1146/annurev.ento.45.1.175.

Laurance, W.F. 2010. Habitat destruction: death by a thousand cuts. *In* Conservation Biology for All. *Edited by* N.S. Sodhi and P.R. Ehrlich. Oxford University Press, pp. 73–87. doi: 10.1093/acprof:oso/9780199554232.001.0001.

Lázaro, A., and Totland, Ø. 2010. Local floral composition and the behaviour of pollinators: attraction to and foraging within experimental patches. Ecological Entomology, **35**: 652–661. doi: 10.1111/j.1365-2311.2010.01223.x.

LaZerte, S. 2021. GLMs and non-parametric stats. [PowerPoint slides]. Study Design and Quantitative Methods for Resource and Environmental. University of Manitoba.

Lenth, R.V. 2024. emmeans: estimated marginal means, aka least-squares means. doi: 10.32614/CRAN.package.emmeans.

Lewandowski, S. 1987. Diohe'ko, the Three Sisters in Seneca life: implications for a native agriculture in the finger lakes region of New York State. Agriculture and Human Values, **4**: 76–93. doi: 10.1007/BF01530644.

Li, D. 2018. hillR: taxonomic, functional, and phylogenetic diversity and similarity through Hill Numbers. Journal of Open Source Software, **3**: 1041. doi: 10.21105/joss.01041.

Liao, H., Zhou, Z., Liu, Y., Luo, Y., Zhang, C., Feng, Y., Shu, Y., and Wang, J. 2024. 'The Three Sisters' (maize/bean/squash) polyculture promotes the direct and indirect defences of maize against herbivores. European Journal of Agronomy, **155**: 1–11. doi: 10.1016/j.eja.2024.127118.

Liebman, M., and Mohler, C.L. 2009. Weeds and the soil environment. *In* Ecological Management of Agricultural Weeds. Cambridge University Press, pp. 210–268. doi: 10.1017/cbo9780511541810.006.

Lister, B.C., and Garcia, A. 2018. Climate-driven declines in arthropod abundance restructure a rainforest food web. Proceedings of the National Academy of Sciences, **115**: E10397–E10406. doi: 10.1073/pnas.1722477115.

Liu, Y., Francis, R.A., Wooster, M.J., Grosvenor, M.J., Yan, S., and Roberts, G. 2022. Systematic mapping and review of landscape fire smoke (LFS) exposure impacts on insects. Environmental Entomology, **51**: 871–884. doi: 10.1093/ee/nvac069.

Lövei, G.L., and Sunderland, K.D. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology, **41**: 231–256. https://doi.org/10.1146/annurev.en.41.010196.001311.

Lüdecke, D., Ben-Shachar, M., Patil, I., Wiernik, B., Bacher, E., Thériault, R., and Makowski, D. 2022. easystats: an R framework for easy statistical modeling, visualization, and reporting. https://doi.org/10.32614/CRAN.package.easystats.

Magura, T., Lövei, G.L., and Tóthmérész, B. 2017. Edge responses are different in edges under natural versus anthropogenic influence: a meta-analysis using ground beetles. Ecology and Evolution, **7**: 1009–1017. doi: 10.1002/ece3.2722.

Ministry of Agriculture Food and Agribusiness. 2022. Climate zones and planting dates for vegetables in Ontario. Available from https://www.ontario.ca/page/climate-zones-and-planting-dates-vegetables-ontario.

Morán, A., Lihoreau, M., Escudero, A.P., and Gautrais, J. 2023. Modeling bee movement shows how a perceptual masking effect can influence flower discovery. PLOS Computational Biology, **19**: 1–21. doi: 10.1371/journal.pcbi.1010558.

Mrdja, J., Crnobarac, J., Radić, V., and Miklič, V. 2012. Sunflower seed quality and yield in relation to environmental conditions of production region. Helia, **35**: 123–134. doi: 10.2298/HEL1257123M.

Munson-Scullin, W., and Scullin, M. 2005. Potential productivity of midwestern native American gardens. Plains Anthropologist, **50**: 9–21. doi: 10.1179/pan.2005.004.

Nerson, H. 2005. Effects of fruit shape and plant density on seed yield and quality of squash. Scientia Horticulturae, **105**: 293–304. doi: 10.1016/j.scienta.2004.11.008.

NeSmith, D.S. 1993. Transplant age influences summer squash growth and yield. HortScience, **28**: 618–620. Available from https://journals.ashs.org.

Nguyen, H.D.D., and Nansen, C. 2018. Edge-biased distributions of insects. a review. Agronomy for Sustainable Development, **38**: 1–13. doi: 10.1007/s13593-018-0488-4.

Nicholls, E., and Hempel de Ibarra, N. 2017. Assessment of pollen rewards by foraging bees. Functional Ecology, **31**: 76–87. doi: 10.1111/1365-2435.12778.

Noriega, J.A., Hortal, J., Azcárate, F.M., Berg, M.P., Bonada, N., Briones, M.J.I., Del Toro, I., Goulson, D., Ibanez, S., Landis, D.A., Moretti, M., Potts, S.G., Slade, E.M., Stout, J.C., Ulyshen, M.D., Wackers, F.L., Woodcock, B.A., and Santos, A.M.C. 2018. Research trends in ecosystem services provided by insects. Basic and Applied Ecology, **26**: 8–23. doi: 10.1016/j.baae.2017.09.006.

Norris, S.L., Blackshaw, R.P., Critchley, C.N.R., Dunn, R.M., Smith, K.E., Williams, J., Randall, N.P., and Murray, P.J. 2018. Intercropping flowering plants in maize systems increases pollinator diversity. Agricultural and Forest Entomology, **20**: 246–254. doi: 10.1111/afe.12251.

Ogle, D.H., Doll, J.C., Wheeler, A.P., and Dinno, A. 2025. FSA: simple fisheries stock assessment methods. Available from https://fishr-core-team.github.io/FSA/.

Okasha, E.M., Hashem, F.A., and El-Metwally, I.M. 2020. Effect of irrigation system and irrigation intervals on the water application efficiency, growth, yield, water productivity and quality of squash under clay soil conditions. Plant Archives, **20**: 3266–3275.

Olynyk, M., Westwood, A.R., and Koper, N. 2021. Effects of natural habitat loss and edge effects on wild bees and pollination services in remnant prairies. Environmental Entomology, **50**: 732–743. doi: 10.1093/ee/nvaa186.

Ontario Ministry of Agriculture Food and Rural Affairs. 2022. Agronomy guide for field crops - publication 811. *Edited by* C. Brown, J. Follings, M. Moran, and B. Rosser. Ministry of Agriculture, Food and Rural Affairs, Toronto, Canada. Available from https://www.ontario.ca/files/2022-10/omafra-agronomy-guide-for-field-crops-en-2022-10-13.pdf.

Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J., and Sanderson, R.A. 2008. Bumblebee flight distances in relation to the forage landscape. Journal of Animal Ecology, **77**: 406–415. doi: 10.1111/j.1365-2656.2007.01333.x.

Paris, H.S., and Nerson, H. 2003. Seed dimensions in the subspecies and cultivar-groups of *Cucurbita pepo*. Genetic Resources and Crop Evolution, **50**: 615–625. https://doi.org/10.1023/A:1024464831595.

Pleasant, J.M. 2006. The science behind the Three Sisters mound system: an agronomic assessment of an Indigenous agricultural system in the northeast. *In* Histories of Maize: multidisciplinary approaches to the prehistory, linguistics, biogeography, domestication, and evolution of maize. *Edited by* J. Staller, R. Tykot, and B. Benz. Left Coast Press, New York. pp. 529–537. Available from https://www.ebsco.com/terms-of-use.

Pleasant, J.M. 2016. Food yields and nutrient analyses of the Three Sisters: a Haudenosaunee cropping system. Ethnobiology Letters, **7**: 87–98. doi: 10.14237/ebl.7.1.2016.721.
Pleasant, J.M., and Burt, R.F. 2010. Estimating productivity of traditional Iroquoian cropping systems from field experiments and historical literature. Journal of Ethnobiology, **30**: 52–79. https://doi.org/10.2993/0278-0771-30.1.52.
Purvis, E.E.N., Meehan, M.L., and Lindo, Z. 2020. Agricultural field margins provide food and nesting resources to bumble bees (*Bombus* spp., Hymenoptera: Apidae) in southwestern Ontario, Canada. Insect Conservation and Diversity, **13**: 219–228. doi: 10.1111/icad.12381.
R Core Team. 2024. R: a language and environment for statistical computing. Available from https://www.r-project.org/.
Rainio, J., and Niemela, J. 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity and Conservation, **12**: 487–506. https://doi.org/10.1023/a:1022412617568.
Rey Benayas, J.M., and Bullock, J.M. 2012. Restoration of biodiversity and ecosystem services on agricultural land. Ecosystems, **15**: 883–899. doi: 10.1007/s10021-012-9552-0.
Ruiz, A., Archontoulis, S. V., and Borrás, L. 2022. Kernel weight relevance in maize grain yield response to nitrogen fertilization. Field Crops Research, **286**: 1–10. doi: 10.1016/j.fcr.2022.108631.
Sanderfoot, O. V., Bassing, S.B., Brusa, J.L., Emmet, R.L., Gillman, S.J., Swift, K., and Gardner, B. 2022. A review of the effects of wildfire smoke on the health and behavior of wildlife. Environmental Research Letters, **16**: 1–24. doi: 10.1088/1748-9326/ac30f6.
Sardiñas, H.S., Tom, K., Ponisio, L.C., Rominger, A., and Kremen, C. 2016. Sunflower (*Helianthus annuus*) pollination in California's Central Valley is limited by native bee nest site location. Ecological Applications, **26**: 438–447. doi: 10.1890/15-0033.
Schindler, M., Diestelhorst, O., Härtel, S., Saure, C., Schanowski, A., and Schwenninger, H.R. 2013. Monitoring agricultural ecosystems by using wild bees as environmental indicators. BioRisk, **8**: 53–71. doi: 10.3897/biorisk.8.3600.
Sharma, I.P., Kanta, C., Dwivedi, T., and Rani, R. 2020. Indigenous agricultural practices: a supreme key to maintaining biodiversity. *In* Microbiological Advancements for Higher Altitude Agro-Ecosystems & Sustainability. Springer Singapore, Singapore. pp. 91–112. doi: 10.1007/978-981-15-1902-4_6.
Shen, L., Huang, Y., and Li, T. 2017. Top-grain filling characteristics at an early stage of maize (*Zea mays* L.) with different nitrogen use efficiencies. Journal of Integrative Agriculture, **16**: 626–639. doi: 10.1016/S2095-3119(16)61457-0.
Shorthouse, D.P. 2010. SimpleMappr, an online tool to produce publication-quality point maps. Available from https://www.simplemappr.net/ [accessed 13 October 2023].
Soga, M., Kanno, N., Yamaura, Y., and Koike, S. 2013. Patch size determines the strength of edge effects on carabid beetle assemblages in urban remnant forests. Journal of Insect Conservation, **17**: 421–428. doi: 10.1007/s10841-012-9524-x.
Susko, D.J., and Lovett-Doust, L. 2000. Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). American Journal of Botany, **87**: 56–66. doi: 10.2307/2656685.
Terry, P.A., Pearson, D., and Holder, G. 2020. Sustainable agriculture: nutrition of Indigenous American 3 sisters garden compared to monoculture corn production and a cool old squash. Journal of Scientific Research and Reports, **26**: 99–108. doi: 10.9734/jsrr/2020/v26i830299.

Tilman, D. 1999. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences, **96**: 5995–6000. https://doi.org/10.1073/pnas.96.11.5995.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., and Thies, C. 2005. Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. Ecology Letters, **8**: 857–874. doi: 10.1111/j.1461-0248.2005.00782.x.

Uddin, Z., Suppakul, P., and Boonsupthip, W. 2016. Effect of air temperature and velocity on moisture diffusivity in relation to physical and sensory quality of dried pumpkin seeds. Drying Technology, **34**: 1423–1433. doi: 10.1080/07373937.2015.1119840.

Wardhaugh, C.W. 2015. How many species of arthropods visit flowers? Arthropod-Plant Interactions, **9**: 547–565. doi: 10.1007/s11829-015-9398-4.

Warren, D.L., and Seifert, S.N. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecological Applications, **21**: 335–342. doi: 10.1890/10-1171.1.

Westphal, C., Steffan-Dewenter, I., and Tscharntke, T. 2006. Bumblebees experience landscapes at different spatial scales: possible implications for coexistence. Oecologia, **149**: 289–300. doi: 10.1007/s00442-006-0448-6.

White, J.W., and González, A. 1990. Characterization of the negative association between seed yield and seed size among genotypes of common bean. Field Crops Research, **23**: 159–175. doi: 10.1016/0378-4290(90)90052-D.

Willey, R.W. 1979. Intercropping - its importance and research needs. part 1. competition and yield advantages. Field Crop Abstracts, **32**: 1–10.

Zhang, C., Postma, J.A., York, L.M., and Lynch, J.P. 2014. Root foraging elicits niche complementarity-dependent yield advantage in the ancient “Three Sisters” (maize/bean/squash) polyculture. Annals of Botany, **114**: 1719–1733. doi: 10.1093/aob/mcu191.

Zhang, W., Ricketts, T.H., Kremen, C., Carney, K., and Swinton, S.M. 2007. Ecosystem services and dis-services to agriculture. Ecological Economics, **64**: 253–260. doi: 10.1016/j.ecolecon.2007.02.024.

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., and Dorn, S. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. Biological Conservation, **143**: 669–676. doi: 10.1016/j.biocon.2009.12.003.

Supplementary Tables and Figures

Table S 8. A visual outline of a traditional Three Sisters planting schedule compared to the adjusted and actual observed schedule followed for the 2023 field season. The traditional Three Sisters timeline was based on a combination of commonly referenced guidelines for growing the Haudenosaunee Three Sisters and select ethnobotanical literature due to lack of formal records. A solid-coloured bar represents the respective activity was performed directly in the field. A cross-hatched bar represents the respective activity was performed indoors.

		Anticipated Last Frost Dates: May 21 - May 31							
Planting Schedule	**Activity**	**May 21-27**	**May 28-June 3**	**June 4-10**	**June 11-17**	**June 18-24**	**June 25-July 1**	**June 26-July 2**	**July 3-9**
Traditional Three Sisters	Plant Corn								
	Plant Bean								
	Plant Squash								
Adjusted Three Sisters	Plant Corn								
	Plant Sunflower								
	Plant Bean								
	Plant Squash								
	Transplant Squash								
Observed Three Sisters	Plant Corn								
	Plant Sunflower								
	Plant Bean								
	Plant Squash								
	Transplant Squash								

Table S 9. 2023 Field season study sites and corresponding dates of insect sampling procedures. Table does not include dates of sampling preformed outside the planned sampling window. Dashes (–) indicate no sampling was performed. NA indicates sampling was performed but were unable to be processed.

	Dates sampled				
Study Site	**June**	**July**	**August**	**September**	**Total Sampling Rounds**
Bee Bowls and Blue Vane Traps					
Brokenhead	30	–	04	01	3
Glenlea	–	03	03	30	3
New Liskeard	–	25	16	01	3
Sudbury	–	31	01	03	3
Emo	NA	NA	NA	NA	NA
Targeted Aerial Netting					
Brokenhead	–	–	11, 14, 30	01, 06	5
Glenlea	–	–	10, 14, 24, 28	07	5
New Liskeard	–	–	15, 23	06	3
Sudbury	–	–	08, 21	03	3
Emo	NA	NA	NA	NA	NA
Pitfall Traps					
Brokenhead	30-07(Jul)	14-21, 28-04(Aug)	11-18, 25-01(Sept)	06-13	6
Glenlea	26-03(Jul)	10-17, 24-31	07-14, 21-28	05-12	6
New Liskeard	–	10-17, 25-01(Aug)	09-16, 23-30	–	4
Sudbury	–	10-17, 24-31	07-14, 21-28	05-12	5
Emo	NA	NA	NA	NA	NA

Table S 10. Results of collinearity diagnostics for wild bee and ground beetle abundance and species richness models. Variance inflation factor (VIF) values >5 are in bold. Dashes (–) indicate that the variable was not included in the model.

		Response Variable	
		Abundance	Species Richness
Insect Taxa	**Predictor Variables**	**VIF**	**VIF**
Wild Bee	Position	1.00	–
	Daily Average Temperature (°C)	**419.47**	–
	Daily Maximum Temperature (°C)	**265.71**	–
	Daily Minimum Temperature (°C)	**51.36**	–
	Daily Average Wind Speed (km/h)	**105.75**	–
	Daily Maximum Wind Speed (km/h)	**147.79**	–
	Daily Minimum Wind Speed (km/h)	3.19	–
	Floral Richness	**11.78**	–
Carabid Beetle	Area	1.00	1.00
	Position	1.00	1.00
	Weekly Average Temperature (°C)	**29.75**	**28.78**
	Weekly Maximum Temperature (°C)	**8.28**	**7.31**
	Weekly Minimum Temperature (°C)	**17.83**	**19.57**
	Bare Ground (%)	1.70	1.75
	Grass Cover (%)	2.14	2.04

Table S 11. List of wild bee species caught during the 2023 field season. Sorted alphabetically by genus and species. Abundances presented by sampling method. List does not include excluded data. The following abbreviations are used for diet breadth (lecty): oligo., oligolectic; and poly., polylectic. No lecty information is provided for parasitic bee species. The following abbreviations are used for sampling method: BB., bee bowls; BVT., blue vane traps; and TAN., targeted aerial netting. Rare records for Manitoba are in bold, and for Canada include an asterisk.

Species	Lecty	Floral Host	Authority	BB	BVT	TAN	Total
Agapostemon (Agapostemon) texanus	poly.		Cresson 1872	0	1	0	1
Anthophora (Clisodon) terminalis	poly.		Cresson 1869	0	4	0	4
Bombus (Pyrobombus) bimaculatus	poly.		Cresson 1863	0	0	2	2
Bombus (Subterraneobombus) borealis	poly.		Kirby 1837	3	48	15	66
Bombus (Thoracobombus) fervidus	poly.		(Fabricius 1798)	1	1	1	3
Bombus (Cullumanobombus) griseocollis	poly.		(De Geer 1773)	2	5	255	262
Bombus (Pyrobombus) huntii	poly.		Greene 1860	0	0	1	1
Bombus (Pyrobombus) impatiens	poly.		Cresson 1863	0	3	92	95
Bombus (Bombias) nevadensis	poly.		Cresson 1874	0	0	2	2
Bombus (Thoracobombus) pensylvanicus	poly.		(De Geer 1773)	0	0	1	1
Bombus (Cullumanobombus) rufocinctus	poly.		Cresson 1863	0	11	24	35
Bombus (Pyrobombus) sandersoni	poly.		Franklin 1913	0	0	2	2
Bombus (Pyrobombus) ternarius	poly.		Say 1837	1	20	17	38
Bombus (Bombus) terricola	poly.		Kirby 1837	0	0	1	1
Bombus (Pyrobombus) vagans	poly.		Smith 1854	1	10	179	190
Lasioglossum (Lasioglossum) athabascense	poly.		(Sandhouse 1933)	0	1	0	1
Lasioglossum (Lasioglossum) leucozonium	poly.		(Schrank 1781)	0	2	0	2
Lasioglossum (Dialictus) occidentale	poly.		(Crawford 1902)	0	1	0	1
Lasioglossum (Dialictus) perpunctatum	poly.		(Ellis 1913)	0	1	0	1
Lasioglossum (Leuchalictus) zonulus	poly.		(Smith 1848)	1	4	0	5
Megachile (Megachile) inermis	poly.		Provancher 1888	1	0	5	6
Megachile (Xanthosarus) latimanus	poly.		Say 1823	0	0	6	6
Melissodes (Eumelissodes) agilis	oligo.	*Helianthus*	Cresson 1878	0	20	65	85

Melissodes (Eumelissodes) confusus	oligo.	Compositae	Cresson 1878	0	5	1	6
Melissodes (Eumelissodes) druriellus	oligo.	*Solidago, Symphyotrichum*	(Kirby 1802)	0	3	0	3
Melissodes (Eumelissodes) trinodis	oligo.	*Helianthus* and related Compositae	Robertson 1901	0	2	26	28
Protandrena (Pterosarus) perlaevis	oligo.	*Helianthus*	(Cresson 1872)	0	0	5	5
Triepeolus helianthi	NA		(Robertson 1897)	0	0	1	1
			Total Specimen	**10**	**142**	**701**	**853**
			Total Genera	**3**	**5**	**5**	**8**
			Total Species	**7**	**18**	**20**	**28**

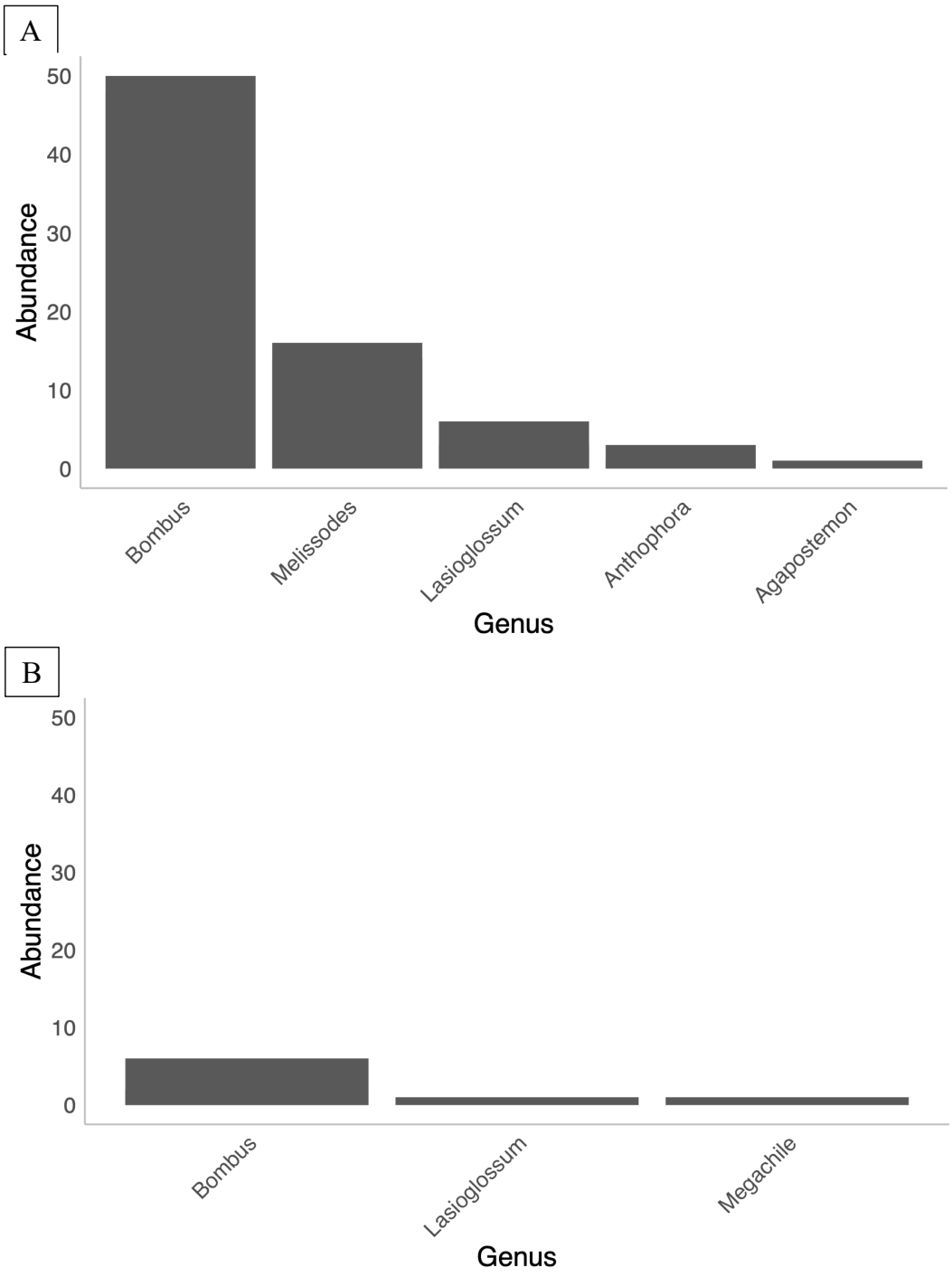

Figure S 4. Wild bee abundance by genus per passive sampling method across both study areas and all sites. Graphs do not include excluded data. (A) Bee genera and abundances associated with BVT captures. *Agapostemon* was the only genera with 1 individual. (B) Bee genera and abundances associated with bee bowl captures. *Lasioglossum* and *Megachilie* had 1 individual.

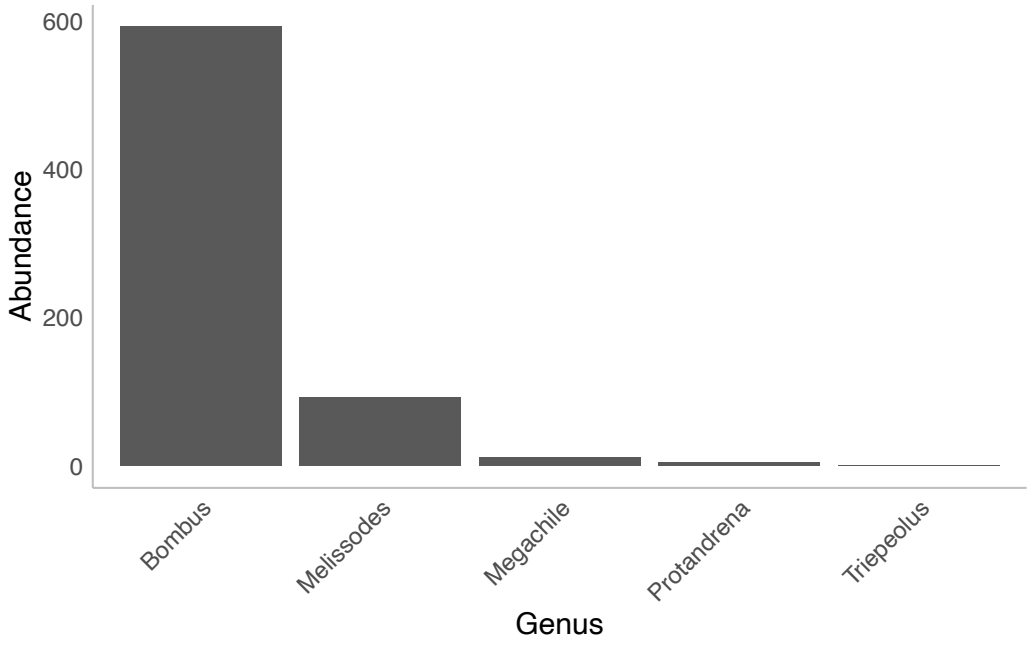

Figure S 5. Wild bee abundance by genus caught through targeted aerial netting within the Three Sisters garden area across all sites. Graphs do not include excluded data. *Triepeolus* was the only genera with 1 individual.

Table S 12. List of wild bee species caught through bee bowls and blue vane traps (BVTs) during the 2023 field season. Sorted alphabetically by genus and species. Abundances presented by sampling area. List does not include excluded data. The following abbreviations are used for diet breadth (lecty): oligo., oligolectic; and poly., polylectic. No lecty information is provided for parasitic bee species. The following abbreviations are used for study area: 3S., Three Sisters garden area; and Nat., Natural area. Rare records for Manitoba are in bold.

				Bee Bowls		BVTs		
Species	**Lecty**	**Floral Host**	**Authority**	**3S**	**Nat**	**3S**	**Nat**	**Total**
Agapostemon (Agapostemon) texanus	poly.		Cresson 1872	0	0	0	1	1
Anthophora (Clisodon) terminalis	poly.		Cresson 1869	0	0	1	3	4
Bombus (Subterraneobombus) borealis	poly.		Kirby 1837	2	1	31	17	51
Bombus (Thoracobombus) fervidus	poly.		(Fabricius 1798)	1	0	1	0	2
Bombus (Cullumanobombus) griseocollis	poly.		(De Geer 1773)	2	0	2	3	7
Bombus (Pyrobombus) impatiens	poly.		Cresson 1863	0	0	3	0	3
Bombus (Cullumanobombus) rufocinctus	poly.		Cresson 1863	0	0	2	9	11
Bombus (Pyrobombus) ternarius	poly.		Say 1837	0	1	7	13	21
Bombus (Pyrobombus) vagans	poly.		Smith 1854	1	0	4	6	11
Lasioglossum (Lasioglossum) athabascense	poly.		(Sandhouse 1933)	0	0	1	0	1
Lasioglossum (Lasioglossum) leucozonium	poly.		(Schrank 1781)	0	0	2	0	2
Lasioglossum (Dialictus) occidentale	poly.		(Crawford 1902)	0	0	1	0	1
Lasioglossum (Dialictus) perpunctatum	poly.		(Ellis 1913)	0	0	0	1	1
Lasioglossum (Leuchalictus) zonulus	poly.		(Smith 1848)	0	1	2	2	5
Megachile (Megachile) inermis	poly.		Provancher 1888	0	1	0	0	1
Melissodes (Eumelissodes) agilis	oligo.	*Helianthus*	Cresson 1878	0	0	12	8	20
Melissodes (Eumelissodes) confusus	oligo.	Compositae	Cresson 1878	0	0	1	4	5
Melissodes (Eumelissodes) druriellus	oligo.	*Solidago, Symphyotrichum*	(Kirby 1802)	0	0	2	1	3
Melissodes (Eumelissodes) trinodis	oligo.	*Helianthus* and related Compositae	Robertson 1901	0	0	1	1	2

Total Specimen	**6**	**4**	**73**	**69**	**152**
Total Genera	**1**	**3**	**4**	**5**	**6**
Total Species	**4**	**4**	**16**	**13**	**19**

Table S 13. List of wild bee species caught through targeted aerial netting within the Three Sisters garden area during the 2023 field season. Sorted alphabetically by genus and species. Abundances presented by position. List does not include excluded data. The following abbreviations are used for diet breadth (lecty): oligo., oligolectic; and poly., polylectic. No lecty information is provided for parasitic bee species. The following abbreviations are used for position: Ext., Exterior; and Int., Interior. Rare records for Manitoba are in bold.

				Position		
Species	**Lecty**	**Floral Host**	**Authority**	**Ext**	**Int**	**Total**
Bombus (Pyrobombus) bimaculatus	poly.		Cresson 1863	2	0	2
Bombus (Subterraneobombus) borealis	poly.		Kirby 1837	5	10	15
Bombus (Thoracobombus) fervidus	poly.		(Fabricius 1798)	0	1	1
Bombus (Cullumanobombus) griseocollis	poly.		(De Geer 1773)	128	127	255
Bombus (Pyrobombus) huntii	poly.		Greene 1860	0	1	1
Bombus (Pyrobombus) impatiens	poly.		Cresson 1863	40	52	92
Bombus (Bombias) nevadensis	poly.		Cresson 1874	0	2	2
Bombus (Thoracobombus) pensylvanicus	poly.		(De Geer 1773)	1	0	1
Bombus (Cullumanobombus) rufocinctus	poly.		Cresson 1863	12	12	24
Bombus (Pyrobombus) sandersoni	poly.		Franklin 1913	1	1	2
Bombus (Pyrobombus) ternarius	poly.		Say 1837	7	10	17
Bombus (Bombus) terricola	poly.		Kirby 1837	0	1	1
Bombus (Pyrobombus) vagans	poly.		Smith 1854	83	96	179
Megachile (Megachile) inermis	poly.		Provancher 1888	0	5	5
Megachile (Xanthosarus) latimanus	poly.		Say 1823	3	3	6
Melissodes (Eumelissodes) agilis	oligo.	*Helianthus*	Cresson 1878	33	32	65
Melissodes (Eumelissodes) confusus	oligo.	Compositae	Cresson 1878	0	1	1

Melissodes (Eumelissodes) trinodis	oligo.	*Helianthus*	Robertson 1901	8	18	26
Protandrena (Pterosarus) perlaevis	oligo.	*Helianthus*	(Cockerell 1896)	2	3	5
Triepeolus helianthi	NA		(Robertson 1897)	1	0	1
			Total Specimen	**326**	**375**	**701**
			Total Genera	**5**	**4**	**5**
			Total Species	**14**	**17**	**20**

Table S 14. List of carabid beetle species caught through pitfall trap during the 2023 field season. Sorted alphabetically by genus and species. Abundances presented by position and sampling area. List does not include excluded data. The following abbreviations are used for position: Ext., Exterior; Mid., Middle; and Int., Interior.

		Three Sisters Garden Area			**Natural Area**			
Species	**Authority**	**Ext**	**Mid**	**Int**	**Ext**	**Mid**	**Int**	**Total**
Agonum (Olisares) corvus	(LeConte 1860)	0	0	1	0	1	0	2
Agonum (Olisares) cupreum	Dejean 1831	0	0	0	2	2	1	5
Agonum (Olisares) cupripenne	(Say 1823)	0	0	0	1	0	0	1
Agonum (Agonum) placidum	(Say 1823)	3	5	2	0	2	1	13
Agonum (Olisares) trigeminum	Lindroth 1954	0	0	0	1	0	0	1
Amara (Curtonotus) carinata	LeConte 1848	1	1	6	0	0	0	8
Amara (Amara) coelebs	Hayward 1908	0	0	0	0	1	0	1
Amara (Amara) confusa	LeConte 1848	3	4	4	4	1	1	17
Amara (Amara) cupreolata	Putzeys 1866	1	0	0	0	5	1	7
Amara (Amarocelia) farcta	LeConte 1855	3	1	4	2	0	3	13
Amara (Amara) impuncticollis	(Say 1823)	5	8	0	1	1	0	15
Amara (Curtonotus) lacustris	LeConte 1855	3	1	4	0	2	1	11
Amara (Amara) littoralis	Mannerheim 1843	6	6	7	1	1	2	23
Amara (Percosia) obesa	(Say 1823)	2	2	2	3	3	3	15

Amara (Celia) pseudobrunnea	Lindroth 1968	0	0	0	2	1	0	3
Amara (Curtonotus) torrida	(Panzer 1797)	5	1	2	3	2	0	13
Anchomenus quadratum	(LeConte 1854)	1	0	0	0	0	0	1
Bembidion (Trepanedoris) canadianum	Casey 1924	0	0	0	1	0	1	2
Bembidion (Furcacampa) mimus	Hayward 1897	10	19	29	0	0	0	58
Bembidion nudipenne	Lindroth 1963	6	3	4	0	0	1	14
Bembidion (Bembidion) quadrimaculatum	Say 1823	7	6	5	0	0	0	18
Bembidion (Furcacampa) timidum	(LeConte 1848)	0	4	0	0	0	0	4
Calosoma (Chrysostigma) calidum	(Fabricius 1775)	1	1	2	1	4	8	17
Carabus (Carabus) granulatus	Linné 1758	0	0	0	0	1	0	1
Chlaenius (Agostenus) alternatus	G.H. Horn 1871	0	1	0	1	1	0	3
Clivina (Clivina) collaris	(Herbst 1784)	1	4	5	0	0	0	10
Clivina (Clivina) fossor	(Linné 1758)	7	8	8	0	2	4	29
Clivina (Clivina) impressefrons	LeConte 1844	0	0	1	0	0	0	1
Cymindis (Cymindis) neglecta	Haldeman 1843	0	0	2	0	0	0	2
Dicaelus (Paradicaelus) sculptilis	Ball 1959	0	0	0	1	2	0	3
Diplocheila (Isorembus) obtusa	(LeConte 1848)	0	1	0	0	1	0	2
Diplocheila (Isorembus) striatopunctata	(LeConte 1844)	0	1	0	0	0	0	1
Harpalus (Harpalus) affinis	(Schrank 1781)	1	0	2	0	0	0	3
Harpalus (Harpalomerus) amputatus	Say 1830	7	2	1	0	0	0	10
Harpalus (Anamblystus) fallax	LeConte 1859	1	0	0	0	0	0	1
Harpalus (Anamblystus) herbivagus	Say 1823	1	0	0	1	4	1	7
Harpalus (Pseudoophonus) pensylvanicus	(De Geer 1774)	8	6	7	4	4	1	30
Harpalus (Anamblystus) plenalis	Casey 1914	0	0	0	0	1	0	1
Harpalus (Anamblystus) pleuriticus	Kirby 1837	0	1	2	1	5	2	11
Harpalus (Anamblystus) somnulentus	Dejean 1829	2	2	2	3	2	7	18
Loricera pilicornis	(Fabricius 1775)	1	0	0	0	0	0	1
Microlestes linearis	(LeConte 1851)	0	0	2	0	0	0	2

Notiophilus aquaticus	(Linné 1758)	1	1	1	1	0	1	5
Oxypselaphus pusillus	(LeConte 1854)	1	0	0	3	1	0	5
Patrobus (Fossifrons) lecontei	Chaudoir 1871	0	0	1	0	0	0	1
Poecilus corvus	(LeConte 1873)	17	4	5	0	1	1	28
Poecilus lucublandus	(Say 1823)	10	3	4	4	8	21	50
Poecilus scitulus	LeConte 1848	0	2	1	2	0	1	6
Pterostichus (Phonias) femoralis	(Kirby 1837)	0	0	1	0	1	2	4
Pterostichus (Morphnosoma) melanarius	(Illiger 1798)	44	28	31	61	24	48	236
Stenolophus (Agonoderus) comma	(Fabricius 1775)	2	0	0	0	0	0	2
Synuchus impunctatus	(Say 1823)	3	4	3	9	4	12	35
	Total Specimen	**164**	**130**	**151**	**113**	**88**	**124**	**770**
	Total Genera	**14**	**12**	**13**	**12**	**13**	**10**	**21**
	Total Species	**31**	**29**	**31**	**24**	**29**	**23**	**52**

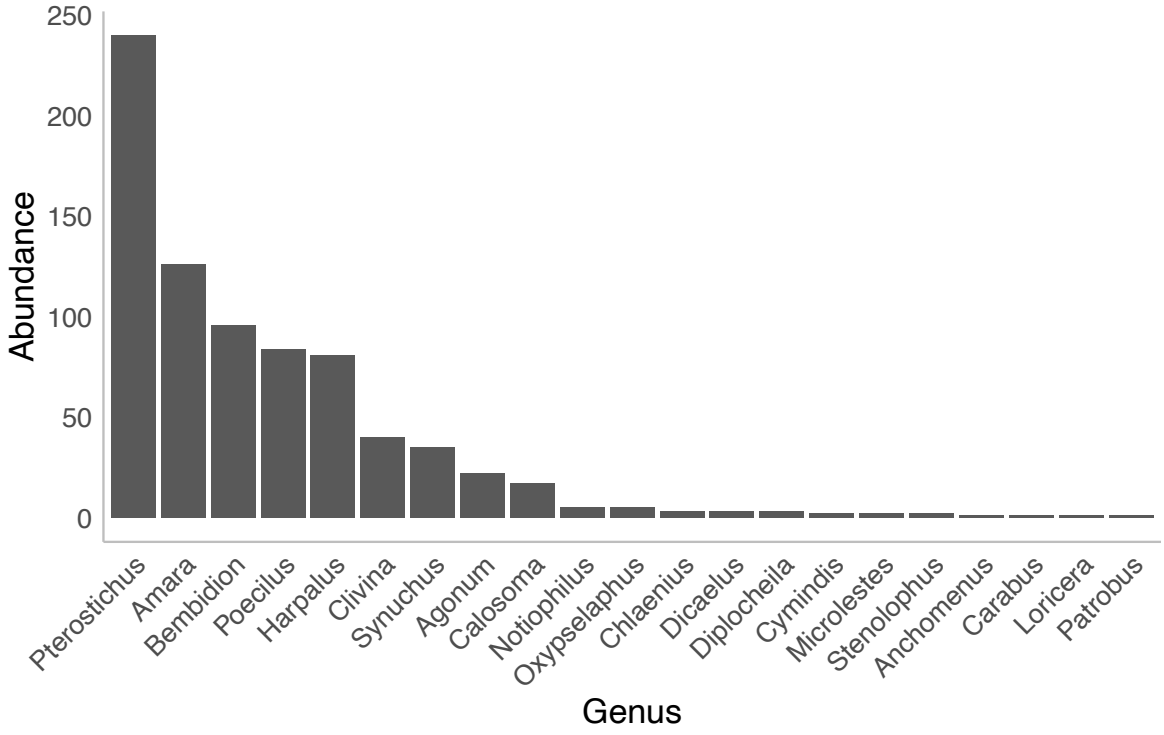

Figure S 6. Pitfall trap collected carabid beetle abundance per genus across both study areas and all sites. Graph does not include excluded data. *Anchomenus* (1)*, Cymindis* (2)**,** *Loricera* (1), *Microlstes* (2), *Patrobus* (1), and *Stenolophus* (2) had ≤2 individuals.

CONCLUSION AND IMPLICATIONS OF FINDINGS

In this study, I explored the effects of the Three Sisters intercropping system on wild bee and ground beetle biodiversity, as well as crop yield and quality, in southern Manitoba and northern Ontario. I investigated these dynamics by comparing the Three Sisters to different planting variations (e.g., mono- and di-crops) of select Indigenous-specific crops (Chapter two), and the surrounding natural area (Chapter three). My objectives were: (i), document wild bee and ground beetle diversity associated with the Indigenous significant crops in monocrop and intercrops, (ii) document a baseline of insect abundance, species diversity and associated ecological function related to the Indigenous planting method of the Three Sisters, and contribute to provincial and national species records, (iii) gather insights on Three Sisters crop yield and quality and determine wild bee ecological function influence, and (iiii) use the findings to inform and guide future Three Sisters focused projects and land management practices for increasing insect biodiversity.

I met my first objective by documenting wild bee and ground beetle diversity in different planting combinations of Indigenous significant crop's (monocrop, dicrop, and Three Sisters intercrop) in southern Manitoba and northern Ontario, capturing a total of 56 wild bee and 57 ground beetle species in 2022. However, inconsistencies in data limited analysis to 124 bees captured via targeted aerial netting and 8,557 ground beetles captured via pitfall traps. Consequently, it was not possible to assess the intended additional bee diversity indices or ecological function. Despite these limitations, my results provide robust insights into Indigenous significant crop combination effects on bee abundances and ground beetle diversity, forming a foundation for future Three-Sisters-focused studies with improved sampling protocols. I found wild bee abundances to be positively affected by the Three Sisters and sunflower-containing treatments. This pattern highlights the role of intercropping pollinator-attractive crops in enhancing wild bee activity, likely due to the complementarity in floral traits and extended temporal and morphological availability of resources (Jones and Gillett 2005, Ghazoul 2006, Guzman et al. 2019). Furthermore, I found ground beetle abundances, richness, and Hill-Shannon diversity did not differ across treatments, suggesting that they may respond more strongly to soil conditions, canopy structure, and other biotic and abiotic factors than crop diversity alone (Thiele 1997, Kromp 1999, Hummel et al. 2012). Overall, these findings indicate

that while intercropping can create high-quality niches for pollinators, its effects on ground-dwelling predators may be less immediate or may require additional habitat consideration to detect treatment-specific responses.

I met my second objective by providing the first baseline recordings of 39 wild bee and 61 ground beetle species out of 1,070 and 1,538 individuals, respectively, associated with the Three Sisters intercrop and the surrounding natural area in southern Manitoba and northern Ontario in 2023. After exclusion of data that did not meet the study criteria, 701 wild bees representing 20 species and 770 ground beetles representing 52 species were assessed. Regarding wild bee captures, bee bowl and BVT data were excluded, removing the ability to compare against the surrounding natural area; however, my results suggest the potential for the Three Sisters to contribute to a more diverse habitat. Although within a Three Sisters garden area, bee abundance and species richness indices were comparable between the interior and exterior positions, *Bombus* spp. dominated captures, followed by *Melissodes (Eumelissodes) agilis* Cresson and *Melissodes (Eumelissodes) trinodis* Robertson*,* prominent sunflower specialists, and one rare capture of a kleptoparasite species, *Triepeolus helianthi* (Robertson)*,* observed in the exterior. Most notably, I found one species, *Bombus (Thoracobombus) pensylvanicus* (De Geer), representing only the second record in Manitoba since 1948, highlighting the potential of this system to support rare or endangered taxa. Ground beetle diversity was significantly higher within the Three Sisters garden area than in the surrounding natural area, although within-spatial differences were minimal. Together, these results demonstrate the Three Sisters system can support abundant and diverse insect assemblages, including rare or specialist species, highlighting its potential to enhance pollinator and predator populations in small-scale agricultural areas.

I met my third objective by evaluating crop yield, quality (in terms of weight), and ecological function within the Three Sisters system. Comparisons among treatments and spatial positions revealed no consistent advantage for yield or quality, likely due to early-season stressors such as excessive soil moisture, weed competition, and establishment challenges that limited potential for complementary interactions (Fausey and Lal 1990, Liebman and Dyck 1993, Daramola 2020). However, I found differences in these metrics at the site level, indicating that local environmental conditions and management history strongly influenced crop production and

seed quality (Munson-Scullin and Scullin 2005, Cuddington 2011). Regarding the provisioning of proxied ecosystem services, I found pollination and pest control services mirrored these trends: no effect of position was found, but mean seasonal abundances varied across sites, both outcomes likely due to the wide foraging and activity ranges of the taxa (Ewers and Didham 2008, Olynyk et al. 2021). Together, these results highlight the context-dependent nature of productivity and ecological function in small-scale Three Sisters gardens, demonstrating that while crop complementarity may not always translate into measurable yield gains under sub-optimal conditions, the Three Sisters system consistently provides habitat and resources to support diverse and functional insect assemblages (Hooper et al. 2005).

This study provides one of the first steps towards understanding how the Three Sisters system supports insect diversity, influences crop performance, and may be a key tool in mitigating global biodiversity losses associated with intensive agriculture. Although my findings represent only a small contribution to a much larger body of work that is still needed, in conjunction with Gibson (2024), the number of wild bee species associated with the Three Sisters Indigenous significant crops has increased to 61 in the few areas sampled in Manitoba and Ontario. Future research should prioritize identifying heirloom crop varieties or varieties that are complementary to local environmental conditions, as a choice of cultivar landrace strongly influences crop growth, resource sharing, and the delivery of ecosystem services. Selecting varieties that maximize mutual benefits, for example, by aligning bloom times for pollinator support, ensuring effective weed suppression, or optimizing nutrient use, could enhance both harvest outcomes and ecological functions. Also, integrating functional traits of insects (e.g., nesting guilds, body size, phenology) will be essential to link diversity more directly to ecosystem services. Finally, future studies must account for the strong context-dependence revealed in this work by expanding across environmental gradients, soil types, and management regimes. Beyond insect diversity and crop performance, the Three Sisters system holds promise as a platform for a variety of future research, such as a long-term climate change monitoring system, conservation-focused studies, and investigations into multifunctional agroecosystem management practices.

While new directions for Three Sisters focused research are promising, implementing and sustaining these studies appearing straightforward with studies similar in design such as Tilman

and Downing (1994) being effective, they are slow and costly to implement as they require global cooperation, paradigm shifts in government, and coordination among scientists, non-government organizations (NGO), policy makers, funding agencies, science communicators, and citizens (Pretty 1997, Rey Benayas and Bullock 2012, Petersen and Snapp 2015, Forister et al. 2019, Bali and Kaleka 2022, Harvey et al. 2023, Leandro 2023, Gebreegziabher 2024). Most current existing public policies or funding incentives are biased towards select species or allow exceptions in favour of certain high-value groups or agribusinesses (Petersen and Snapp 2015, Leandro 2023). However, an example of successful collaboration is the Kunming-Montreal Global Biodiversity Framework (GBF), a tool to advance global agreements to protect nature, restore ecosystems, and ensure equal sharing of resources (Convention on Biological Diversity (CBD) 2022, Leandro 2023). The GBF was built on the Strategic Plan for Biodiversity 2011-2020 and considerations from various groups, including Indigenous and local communities, United Nations organizations and programs, multilateral environmental agreements, women and youth groups, local businesses, faith-based organizations, and concerned citizens (Convention on Biological Diversity (CBD) 2022). The amount of care, effort, funds, and long-term commitment put into implementing a single policy like the GBF emphasizes the most common challenges experts have determined to bottleneck agricultural shifts towards mitigation strategies or associated research (Petersen and Snapp 2015, Leandro 2023).

Although short-term studies have their place and can be effective when creating financial supports, awareness campaigns, and education programs, advancing the Three Sisters further into the research field will require collaboration with local farmers, which has been proven difficult without sufficient funding. Reluctancy towards adopting environmentally friendly and restorative agricultural practices is not solely based on increased work and restrictions, but also on the lack of financial compensation for the loss of land, additional supports, and training (Rey Benayas and Bullock 2012, Petersen and Snapp 2015, Chapman et al. 2019, Gebreegziabher 2024). Land owners must be explicitly rewarded for restorative actions, not only long-term investments but at the time when society demands more than food, fibre, and fuel production from their agricultural lands (Rey Benayas and Bullock 2012). Although direct financing measures for ecosystem services have been established worldwide, such as tax deductions, those concerning mitigation or restoration mostly focus on afforestation (Rey Benayas and Bullock 2012).

Beyond farmers, citizen science and education will be crucial for integrating insect-driven Three Sisters projects. The widespread perception of insects as "creepy" is a reflection of political organizations, given the current lack of policy for insects beyond bees and large pollinators, but research suggests rehabilitation of feelings towards insects is possible (Donkersley et al. 2022, Lampert et al. 2023). Environmental education with hands-on experiences and question-based learning has shown to promote a positive attitude, confidence, and increased knowledge and acceptance of insects and allow for real-world actions individuals can take toward small differences (Petersen and Snapp 2015, Donkersley et al. 2022, Lampert et al. 2023).

Arguably, the most important aspect to consider for future Three Sisters research is not to separate economic or ecological outcomes from cultural significance (Herrighty 2022). This planting tradition is a rich body of traditional ecological knowledge (TEK), where crops were selected, managed, and maintained for generations based on their complementarity and their role in sustaining communities, landscapes, and all living beings. Continued research must therefore be conducted in genuine partnership with Indigenous communities, ensuring that study designs respect cultural context, seed sovereignty, and place-based knowledge while centring Indigenous leadership and priorities. By weaving together ecological science with TEK, the Three Sisters can be better understood not only as a multifunctional intercrop supporting biodiversity and food production, but also as a living cultural practice that sustains relationships between people, land, and animals, reflecting a philosophy of stewardship, reciprocity, and interconnectedness.

References

Bali, G.P.K., and Kaleka, A.S. 2022. Potential reasons for insect decline. *In* Global Decline of Insects. *Edited by* H.A. Farag El-Shafie. IntechOpen, London, United Kingdom. pp. 25–42.

Chapman, M., Satterfield, T., and Chan, K.M.A. 2019. When value conflicts are barriers, can relational values help explain farmers' participation in conservation incentive programs? Land Use Policy, **82**: 464–475. doi: 10.1016/j.landusepol.2018.11.017.

Convention on Biological Diversity (CBD). 2022, December 7–19. Kunming-Montreal global biodiversity framework. *In* Conference of the Parties to the Convention on Biological Diversity. Montreal, Canada.

Cuddington, K. 2011. Legacy effects: the persistent impact of ecological interactions. Biological Theory, **6**: 203–210. doi: 10.1007/s13752-012-0027-5.

Daramola, O.S. 2020. Timing of weed management and yield penalty due to delayed weed management in soybean. Planta Daninha, **38**: 1–7. doi: 10.1590/S0100-83582020380100072.

Donkersley, P., Ashton, L., Lamarre, G.P.A., and Segar, S. 2022. Global insect decline is the result of wilful political failure: a battle plan for entomology. Ecology and Evolution, **12**: 1–13. doi: 10.1002/ece3.9417.

Ewers, R.M., and Didham, R.K. 2008. Pervasive impact of large-scale edge effects on a beetle community. Ecology, **105**: 5426–5429. doi: 10.1073/pnas.0800460105.

Fausey, N.R., and Lal, R. 1990. Soil wetness and anaerobiosis. *In* Advances in Soil Science (Volume 11): Soil Degradation. *Edited by* R. Lal and B.A. Stewart. Springer, New York, NY. pp. 173–186.

Forister, M.L., Pelton, E.M., and Black, S.H. 2019. Declines in insect abundance and diversity: we know enough to act now. Conservation Science and Practice, **1**: 1–8. doi: 10.1111/csp2.80.

Gebreegziabher, H.G. 2024. A systematic review of insect decline and discovery: trends, drivers, and conservation strategies over the past two decades. Psyche: A Journal of Entomology, **2024**: 1–16. doi: 10.1155/2024/5998962.

Gibson, S.D., Onuferko, T.M., Myers, L., and Colla, S.R. 2024. Determining the plant-pollinator network in a culturally significant food and medicine garden in the Great Lakes region. PeerJ, **12**: 1–24. doi: 10.7717/peerj.17401.

Guzman, A., Chase, M., and Kremen, C. 2019. On-farm diversification in an agriculturally-dominated landscape positively influences specialist pollinators. Frontiers in Sustainable Food Systems, **3**: 1–9. doi: 10.3389/fsufs.2019.00087.

Harvey, J.A., Tougeron, K., Gols, R., Heinen, R., Abarca, M., Abram, P.K., Basset, Y., Berg, M., Boggs, C., Brodeur, J., Cardoso, P., de Boer, J.G., De Snoo, G.R., Deacon, C., Dell, J.E., Desneux, N., Dillon, M.E., Duffy, G.A., Dyer, L.A., Ellers, J., Espíndola, A., Fordyce, J., Forister, M.L., Fukushima, C., Gage, M.J.G., García-Robledo, C., Gely, C., Gobbi, M., Hallmann, C., Hance, T., Harte, J., Hochkirch, A., Hof, C., Hoffmann, A.A., Kingsolver, J.G., Lamarre, G.P.A., Laurance, W.F., Lavandero, B., Leather, S.R., Lehmann, P., Le Lann, C., López-Uribe, M.M., Ma, C. Sen, Ma, G., Moiroux, J., Monticelli, L., Nice, C., Ode, P.J., Pincebourde, S., Ripple, W.J., Rowe, M., Samways, M.J., Sentis, A., Shah, A.A., Stork, N., Terblanche, J.S., Thakur, M.P., Thomas, M.B., Tylianakis, J.M., Van Baaren, J., Van de Pol, M., Van der Putten, W.H., Van Dyck, H., Verberk, W.C.E.P., Wagner, D.L., Weisser, W.W., Wetzel, W.C., Woods, H.A., Wyckhuys, K.A.G., and Chown, S.L. 2023. Scientists' warning on climate change and insects. Ecological Monographs, **93**: 1–37. doi: 10.1002/ecm.1553.

Herrighty, E.M. 2022. Seed sovereignty, rematriation, and Three Sisters intercropping in Native American communities. [M.Sc.] thesis, Iowa State University, Ames, Iowa.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., and Wardle, D.A. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs, **75**: 3–35. doi: 10.1890/04-0922.

Hummel, J.D., Dosdall, L.M., Clayton, G.W., Harker, K.N., and O'Donovan, J.T. 2012. Ground beetle (Coleoptera: Carabidae) diversity, activity density, and community structure in a diversified agroecosystem. Environmental Entomology, **41**: 72–80. doi: 10.1603/EN11072.

Jones, G.A., and Gillett, J.L. 2005. Intercropping with sunflowers to attract beneficial insects in organic agriculture. Florida Entomologist, **88**: 91–96. https://doi.org/10.1653/0015-4040(2005)088[0091:IWSTAB]2.0.CO;2.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture, Ecosystems & Environment, **74**: 187–228. https://doi.org/10.1016/S0167-8809(99)00037-7.

Lampert, P., Goulson, D., Olsson, D., Piccolo, J., and Gericke, N. 2023. Sustaining insect biodiversity through action competence — an educational framework for transformational change. Biological Conservation, **283**: 1–9. doi: 10.1016/j.biocon.2023.110094.

Leandro, C. 2023. Insect and arthropod conservation policies: the need for a paradigm shift. Current Opinion in Insect Science, **58**: 1–7. doi: 10.1016/j.cois.2023.101075.

Liebman, M., and Dyck, E. 1993. Crop rotation and intercropping strategies for weed management. Ecological Applications, **3**: 92–122. https://www.jstor.org/stable/1941795.

Munson-Scullin, W., and Scullin, M. 2005. Potential productivity of midwestern native American gardens. Plains Anthropologist, **50**: 9–21. doi: 10.1179/pan.2005.004.

Olynyk, M., Westwood, A.R., and Koper, N. 2021. Effects of natural habitat loss and edge effects on wild bees and pollination services in remnant prairies. Environmental Entomology, **50**: 732–743. doi: 10.1093/ee/nvaa186.

Petersen, B., and Snapp, S. 2015. What is sustainable intensification? views from experts. Land Use Policy, **46**: 1–10. doi: 10.1016/j.landusepol.2015.02.002.

Pretty, J.N. 1997. The sustainable intensification of agriculture. Natural Resources Forum, **21**: 247–256. doi: 10.1111/j.1477-8947.1997.tb00699.x.

Rey Benayas, J.M., and Bullock, J.M. 2012. Restoration of biodiversity and ecosystem services on agricultural land. Ecosystems, **15**: 883–899. doi: 10.1007/s10021-012-9552-0.

Thiele, H.-U. 1997. Carabid beetles in their environments: a study on habitat selection by adaptations in physiology and behaviour. *Edited by* W.S. Hoar, B. Hoelldobler, H. Langer, and M. Lindauer. Springer, New York.

Tilman, D., and Downing, J.A. 1994. Biodiversity and stability in grasslands. Nature, **367**: 363–365. https://doi.org/10.1038/367363a0.