

INVESTIGATING THE FEEDBACKS BETWEEN SOIL HEALTH AND
PEST MANAGEMENT PRACTICES IN FIELD CROPS

A Thesis

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ABSTRACT

Field crop growers are increasingly aware of the benefits for managing healthy biologically active soils, such as improved nutrient cycling and decomposition, and soil structure. Of equal importance to growers is the management of crop pests, which may include pesticides that have unintended effects on soil biological function. Historically, soil health and pest management are rarely integrated, despite that both practices are aimed at enhancing and maximizing productivity. The goal of this research was to explore the feedbacks between soil health and pest management tactics in field crops, and to quantify the influence of pest management on soil biological function, specifically biological control and decomposition. A three-year field experiment (2017-2019) was conducted in a corn-soybean-corn rotation, and evaluated the influence of two management practices: (1) preventative pest management, with pesticide-treated seed and foliar sprays, and a scouting-based IPM, on pest regulation (chapter one), and soil fertility and decomposition (chapter two). To assess pest regulation, I measured predator activity-density and predation at the soil surface, and the biological control potential of soil-borne entomopathogenic fungi (EPF). Aboveground predators were measured via pitfall trapping and predation dynamics were assessed with a standard sentinel-bait assay. Belowground biological control potential was determined using a sentinel-bait bioassay to quantify the abundance of entomopathogenic fungi. To determine decomposition and nutrient cycling, I measured soil fertility (pH, organic matter, macro- and micro- nutrients) and % wet aggregate stability. Soil microarthropods were measured via Berlese funnel extraction and decomposer activity was determined through microbial extracellular enzymes and bait lamina strips. Over the course of the experiment, I found that the use of seed treatments and foliar applications together negatively suppressed total predator activity-density, spider activity-density, predation, and the infection

potential of soil-borne entomopathogenic fungi, compared with no pesticide use in one out of three years of the field study. I also found that pest management practices had minimal impacts on the soil physicochemical properties compared to biological factors. I observed increased microbial enzyme activity and feeding activity in preventatively managed fields, and both practices increased the abundance of decomposer microarthropods, depending on taxa and pesticide input. There were higher abundances of two collembolan families (Isotomidae and Entomobryidae) and three mite taxa (Oribatida, Mesostigmata, and Astigmatina) in IPM and preventative practices, and a suppression of one mite taxa (Prostigmata) in preventative management in all three years of the study. Overall, the use of pesticides did not improve crop yield in either corn or soybean. These results highlight that the use of seed treatments within an IPM practice may not always be necessary for maximum crop productivity. Taken together, my findings suggest pest management practices may have unintended consequences for above and below ground soil biological control, and diverse impacts on decomposer community composition and function, with responses varying by biotic compartment, season, and the specific active ingredients used. These results emphasize the need for further research exploring the mechanisms underlying the responses of soil biota to pesticidal seed treatments and foliar applications to better align practices for managing both soil health and agricultural pests in field crops.

BIOGRAPHICAL SKETCH

Lindsay Fennell grew up in the small town of Pepperell in northern Massachusetts, once known for its good soil, plentiful orchards, and robust paper mills. There she was educated in the public school system and graduated from North Middlesex Regional High School (NMRHS) in 2001. She was an avid soccer player throughout her high school tenure and played both the clarinet and saxophone in jazz band. After graduation, she attended Simmons College in Boston, MA for three years where she studied Creative Writing and Gender Studies before deciding to explore and travel the country.

She returned to Massachusetts in 2010 and enrolled at Holyoke Community College (HCC) in Holyoke, MA where she earned an Associate Degree in Sustainable Agriculture. She then transferred to Cornell University in Ithaca, NY and in 2012, graduated *magna cum laude* with a Bachelor of Science degree in Agricultural Sciences. For the following three years, she worked for Dr. Harold van Es in the Cornell Soil Health Laboratory as a Research Technician, and in 2017, she was accepted into a graduate program in the Department of Entomology at Cornell University as an advisee of Dr. Kyle Wickings. During her time as a graduate student at Cornell, she was actively involved in the Jugatae Entomology Graduate Student Club and participated in several local Extension/Outreach events. Upon receiving her degree, Lindsay plans to spend the summer farming in the Finger Lakes outside of Ithaca.

Dedicated to Daniel J. Fennell and Denise M. Quinn

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Introduction

Agricultural producers face the enormous challenge of being able to feed a growing world population, projected to reach 10 billion by 2050 (United Nations, 2020), while conserving soil, water, and the biodiversity that are needed to ensure that farming is possible and sustainable. An essential way to make farming more sustainable is an improved knowledge of biological processes in order to optimize services such as pest regulation and nutrient cycling and to provide insight into the ways that we can bolster soils to require fewer external inputs, while not compromising on productivity. It is widely recognized that healthy biologically active soil is the foundation for sustainable farming in the future (McLeman et al., 2014; Reganold et al., 1990; Wachira et al., 2015; Welbaum et al., 2004).

In recent decades, it has been increasingly recognized that the sustainable, effective management of soils requires a more comprehensive understanding of both above and belowground soil biological processes. While many use the term ‘soil quality’ and ‘soil health’ interchangeably, the phrase ‘soil health’ portrays soil as a living community whose functions depend on the activity of biota, such as bacteria, fungi, nematodes, collembola, mites, and earthworms, that require conservation through proper management (Doran and Zeiss, 2000; Kibblewhite et al., 2008). These organisms directly and indirectly improve nutrient cycling, soil structure and stability, increase soil water infiltration, and regulate pathogen pressure (Holloway et al., 2008; Pretorius et al., 2018). Yet, quantifying and monitoring soil health remains somewhat elusive, stemming partly from the many knowledge gaps in the understanding of community and ecosystem processes in soil and how they respond to different agricultural practices (Eisenhauer et al., 2017). However, there is a general agreement that soil health is driven by at least four major components: soil organic matter, structure and stability, nutrient

availability, and pest resistance and resilience (van Es and Magdoff, 2009); all factors determined directly or indirectly by biological processes.

Soil organic matter accumulates through the continual deposition of organic carbon which is comprised primarily of both living and dead components, such as senesced microbes, plant tissues, root exudates and detritus (Sokol et al., 2019). These inputs undergo biotic decay via physical fragmentation by soil animals, degradation by microbial extracellular enzymes, and ingestion and assimilation into microbial and animal biomass. This biomass is then released as waste products that can be stored in free or mineral-associated belowground organic matter pools (Cotrufo et al., 2013). Both microbial and faunal communities also determine a soil's physical structure and stability. For example, arbuscular mycorrhizal fungi contribute to the stability of soil aggregates through the production of glycoproteins and glomalin deposited on hyphal walls and soil particles (Miller and Jastrow, 2000). These sticky substances enmesh and entangle soil particles that create networks of macroaggregates of various sizes that contribute to aggregate stabilization and increased water infiltration (Lynch and Bragg, 1985). Soil macrofauna, such as earthworms, contribute to the bioturbation and redistribution of organic matter through the soil profile, and loosen compacted soil by boring holes, also leading to improved porosity (Shipitalo and Le Bayon, 2004).

Soil microbes, i.e. bacteria, fungi, viruses, and Archaea, are the primary agents in nutrient mineralization (Coleman et al., 2018). Microbes produce extracellular enzymes that mineralize organic molecules into less complex inorganic forms that be assimilated. Although microbes are the main drivers of nutrient mineralization, soil fauna play an important role in decomposition and nutrient cycling through their influence on microbial communities, either directly, through grazing and feeding, or indirectly, through fragmenting plant detritus. For example, litter

consumption and microbivory by soil arthropods can stimulate the production of microbial extracellular enzymes (Wickings et al., 2011). Additionally, soils that contain a diversity of entomopathogenic and antagonistic organisms, i.e. fungi and nematodes (Hajek and Eilenberg, 2018; Kaya and Gaugler, 1993), and epigeic predatory invertebrates that can aid in pest regulation by actively suppressing crop pests (Lang et al., 1999; Michalko et al., 2019), may require less synthetic inputs.

Entomopathogenic fungi (EPF) are widespread in most terrestrial settings and their capability as biological control agents is largely determined by biotic and abiotic conditions (Meyling and Eilenberg, 2007). Species in the group Ascomycota, including the commonly occurring *Metarhizium* spp. and *Beauveria* spp., are facultative saprophytes that can contribute considerably to arthropod mortality, due to their naturally occurring conidia that persist in the soil (Hajek and Eilenberg, 2018). Generalist arthropod predators, such as ground beetles and spiders, are among the dominant epigeal predators in agricultural systems (Lang et al., 1999). Carabid beetles contribute to the control of aphids and slugs, and spiders, both cursorial and web-building, can exert additional pressure on a variety of crop pests (Michalko et al., 2019; Sunderland, 1999).

To build soil health and take advantage of these ecological services, farmers are encouraged to adopt practices such as conservation tillage, crop rotations, and the incorporation of perennials (Doran, 2002). Tillage practices, such as moldboard plowing, are well known to have detrimental impacts on the biological properties of soil (Hobbs et al., 2008); however, practices such as reduced tillage maintains crop residues at the soil surface. This organic matter provides a resource for both above and belowground biota, as an additional input of organic carbon as well as habitat for generalist predators, such as ground beetles and spiders (Clark et al.,

1993; Miura et al., 2008; Pearsons et al., 2017). Crop rotations are a crucial management tool to minimize the application of synthetic inputs, by increasing soil nutrients through the enhancement of microbial biomass (McDaniel et al., 2014), and by offsetting pest life cycles such as corn rootworm (*Diabrotica* spp.). Perennial grasses and hay can also bring numerous benefits to agricultural systems. Perennials allow for greater periods of reduced disturbance and the subsequent stabilization of soil (Karlen et al 2006). For example, the incorporation of perennials into corn rotations can improve soil bulk density, nitrogen and carbon stocks and even increase yields compared to annual rotations (Olmstead and Brummer, 2008; Riedell et al., 2013). In addition to physicochemical traits, incorporating perennials into crop rotation have also been shown to increase densities of epigeic predators and decomposers (Cardoza et al., 2015). Concurrently, while farmers are increasingly adopting practices to improve soil biological health, many are simultaneously needing to implement pest management practices that limit biotic activity. These two goals are directly at odds with one another and pesticides that are used in field crops may be over-used and influence soil biological communities more strongly than is currently realized.

Pest management in field crops, such as corn as soybean, became heavily reliant on synthetic pesticides following World War I, resulting in adverse consequences for non-target organisms, increased environmental pollution, and pesticide resistance. In 1959, Stern et al. introduced the concept of Integrated Pest Management as a strategy to reduce the harmful effects of pesticides by scouting for pests in the field and accounting for the severity of pest damage prior to the use of chemicals (Stern et al., 1959). Although the definition of Integrated Pest Management (IPM) has evolved over the past 60 years (Kogan, 1998), an ideal IPM program for corn and soybean would involve monitoring and management of important pests (insects and

fungus pathogens) and the judicious use of pesticides when thresholds of economic importance are met (Ehler, 2006). In 2019, 36.3 and 30.8 million hectares of corn and soybean respectively were grown in the U.S. (USDA/NASS, 2019). In practice, monitoring for crop pests in such largescale cropping systems is difficult and many of the major pests are sporadic in time and hard to predict (Hodgson et al., 2012; Krupke et al., 2017). Crop rotation has generally been an effective management tactic against corn rootworm (CRW) (*Diabrotica virgifera* LeConte and *D. barberi* Smith & Lawrence), but is not always a practical or economically viable option (Gray et al., 2009). Moreover, because rootworm larvae inhabit the soil, they are difficult to monitor especially in large-scale commodity crops. Soybean aphid (*Aphis glycines* Matsumura) is a major pest of soybean, but often occurs sporadically in fields at densities that rarely impact yield (Seagraves and Lundgren, 2012). Fungal pathogens, e.g. *Fusarium* spp., are also a major constraint for soybean production, depending on year and location (Esker and Conley, 2012), with populations that are spatially variable and difficult to predict (Boerma et al., 2004). Recent studies in plant pathology have indicated that crop rotation, diversification, and cultivar choice may be the most suitable management practices for avoiding fungal disease (Marburger et al., 2016; Speilman et al., 2002). Given these constraints and the unpredictability of major pests, preventative pest management tactics that are risk-adverse, such as the prophylactic use of pesticidal seed treatments and foliar sprays, are often more attractive than scouting fields and using thresholds to determine if and when to make pesticide applications.

Pesticidal seed treatments are comprised mainly of the systemic neonicotinoid insecticides imidacloprid and thiamethoxam (Elbert et al., 2008) in combination with fungicides, like mefenoxam and fludioxonil. These active ingredients are meant to target early-season insect pests and pathogens in corn and soybean, and have a more targeted delivery compared to

conventional alternatives that are toxic to vertebrates, i.e. foliar-applied organophosphates. Yet, there is a growing body of evidence about the extensive negative impacts of pesticidal seed treatments on pollinators (Anderson and Harmon-Threatt, 2019; Krupke et al., 2012), migratory birds (Eng et al., 2017; Lopez-Antia et al., 2016), aquatic systems (Basley and Goulson, 2018), and importantly, invertebrates in agroecosystems (Douglas et al., 2015; Nettles et al., 2016). Moreover, pesticidal seed treatments may pose limits on IPM principles, such as scouting and economic thresholds, and constrain the ability of growers to meet pest management goals without introducing unintentional consequences on soil biota.

Foliar sprays of pyrethroid insecticides and strobilurin fungicides are frequently used together in corn and soybean to target insects pests such as corn earworm (*Helicoverpa zea* Boddie), fall armyworm (*Spodoptera frugiperda* J.E. Smith), and bean leaf beetle (*Cerotoma trifurcate* Forster), and fungal diseases such as downy mildew (*Peronospora manshurica* Naumov), septoria brown spot (*Septoria glycines*) and Phytophthora root and stem rot (*Phytophthora* spp.). It is also well documented that foliar sprays have unintended consequences on beneficial biota in agricultural systems (El-Wakeil et al., 2013; Geiger et al., 2010; Mahoney et al., 2015; Pfiffner and Luka, 2003).

Despite a large body of literature on the negative impact of pesticides on beneficial organisms, the ecological feedbacks between managing soil health and the influence of differing pest management tactics on soil biological function are less understood. These two strategies are rarely integrated, despite the potential for enhanced yield and sustainability. For example, increased populations of predators and entomopathogens could lead to improved biological control potential and reduce the need for insecticides. Additionally, the proper timing of pesticide input could limit impacts on beneficial decomposers and improve nutrient cycling or

oppositingly, the overuse of pesticides or poor timing of pesticides could suppress beneficial soil biota and their functioning. Harmonizing soil health and pest management is challenging, and it requires a better understanding of soil biological processes above and belowground.

The main objective of this thesis was to determine how the preventative use of insecticides and fungicides alter benchmarks of soil health compared to a scouting-based IPM approach. To achieve this objective, I conducted a three-year field study (2017-2019) in a corn-soy-corn rotation in fields that were in alfalfa, a perennial legume, for four consecutive years prior to the establishment of the experiment. Soil health was assessed through biological control, in Chapter 1, and decomposition, in Chapter 2. Additionally, I quantified yield to determine if productivity differed between pest management practices.

Together, these studies contribute to our understanding how to best integrate soil and pest management practices to advance both the effectiveness and sustainability of productivity in field crops.

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Chapter One

Preventative pest management in field crops influences biological control potential of epigeal arthropods and soil-borne entomopathogenic fungi

Abstract

Field crop growers in the U.S. are increasingly adopting prophylactic pest management strategies to control sporadic crop pests that are spatially and temporally difficult to predict. While evidence shows these preventative management practices, specifically pesticidal seed treatments, can be important in regions of the U.S. with more predictable pest pressures, employing them ubiquitously in field crops across the Northeast can have variable returns on productivity.

Prophylactics may also pose limitations to core principles of Integrated Pest Management (IPM), such as scouting and the use of economic thresholds to make pest control decisions, and may negatively impact beneficial soil biota both above and belowground. I conducted a three-year field experiment (2017-2019) to compare the influence of a preventative pest management practice and a scouting-based IPM practice on biological control organisms. Above-ground predators were measured via pitfall trapping and predation dynamics were assessed with a standard sentinel-bait assay. Belowground biological control potential was determined using a sentinel-bait bioassay to quantify the abundance of soil-borne entomopathogenic fungi (EPF).

The use of seed treatments and foliar sprays together negatively influenced total predator activity-density, spider (order Araneae) activity-density, predation, and the biological control potential of EPF, in one out of three years of the study. In the third year, there was an increase in the activity-density of spiders in preventative plots that included seed treatments. Overall, the use

of pesticides did not improve crop yield in either corn or soybean. These findings highlight that the use of a prophylactic pesticide program may not always be necessary for maximum crop productivity and that this management approach can occasionally have unintended negative consequences on above and below ground soil biota and the ecosystem services they provide.

1. Introduction

Since the mid-20th century there has been a rise in agricultural simplification, as evidenced by the millions of hectares of corn (*Zea mays* L.) and soybean (*Glycine max* L.) grown across the U.S. In 2018, there were 36.1 million hectares and 36.3 million hectares of corn and soybean, respectively (Barrett, 2018). This simplification was expanded by the “Green Revolution” in the 1950s and 1960s, which increased food production with the onset of synthetic fertilizers and pesticides (Matson et al. 1997). Yet, these advancements also led to an increase in insecticide resistance, environmental pollution, the overuse of highly toxic broad-spectrum chemicals, and negative impacts on non-target organisms (Howarth, 2008; Pimentel et al., 1992; Smith, 1972). In response to these negative outcomes, entomologists introduced the concept of Integrated Pest Management (IPM) as an evidence-based paradigm involving scouting for pests in the field and accounting for the severity of pest damage prior to the use of chemical control (Stern et al., 1959). A fundamental component of this new strategy was to support diverse populations of natural enemies in cropping systems in order to maintain pest populations below economic injury levels, thus applying pesticides only when necessary.

Despite growing interest in IPM practices, in recent years farmers have been progressively adopting prophylactic strategies towards pest management such as pesticidal seed treatments comprised mainly of the systemic neonicotinoid insecticides imidacloprid and thiamethoxam (Elbert et al., 2008) in combination with fungicides like mefenoxam and fludioxonil. In field crops like corn and soybean, these seed treatments are aimed at controlling belowground soil pests, such as white grubs (family: Scarabaeidae), wireworm (*Melanotus communis* Gyllenhal) and seedcorn maggot (*Delia platura* Meigen), and seedling diseases caused

by *Fusarium* spp., all of which are difficult to monitor as their populations are spatially variable and difficult to predict (Boerma et al., 2004; Sappington et al., 2018). Systemic neonicotinoid seed treatments are also meant to target corn rootworm (CRW) (*Diabrotica virgifera* LeConte and *D. barberi* Smith & Lawrence), and protect young soybean plants from foliar pests such as soybean aphid (*Aphis glycines* Matsumura) (Hodgson et al., 2012). However, the insecticide only remains in plant tissue for roughly two to three weeks (Alford and Krupke, 2017), and soybean aphids typically only exceed economic thresholds in late July or August and occur sporadically in fields at levels that impact yield (Seagraves and Lundgren, 2012); therefore, it has a narrow early season control window. Nevertheless, the marked increase in seed treatment usage is partly due to the rising demand for field crop commodities, their ease of application, and targeted delivery compared to conventional alternatives that are more toxic to vertebrates, i.e. foliar-applied organophosphates and pyrethroids (Jeschke et al., 2011; Simon-Delso et al., 2015).

While studies have shown yield benefits of seed treatments in certain regions of the U.S. with more predictable pest pressures (North et al., 2016), employing them ubiquitously in field crops has proven to have variable returns on productivity (Bredeson and Lundgren, 2015; Hurley and Mitchell, 2017; Mourtzinis et al., 2019). There is also mounting evidence of unintended consequences of pesticidal seed treatments on pollinators (Krupke et al., 2012; Mullin et al., 2010; Wu et al., 2011), aquatic systems (Bonmatin et al., 2015; Morrissey et al., 2015), and importantly, natural enemies in agroecosystems (Douglas et al., 2015; Jin et al., 2013; Krupke et al., 2017; Nettles et al., 2016).

Vital entomopathogens and predatory arthropods that aid in pest regulation are particularly sensitive to agricultural management practices (Clifton et al., 2015; Patterson et al., 2019; Pretorius et al., 2018). Soilborne entomopathogenic fungi (EPF) are widespread in most

terrestrial settings and their capability as biological control agents is largely determined by the density and movement of insect hosts, and abiotic conditions, i.e. temperature and moisture (Meyling and Eilenberg, 2007). Their distribution may also be altered by plant identity (Randhawa et al., 2018) as they also function as endophytes (Behie et al., 2015; Gan et al., 2017) and show rhizosphere competence (Wyrebek et al., 2011). In laboratory settings, fungicides have proven to have deleterious effects on the growth and sporulation of EPF (Celar and Kos, 2016), but the influence of insecticidal and fungicidal foliar sprays and seed treatments on soilborne EPF in the field remains largely unknown. Generalist arthropod predators, such as carabid beetles, greatly contribute to the control of aphids and slugs, and epigeal spiders can exert additional pressure on pests in combination with other natural enemies (Michalko et al., 2019; Sunderland, 1999). A meta-analysis revealed that the neonicotinoid portion of the seed treatment package can reduce arthropod predator populations in corn and soybean by up to 16% (Douglas and Tooker, 2016). These results are comparable to the negative effects that foliar pyrethroid insecticides had on predator populations, which generally have been well documented (El-Wakeil et al., 2013; Geiger et al., 2010; Pfiffner and Luka, 2003). A more recent meta-analysis demonstrated that neonicotinoids reduced non-target arthropod performance across broad ecological metrics including abundance, behavior, and reproductive success (Main et al., 2018).

While pesticidal seed treatments can play an important role in insect pest management within an IPM framework, their current use pattern in corn and soybean across the U.S. poses limits on other IPM principles such as scouting and economic thresholds. This complexity of ubiquitously employing seed treatments within a field crop IPM program is poorly understood and may restrict the ability of practitioners to meet pest management goals without introducing non-target effects on beneficial organisms both above and belowground. The aim of this study

was to determine the ways in which preventative deployment of insecticides and fungicides impact biological control compared to a scouting-based IPM approach. I expected that a preventative management approach, involving pesticidal seed treatments and foliar sprays, would limit the activity-density and abundance of beneficial soil surface predators and microbes more than a scouting-based IPM approach that would use pesticide applications only when necessary. I also expected that untreated controls, with no pesticide inputs, would have higher activity-densities and abundances of surface predators and soil microbes.

2. Materials and methods

2.1. Site description and experimental design

The field experiment was located at the Cornell AgriTech Research Farm in Geneva, New York (42° 86' N, 77° 02' W, 135 m elevation). The climate of central NY is continental with a mean annual precipitation of 850 mm and mean monthly temperatures ranging from -0.3 C° (January) to 21.7°C (July). The dominant soil type at the research farm is Lima (Fine-loamy, mixed, semi-active, mesic Oxyaquic Hapludalfs) (Carr et al., 1910). Soil texture is loam with spatial variability in sand (range of 32 -37%), silt (range of 41-43%) and clay (range of 21-24%).

The experiment was conducted across three growing seasons (2017-2019) in a corn – soybean – corn rotation, a typical dairy production system in the Northeastern United States. Three treatments were evaluated:

- (1) Preventative pest management (Prev) with pesticide-treated seed and foliar pesticide applications
- (2) Integrated pest management (IPM) with untreated seed and foliar insecticide or fungicide applications based on scouting and economic thresholds

- (3) Untreated controls receiving untreated seed and no foliar pesticide applications

The study was replicated on three fields that were in alfalfa for four years prior to conversion into corn. Alfalfa has been shown to improve biological and physical aspects of soil quality (Clark, 2007) and is one of the most frequently grown forage legumes and highest yielding perennial forage crop grown in New York State (USDA/NASS, 2018). All fields were managed under conservation tillage practices, a common soil-health building tactic of leaving at least 30% or more of crop plant residue on the soil surface to reduce runoff and soil erosion (Magdoff and van Es, 2000). Each field was approximately 1.01 ha for a total experimental area of 3.03 ha. Field 1 was 2.4 km north of fields 2 and 3, which were separated by roughly 20 m. In field 1 and 2, 30 m x 20 m plots were established and in field 3 the plots were 20 m x 20 m; each plot was comprised of 24 rows of either corn or soybean. In field 1, plots were surrounded by 4 m buffers of bare ground and in fields 2 and 3 by 2 m buffers running east to west. All samples were taken in a defined 10 m x 10 m area within each plot to minimize edge effects. The identity of each plot was maintained over the course of the experiment to allow for the accumulation of treatments over time.

2.2. Crop management practices

2.2.1. *Corn 2017*

On June 6th of 2017 fields were chisel plowed, disked, and cultimulched. Corn (var: 552GR Doeblers, Agrisure® GT) was planted on June 13th at a density of 69,750 seeds per ha⁻¹ with 19 cm seed spacing and 76.2 cm spaced rows, along with an application of 22.7 kg ha⁻¹ starter fertilizer (ammonium sulfate, ammonium phosphate, potassium sulfate). Fertilizer rates were determined via soil sampling and following recommendations in the Cornell Guide for

Integrated Field Crop Management (Cox et al., 2017). Glyphosate (Roundup WeatherMAX[®], Monsanto Company, St. Louis, MO, USA) in the form of potassium salt was applied six weeks after planting at a rate of 0.65 L ha⁻¹ to suppress weeds; fields were side-dressed on July 31st with 19 kg ha⁻¹ of ammonium nitrate.

Preventative plots were planted with seeds precoated with a mixture of the systemic insecticide thiamethoxam (0.25 mg a.i.⁻¹; Cruiser[®] 5FS, Syngenta, Greensboro, NC, USA) and the contact fungicide fludioxonil and the systemic fungicides thiabendazole, mefenoxam, azoxystrobin (Maxim Quattro[®], Syngenta, Greensboro, NC, USA). On August 2nd plots received midseason foliar applications of a pyrethroid insecticide (lambda-cyhalothrin, Warrior II[®], 0.08 L ha⁻¹) and strobilurin fungicide (pyraclostrobin, Headline AMP[®], 0.65 L ha⁻¹).

Both IPM and control plots were planted with untreated seed. Additionally, IPM plots were scouted every 7-10 d throughout the entire growing season to determine if economic thresholds of insect pests and fungal pathogens had been met (Cornell Guide for Integrated Field Crop Management, 2017). These pests included wireworm (*M. communis* Gyllenhal), seed corn maggot (*D. platura* Meigen), white grubs (family: Scarabeidae), black cutworm (*Agrotis ipsilon* Hufnagel), fall armyworm (*Spodoptera frugiperda* J.E. Smith) and corn earworm (*Helicoverpa zea* Boddie); and fungal diseases such as common rust (*Puccinia sorghi* Schr.), northern leaf blight (*Exserohilum turcicum* Pass.), grey spot (*Cercospora zea-maydis* Tehon and Daniels) and anthracnose (*Colletotrichum graminicola* Wilson).

All plots were hand harvested on October 25th. Due to late planting and field conditions, only fields 1 and 2 were harvested at full physiological maturity. I followed a standard protocol: number of plants and ears in two 4.04 m rows chosen at random within the defined sample area, fresh weight of total number of ears per 4.04 m , and a subset of 10 randomly chosen ears to

determine moisture content (Lauer, 2002). Yield was calculated by the formula: [(pounds of harvested ear corn per plot) / (115)] x 1000 = estimated kilograms per hectare. After harvest, I employed a fall tillage practice at a depth of 27.9 cm to alleviate subsurface compaction.

2.2.2. Soybean 2018

On May 26th of 2018 fields were chisel plowed and disked twice. Soybean (var: SG 2125 Early Group II Seedway, Genuity[®] Roundup Ready 2 Yield[®]) was planted on May 31st at a planting density of 322,767 seeds per ha⁻¹ with 19 cm seed spacing and 76.2 cm spaced rows, along with an application of 308 kg ha⁻¹ starter fertilizer (ammonium phosphate, potassium sulfate). Fertilizer rates were determined via soil sampling and following recommendations in the 2017 Cornell Guide for Integrated Field Crop Management (Cox et al., 2017). Glyphosate (Roundup WeatherMAX[®], Monsanto Company, St. Louis, MO, USA) was applied a rate of 0.65 L ha⁻¹ to suppress weeds.

Preventative plots were planted with seeds precoated with CruiserMaxx[®]Vibrance[®] Beans (Syngenta, Greensboro, NC, USA), which includes the systemic insecticide thiamethoxam (class neonicotinoid, 0.25 mg a.i.⁻¹ seed), the fungicides mefenoxam 3.13%, fludioxonil 1.04%, sedaxane 1.04%, and a bacterial inoculant (*Bradyrhizobium japonicum* strain TA-11 Nod+, Urbana Laboratories, St. Joseph, MO, USA). On August 6th preventative plots received midseason foliar applications of a pyrethroid insecticide (lambda-cyhalothrin, Warrior II[®], 0.08 L ha⁻¹) and strobilurin fungicide (pyraclostrobin, Headline AMP[®], 0.65 L ha⁻¹).

Both IPM and control plots were planted with untreated seed and IPM plots were scouted every 7-10 d throughout the growing season for economic thresholds for insect pests and fungal pathogens were followed based on recommendations in the 2017 Cornell Guide for Integrated

Field Crop Management. These pests included wireworm (*M. communis* Gyllenhal), seed corn maggot (*D. platura* Meigen), white grubs (family: Scarabaeidae), and bean leaf beetle (*Cerotoma trifurcate* Forster); and fungal diseases such as downy mildew (*Peronospora manshurica* Naumov), frogeye leaf spot (*Cercospora sojina*) and Phytophthora root and stem rot (*Phytophthora* spp.). Due to a Japanese beetle (*Popillia japonica*) outbreak (30% defoliation after bloom) (Hodgson et al., 2011), IPM plots received a midseason foliar application of pyrethroid insecticide (lambda-cyhalothrin, Warrior II®, 0.08 L ha⁻¹) at the same time as Preventative plots.

All plots were hand harvested on October 19th following a standard protocol for soybean yield estimates (Casteel, 2012). The number of pods were counted along a row length of 53.3 cm (3 to 4 plants) at 3 random locations chosen at random within the defined sample area, and the average number of seeds per pod was calculated from ten random pods. Yield was calculated by the formula: [(number of pods per plot x average number of seeds per pod)]/ (seed size factor of 18) = estimated kilograms per hectare. Soybean crop residue was left on the soil surface over winter to minimize soil disturbance as part of the conservation tillage practice (Shen et al., 2018; Turmel et al., 2015).

2.2.3. Corn 2019

On May 23rd fields were chisel plowed and on June 8th disked. Corn (var: 45T05, Organic Silage Hybrid) was planted on June 10th at a density of 69,750 seeds per ha⁻¹ with 19 cm seed spacing and 76.2 cm spaced rows, along with an application of 36 kg ha⁻¹ starter fertilizer (ammonium sulfate, ammonium phosphate, potassium sulfate). Fertilizer rates were determined via soil sampling and following recommendations in the 2017 Cornell Guide for Integrated Field

Crop Management (Cox et al., 2017). A mixture of pre-emergent herbicides was applied on June 15th, Atrazine[®] (Drexel Chemical Company, Memphis, TN, USA), Prowl[®] H2O (BASF Corporation, NC, USA) and Dual Magnum[®] (Syngenta, Greensboro, NC, USA), at a rate of 1.2 L ha⁻¹ to suppress weeds.

Due to a lack of available untreated glyphosate tolerant seed, pesticide-treated seed was prepared by the Cornell University Seed Improvement Program and based on EPA pesticide labels to achieve the appropriate amount of active ingredient per kernel. Seeds were treated with a mixture of the insecticide thiamethoxam (0.25 mg a.i.⁻¹; Cruiser[®] 5FS, Syngenta, Greensboro, NC, USA) and the contact fungicide fludioxonil and the systemic fungicides thiabendazole, mefenoxam, azoxystrobin (Maxim Quattro[®], Syngenta, Greensboro, NC, USA). Preventative plots were planted on June 10th at the same time as IPM and control plots. On August 7th plots received midseason foliar applications of a pyrethroid insecticide (lambda-cyhalothrin, Warrior II[®], 0.08 L ha⁻¹) and strobilurin fungicide (pyraclostrobin, Headline AMP[®], 0.65 L ha⁻¹).

Both IPM and control plots were planted with untreated seed and IPM plots were scouted for pests as described above (2.2.1). All plots were hand harvested on October 23rd following a standard protocol: number of plants and ears in two 4.04 m rows chosen at random within the defined sample area, fresh weight of total number of ears per 4.04 m , and 10 randomly chosen ears to determine moisture content (Lauer, 2002). Yield was calculated by the formula [(pounds of harvested ear corn per plot) / (115)] x 1000 = estimated kilograms per hectare.

2.3. Pesticide residue analysis

In 2018 and 2019, at eight weeks after planting, five replicate cores per plot were taken at plow depth with an Oakfield Apparatus soil probe and sieved at 8 mm. A 10 g subsample of

fresh soil from each plot was sent to the Cornell University Chemical Ecology Core Facility for analysis (Cornell University, Comstock Hall, Ithaca, NY 14853). Samples were analyzed with a modified QuEChERS method and screened for thiamethoxam and one of its primary metabolites, clothianidin, by liquid chromatography tandem mass spectrometry (LC-MS/ MS) (Appendix A). Values are expressed as $\mu\text{g g}^{-1}$ or ppb.

2.4. Biological control potential

2.4.1. *Epigeic predatory invertebrates*

To assess the influence of the treatments on the composition of soil-surface arthropod predators, pitfall traps were installed (2 per plot, 7 m apart) and opened for 48 h at two time points in the season, either Midseason (MID) or End of Season (END): August 14th and October 18th in 2017, June 27th and September 17th in 2018, and July 19th and September 18th in 2019. The traps were comprised of a 500 ml plastic deli container (11.5 cm diameter x 8 cm height Reynolds Del Pak[®]). Each trap received 250 ml 50:50 propylene glycol solution during trapping events. Pieces of wood (roughly 20 x 30.5 cm²) supported by stainless steel nails (20 cm) were elevated roughly 10 cm above each trap to serve as a cover while traps were open to reduce any possible accumulation of excess water during rain events. After 48 h of exposure, the two samples from each plot were retrieved, combined, strained through a fine mesh (1 mm) and transferred in 95% ethanol into a single composite sample for later identification. This resulted in 15 individual samples per field (one composite per plot) per sampling event.

Depending on the taxon, predatory arthropods were identified to species, family or order. Carabid beetles, representing more than 5% of the total invertebrates collected, were identified to

species (Bousquet, 2010) and spiders were identified to family (Bradley et al., 2012). All other arthropods were identified using Borror and DeLong Key for Insects (Triplehorn et al., 2005).

2.4.2. Predation

To assess predation dynamics at the soil surface, I employed a standard sentinel bait assay using larvae of the greater wax worm, (*Galleria mellonella* L.) (Brust et al., 1986). The timing of this bioassay was aligned with pitfall trap sampling to best link the specimens captured with predation measurements and occurred on August 17th and October 13th in 2017, June 26th and September 12th in 2018, and July 18th and September 16th in 2019. In each plot, eight larvae were pinned to the soil surface with No. 1 insect pins (BioQuip, Rancho Dominguez, CA, USA) through the final abdominal segment and equally spaced approximately 2 - 3 m apart. Every other larva was enclosed in a cylindrical, hard-ware cloth cage (9.5 cm tall, 11.5-cm diameter, mesh size 1.3 cm) topped with a plastic deli container lid to exclude vertebrates, for a total of four caged and four uncaged larvae in each plot (Douglas et al., 2015; Lundgren et al., 2006). This allowed me to assess % predation of larvae by invertebrates compared to vertebrates. On each sampling date, I made two assessments of predator activity from 8:00-11:00 and 20:00-23:00. After each 3-hour period, larvae were recorded as either eaten, partially eaten, or not eaten. The identity of any predators seen near or feeding on larvae at the time of collection was recorded.

2.4.3. Entomopathogenic fungi

Belowground biological control potential was quantified using the ‘*Galleria* bait method’ to quantify the abundance of entomopathogenic fungi (EPF) (Zimmermann, 1986). Baiting with

the larvae of *Galleria mellonella* is a common tool for assessing native populations of EPF in managed and unmanaged systems (Jabbour and Barbercheck, 2009; Randhawa et al., 2018). Soil samples were collected at two time points between June and October in each year of the experiment on August 9th and October 16th in 2017, June 25th and September 14th in 2018, and July 18th and September 17th in 2019. Six cores (6 cm diameter, 6.3 cm depth) were taken using a turf plugger (Turf-Tec International®, Tallahassee, FL, USA) at random locations within the sampling area in each plot. Samples were stored in coolers in the field and air-dried upon return to the laboratory until further analysis.

Approximately 100 g of soil from each plot was placed in 500 ml plastic deli containers (11.5 cm wide, 8 cm tall Reynolds Del Pak®) and rewetted to 40% water holding capacity (16 ml-20 ml distilled water) to avoid infection by entomopathogenic nematodes. Larvae of the 3rd and 4th instar were used for baiting samples. Prior to baiting, larvae were heat treated in a beaker with 500 ml of water at 56°C for 10 seconds to prevent the production of silk webbing (Meyling, 2007). Ten larvae were placed in each container, sealed with a perforated lid, and incubated in the dark at ambient room temperature (20-25° C) for up to 21 days. Throughout the bioassay the containers were inverted daily to ensure adequate soil contact with the larvae. After the first week, the soil was inspected for dead cadavers every 3 days until all 10 larvae were removed. Cadavers were briefly surface sterilized in 95% ethanol for 30 seconds and 1% sodium hypochlorite for 60 seconds to prevent the growth of external saprophytic fungi, rinsed twice in distilled water, and then placed on wetted filter paper in petri dishes to be monitored for infection by EPF. Sporulating cadavers were identified to genus based on morphological characteristics of the fungal conidia, as described by Humber, 1997. The percentage (%) of larvae infected by EPF was used as a proxy for biological control potential in soils (Gan and Wickings, 2017).

2.5. Statistical analyses

The overall experiment was laid out in a randomized block design with five replicates per treatment: 15 plots per field and 45 plots total. Data from each year was analyzed separately (within-year analysis) as well as summed across years to determine cumulative effects (across-year analysis) of management programs across the three-year study.

For within-year analysis, each of the three years was analyzed separately. In 2017 and 2019, due to low pest pressure there were no inputs to the IPM plots, thus they were identical to the untreated controls; these data were combined as the ‘Untreated’ plots when analyzing the first and third year of the study individually. For the cumulative analysis, the three treatments were summed for each replicate plot individually across years: pitfall trap data were summed into a combined total, predation data on caged *G. mellonella* in the morning and evening were summed and the cumulative percentage was calculated by the number of larvae consumed out of 12 (4 per replicate plot each year tallied across all three years), and EPF infection data were summed and the cumulative percentage calculated by the number of infected larvae of 30 (10 per replicate plot each year tallied across three years).

Linear mixed effects models were used for within-year and across-year analyses to determine if treatment influenced predator activity-density, predation, and EPF. For all predator activity-density and EPF models, fixed effects were treatment, field and season with block as a random effect. For within-year predation models, fixed effects were treatment, field, season, cage and time with block as a random effect, and cross-year predation models had treatment, field and season as fixed effects and block as a random effect. For predator activity-density I assumed normally distributed residuals and data were log transformed if assumptions of

normality were unmet. With the predation and EPF models I used a logit transformation on the proportion of *Galleria mellonella* eaten or infected (Warton and Hui, 2011). To identify significant effects, type III analysis of variance (anova) was used with Satterwaite's method to calculate degrees of freedom. All statistical analyses were performed in R 3.6.1 (R Core Team, 2019) using the lme4 and lmerTest packages for linear mixed effects models (Bates et al., 2015; Kuznetsova et al., 2017).

3. Results

3.1. Pest populations for three-year study

In both 2017 and 2019, there were no pests in IPM plots that warranted a pesticide application based on scouting every 7-10 days throughout the growing season. However, in 2018 leaf damage from Japanese beetle (*Popillia japonica*) reached the treatment threshold of 30% defoliation after bloom (Fig. 1.1) and a foliar application of pyrethroid insecticide (lambda-cyhalothrin, Warrior II®, 0.08 L ha⁻¹) was applied in IPM plots at the same time as preventative plots (Hodgson et al., 2011).

3.2. Pesticide residues

Soil pesticide residues were sampled 8 weeks after planting in 2018 and 2019 for the neonicotinoid thiamethoxam and clothianidin, a metabolite of thiamethoxam. Averaged untreated control and IPM plots planted with untreated seed had very low or no detectable amounts of thiamethoxam and clothianidin (Table 1.1). Preventative plots planted with 0.25 mg a.i.⁻¹ seed had values ranging from 0-1 ng g⁻¹ for thiamethoxam and 1 ng g⁻¹ clothianidin in 2018, and 2-4 ng g⁻¹ thiamethoxam and 2 ng g⁻¹ clothianidin in 2019 (Table 1.1).

3.3. Biological control potential for entire three-year study

3.3.1 *Epigeic predatory invertebrates*

A total of 3,292 predatory macro-arthropods were collected in pitfall traps in all three years of the study. The most commonly collected arthropod taxa were Araneae, Carabidae, Formicidae, Opiliones, Staphylinidae, Lithobiomorpha and Anthicidae, which comprised 31.8%, 30%, 15.6%, 9.2%, 6.8%, 3.9%, and 1.2% of the total amount, respectively. I collected 1,033 spiders in total and 94.5% of adult spiders were represented by three families: Lycosidae (78.6%) Linyphiidae (9.6%), and Corinnidae (6.3%). Additional spider families collected included Theridiidae, Thomisidae, and Tetragnathidae and comprised less than 5% of the total. Of the 977 adult carabids collected over the entire study 93.1% were represented by ten species: *Poecilus chalcites* (Say) (32%), *Harpalus pennsylvanicus* (DeGreer) (15.4%), *Clivina fossor* (Linnaeus) (11.3%), *Pterostichus melanarius* (Illiger) (8.2%), *Poecilus lucublandus* (Say) (7.0%), *Bembidion quadrimaculatum* (Say) (5.7%), *Anisodactylus sanctaecrucis* (Fabricius) (5.4%), *Trechus quadristriatus* (Schränk) (3.3%), *Abacids permundus* (Say) (2.8%), and *Tachyura anceps* (LeConte) (2.2%).

Effects of treatment, time of season and field on predator activity-density

There was no cumulative effect of treatment on total predators over the course of the three-year experiment ($F_{2,60} = 0.18$, $P = 0.83$, Table 1.2). There was a significant effect of time of season with higher total predators midseason compared to the end of the season ($F_{1,60} = 227.72$, $P < 0.001$) and a season*field interaction with higher total predators midseason in field 1 ($F_{2,60} = 9.5$, $P < 0.001$). There was a significant treatment effect on the total number of spiders ($F_{2,72} =$

3.05, $P = 0.05$, Table 1.2). I found higher numbers of spiders in untreated control plots compared to preventative plots that received seed treatments and foliar sprays, and significantly higher numbers of spiders midseason overall ($F_{1,72} = 24.49$, $P < 0.001$). I also found significantly higher numbers of centipedes at the end of the season ($F_{1,72} = 6.76$, $P = 0.01$) and a treatment*time of season interaction with significantly higher numbers in untreated controls compared to preventative plots at the end of the season ($F_{2,72} = 3.51$, $P = 0.03$, Table 1.2). There was also a field effect for centipedes with higher numbers in field 1 compared to fields 2 and 3 ($F_{2,72} = 5.89$, $P < 0.01$, Table 1.2).

3.3.2 Predation

The most observed predators found feeding on *G. mellonella* were harvestmen, carabid beetles, spiders and ants. In all three years, there was a significant cumulative effect of cage with higher predation on uncaged larvae vs caged larvae (2017: $F_{1,312} = 40.59$, $P < 0.001$; 2018: $F_{1,252} = 130.06$, $P < 0.001$; 2019: $F_{1,273} = 22.43$, $P < 0.001$). Given this cage effect and that I was interested in the impact of treatment primarily on invertebrate soil surface predators, further analyses on cumulative predation and predation by year were carried out on caged larvae only (cages prevented vertebrate predators, but not invertebrate predators).

There was no cumulative effect of treatment on a.m. predation ($F_{2,60} = 0.64$, $P = 0.52$, Table 1.2), but there was a significant effect of both time of season ($F_{1,60} = 57.72$, $P < 0.001$) and field ($F_{2,12} = 4.78$, $P = 0.03$) and an interaction of time of season*field with higher predation midseason in field 1 ($F_{2,60} = 4.59$, $P = 0.01$). While there was also no overall cumulative effect of treatment ($F_{2,60} = 0.19$, $P = 0.82$, Table 1.2), season ($F_{1,60} = 1.33$, $P = 0.25$) or field ($F_{2,12} = 2.41$, $P = 0.13$) on p.m. predation, I found a treatment*field interaction with higher predation in

preventative plots ($35\% \pm 2.7$) compared to IPM plots ($25\% \pm 2.5$) in field 3 ($F_{4,60} = 2.80$, $P = 0.03$).

3.3.3. *Entomopathogenic fungi*

Over the course of the study there were two genera of soil-borne EPF that were recovered via baiting, *Metarhizium* and *Beauveria*. For *Metarhizium* there was no cumulative treatment ($F_{2,72} = 1.51$, $P = 0.22$, Table 1.2) or field effect ($F_{2,72} = 1.47$, $P = 0.24$), but I found marginally significant higher infection potential midseason compared to the end of the season ($F_{1,72} = 2.87$, $P = 0.09$). I also found no cumulative treatment effects for *Beauveria* ($F_{2,60} = 1.15$, $P = 0.32$, Table 1.2) and the opposite trend in seasonal pathogenicity with higher infection at the end of the season ($F_{1,60} = 8.89$, $P < 0.01$). There was also lower cumulative infection potential of *Beauveria* in field 3 compared to fields 1 and 2. ($F_{2,12} = 3.79$, $P = 0.05$).

3.4 Biological control potential within-years

3.4.1. *Epigeic predatory invertebrates*

I collected 1,076 predators in 2017, 558 in 2018 and 1,658 in 2019. In 2017, there was a significant main effect of time of season on the average total number of predators with higher captures at the end of season ($F_{1,66} = 9.45$, $P = 0.003$, Table 1.3); in 2018 and 2019 I found the opposite trend with significantly higher captures during the midseason sampling event (2018: $F_{1,36} = 137.10$, $P < 0.001$; 2019: $F_{1,78} = 251.28$, $P < 0.001$, Table 1.3). In all three years, I found a significant main effect of season on total average number of spiders with higher captures midseason (2017: $F_{1,78} = 14.89$, $P < 0.001$; 2018: $F_{1,60} = 11.96$, $P = 0.001$; 2019: $F_{1,38} = 253.80$, $P < 0.001$). Spiders and carabids had notably higher activity-densities in 2017 and 2019 (Table 1.3).

The main effect of treatment on the average total number of predators was significant in 2017 ($F_{1,66} = 8.18$, $P = 0.005$, Fig. 1.2a) with lower captures in preventative plots, but this effect was not significant in 2018 or 2019 ($F_{2,36} = 0.04$, $P = 0.96$; $F_{1,78} = 1.98$, $P = 0.16$, Fig. 1.2b-c). In 2017, there was a significant effect of treatment on the total average number of spiders ($F_{1,78} = 10.38$, $P = 0.001$) with lower captures in preventative plots. The interaction of treatment*season was significant in 2017 with higher captures of spiders in untreated plots midseason ($F_{1,78} = 7.53$, $P = 0.007$, Fig. 1.1a). This effect was driven by spiders in the family Lycosidae, which were negatively impacted by treatment midseason ($F_{1,66} = 8.51$, $P = 0.005$). There was also a significant treatment*field interaction for Lycosidae with higher totals in untreated plots in field 3 ($F_{2,66} = 3.29$, $P = 0.04$). There was no effect of treatment on total spiders in 2018 ($F_{2,60} = 0.3$, $P = 0.96$, Table 1.3; Fig. 1.3b).

In 2019, there was a significant main effect of treatment on the total average number of spiders ($F_{1,39} = 4.33$, $P = 0.04$) with higher captures in preventative plots; the inverse of my findings in 2017. The interaction of treatment*season was also significant with higher captures in preventative plots midseason ($F_{1,39} = 5.39$, $P = 0.02$, Fig. 1.3c); again, this trend was driven by Lycosidae, which were higher in preventative plots (17.60 ± 2.33) compared to untreated plots midseason (13.16 ± 1.13) ($F_{1,27} = 5.54$, $P = 0.02$).

3.4.2. Predation

The predation activity of soil surface arthropods varied by time each year with significantly higher predation at night during the 20:00pm-23:00pm time period than in the morning during 8:00am-11:00am time period (2017: $F_{1,312} = 7.04$, $P = 0.008$; 2018: $F_{1,252} = 6.17$, $P = 0.01$; 2019: $F_{1,273} = 41.63$, $P < 0.001$).

In 2017, there were no differences between midseason (0.10 ± 0.01) or end of season (0.14 ± 0.02) predation, while in 2018, I found higher predation at the end of the season (0.12 ± 0.02) compared to mid (0.07 ± 0.01), and in 2019, higher predation midseason (0.41 ± 0.03) compared to the end (0.20 ± 0.02) (2017: $F_{1,136} = 1.7$, $P = 0.18$; 2018: $F_{1,108} = 4.6$, $P = 0.03$; 2019: $F_{1,136} = 46.97$, $P < 0.001$). In 2017, there was a significant treatment*time interaction with a low and similar level of predation in untreated and preventative treatments in the morning compared with a significantly higher overall level of predation at night, with more predation in untreated plots than treated ones ($F_{1,136.7} = 4.14$, $P = 0.04$, Fig. 1.4a). In 2018 and 2019, there was no main effect of treatment on the predation of caged larvae, but overall there was significantly higher predation at night (2018: $F_{1,108} = 59.8$, $P < 0.001$, Fig. 1.4b; 2019: $F_{1,136} = 69.65$, $P < 0.001$, Fig. 1.4c).

In 2019, there was a treatment*field interaction with higher predation in preventative plots in field 3 ($F_{2,126} = 4.63$, $P = 0.01$) and a treatment*time*field interaction with higher predation in preventative plots at night in field 3 ($F_{2,136} = 2.93$, $P = 0.05$). There was also a marginally significant treatment*season*time*field interaction with higher predation in preventative plots midseason at night in field 3 ($F_{2,136} = 2.42$, $P = 0.09$).

3.4.3. *Entomopathogenic fungi*

In 2017, there was a marginally significant treatment*season*field interaction for *Metarhizium* with higher infection of *G. mellonella* in untreated plots midseason in field 2 ($F_{2,78} = 2.66$, $P = 0.07$). In 2018 and 2019, infection did not differ among treatments (2018: $F_{2,72} = 0.37$, $P = 0.68$; 2019: $F_{1,66} = 1.07$, $P = 0.30$). There were no effects of treatment on *Beauveria* in

2017 or 2018 (2017: $F_{1,78} = 0.85$, $P = 0.35$; 2018: $F_{2,72} = 0.44$, $P = 0.64$), and infection was present in very low numbers in 2019, therefore the data were not analyzed.

There were seasonal trends with significantly higher infection by *Metarhizium* midseason (14.22 ± 2.21) compared to end of season (3.55 ± 1.27) in 2017 ($F_{1,78} = 18.26$, $P < 0.001$), although this trend of EPF pathogenicity was not present in 2018 or 2019 (2018: $F_{1,72} = 0.47$, $P = 0.49$; 2019: $F_{1,66} = 1.75$, $P = 0.19$). Conversely, in 2017 there was significantly higher infection by *Beauveria* at the end of season (9.77 ± 1.86) compared to midseason (1.33 ± 0.60), and I found the same trend in 2018, higher infection by *Beauveria* end of season (0.13 ± 0.02) compared to midseason (0.06 ± 0.01) (2017: $F_{1,78} = 20.35$, $P < 0.001$; 2018: $F_{1,72} = 6.51$, $P = 0.01$).

3.5. Overall yield estimates

In each year, there was no effect of treatment on yield (2017: $F_{2,24} = 0.53$, $P = 0.59$; 2018: $F_{2,24} = 0.28$, $P = 0.75$; 2019: $F_{1,27} = 0.03$, $P = 0.95$, Fig. 1.5a-c). The average yield estimate for corn across all treatments was 174 bu/ac in 2017 and 193 bu/ac in 2019. This was higher than the 2017 and 2019 Ontario county, New York, USA average for corn grain; 157 bu/ac and 177 bu/ac, respectively (USDA/NASS, 2019) (Fig. 1.5a-c). In 2018, soybean yield estimates were 32 bu/ac, lower than the 45 bu/ac average for Ontario County in 2018 (Barrett, 2018) (Fig. 1.5b).

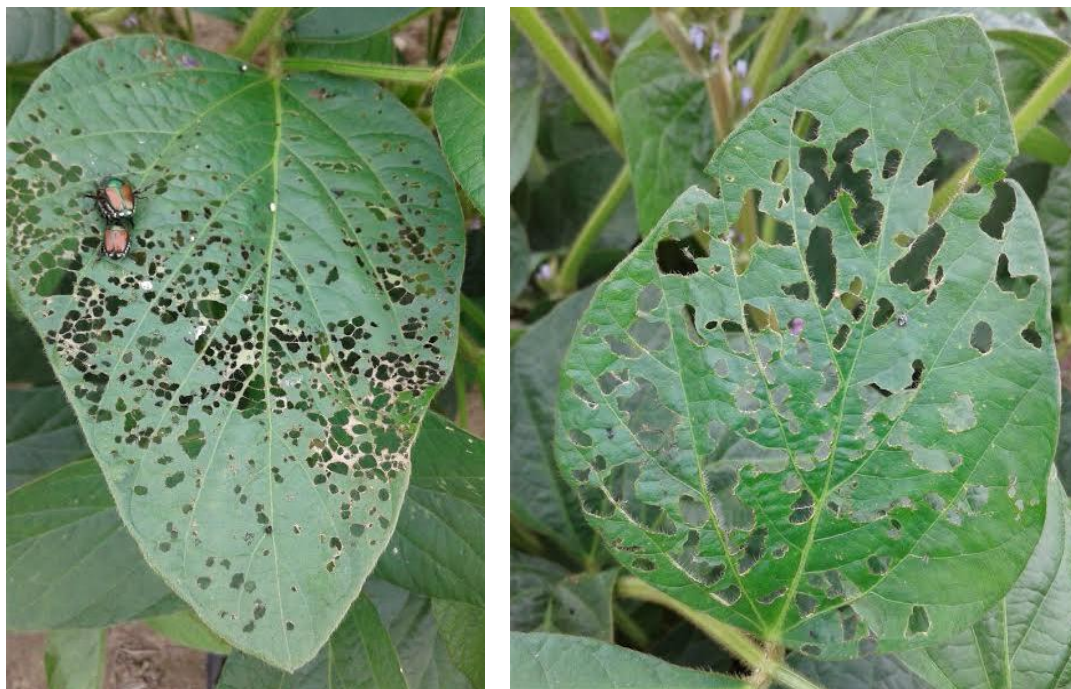


Figure 1.1. Skeletonization of soybean leaves by Japanese beetle in 2018. Fields reached >30% foliar damage post bloom, triggering the use of a foliar insecticide in IPM plots.

Table 1.1. Soil pesticide residues, thiamethoxam (ng g⁻¹) and clothianidin (ng g⁻¹), by year and field (\pm SE). Fields were sampled 8 weeks after planting in 2018 and 2019.

2018	Field	Control	IPM	Preventative
Thiamethoxam	1	0.09 (0.09)	0.13	1.07 (0.13)
	2	0	0	1.12 (0.42)
	3	0	0	0.06 (0.06)
Clothianidin	1	0.28 (0.02)	0.30 (0.05)	0.99 (0.13)
	2	0.14 (0.09)	0.21 (0.07)	1.48 (0.47)
	3	0.04 (0.04)	0.09 (0.05)	0.95 (0.10)
2019				
Thiamethoxam	1	0	0.02 (0.02)	3.31 (1.23)
	2	0.03 (0.03)	0	4.25 (0.51)
	3	0.02 (0.02)	0.02 (0.02)	1.68 (0.55)
Clothianidin	1	0.20 (0.09)	0.29 (0.07)	1.71 (0.34)
	2	0.21 (0.14)	0.14 (0.09)	2.33 (0.43)
	3	0.08 (0.08)	0.08 (0.08)	2.35 (0.25)

Table 1.2. Cumulative averages (individuals two traps⁻¹ \pm SE) of predator activity-densities of major taxa, predation of caged *G. mellonella* in the morning (AM) and at night (PM), and infection potential of two genera of soil-borne entomopathogenic fungi, *Metarhizium* and *Beauveria*, by season and treatment over the course of the three-year experiment (2017-2019). Values marked with different letters within a taxon are significantly different based on Tukey's post-hoc tests ($p < 0.05$).

	TAXA	Midseason			End of Season		
		Untreated	IPM	Prev	Untreated	IPM	Prev
Predator Activity-Density	Lycosidae	15.40 (1.99)	15.33 (1.77)	18.40 (2.28)	1.07 (0.28)	0.87 (0.29)	1.13 (0.27)
	Linyphiidae	1.93 (0.37)	1.80 (0.38)	1.67 (0.27)	0.27 (0.15)	0.53 (0.22)	0.47 (0.19)
	Corinnidae	1.60 (0.29)	1.67 (0.49)	1.00 (0.32)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Total Araneae	7.73 (1.33) a	6.67 (0.71) ab	4.73 (0.46) b	3.73 (0.47)	3.53 (0.51)	3.27 (0.46)
	Carabidae	14.87 (1.51)	15.60 (2.30)	15.53 (2.00)	7.40 (0.88)	6.07 (0.80)	6.73 (0.64)
	Formicidae	18.33 (1.63)	19.87 (1.61)	23.13 (1.76)	3.87 (0.82)	3.33 (0.59)	3.27 (0.70)
	Opiliones	3.80 (0.66)	4.07 (0.73)	4.07 (0.56)	4.87 (0.98)	4.27 (0.78)	3.73 (0.69)
	Staphylinidae	2.80 (0.45)	1.93 (0.42)	1.73 (0.43)	0.87 (0.27)	1.27 (0.32)	0.60 (0.16)
	Lithobiidae	0.60 (0.21)	1.13 (0.17)	1.07 (0.30)	2.73 (0.90) a	1.67 (0.43) ab	1.07 (0.34) b
	Anthicidae	0.60 (0.21)	1.00 (0.14)	0.93 (0.28)	0.13 (0.09)	0.00 (0.00)	0.00 (0.00)
	Total Predators	48.93 (2.57)	50.27 (3.28)	51.60 (3.31)	24.27 (2.23)	20.53 (1.68)	19.07 (1.49)
Predation	% consumed	Untreated	IPM	Prev	Untreated	IPM	Prev
	AM	14.44 (2.87)	13.33 (3.13)	12.78 (1.97)	3.89 (1.60)	1.11 (0.76)	2.78 (1.33)
	PM	25.56 (4.18)	27.22 (2.50)	26.11 (3.24)	30.00 (3.33)	29.44 (1.97)	27.22 (3.40)
EPF	% infection	Untreated	IPM	Prev	Untreated	IPM	Prev
	<i>Metarhizium</i>	9.78 (1.54)	10.00 (1.69)	8.89 (1.83)	10.67 (2.04)	5.33 (1.48)	7.56 (1.64)
	<i>Beauveria</i>	3.56 (0.95)	2.89 (1.02)	8.89 (0.90)	6.67 (1.72)	4.67 (1.55)	7.56 (1.36)

Table 1.3. Seasonal averages (individuals two traps⁻¹ \pm SE) of major arthropods captured via pitfall trapping by year. Carabid beetle species representing >5% of the total amount are included in the table. Values marked with different letters within a taxon and within each year are significantly different based on Tukey's post-hoc tests ($p < 0.05$).

	2017		2018		2019	
TAXA	MID	END	MID	END	MID	END
Lycosid	1.38 (0.22) a	0.38 (0.09) b	0.36 (0.09)	0.33 (0.10)	14.64 (1.12) a	0.31 (0.08) b
Linyphiidae	1.40 (0.19) a	0.24 (0.08) b	0.11 (0.05)	0.09 (0.04)	0.29 (0.08) a	0.09 (0.06) b
Corinnidae	0.31 (0.08) a	0.00 (0.00) b	0.40 (0.12) a	0.00 (0.00) b	0.71 (0.17) a	0.00 (0.00) b
Carabidae	2.02 (0.25)	2.67 (0.29)	3.20 (0.32) a	0.64 (0.11) b	10.11 (1.00) a	3.42 (0.32) b
<i>Pterostichus melan.</i>	0.62 (0.15)	0.58 (0.11)	0.11 (0.05)	0.18 (0.07)	0.07 (0.05) b	0.22 (0.06) a
<i>Bembidion quad. opp.</i>	0.27 (0.09)	0.27 (0.09)	0.53 (0.10) a	0.00 (0.00) b	0.16 (0.06)	0.02 (0.02)
<i>Poecilus lucublandus</i>	0.31 (0.08)	0.11 (0.06)	0.47 (0.12) a	0.00 (0.00) b	0.60 (0.11) a	0.02 (0.02) b
<i>Clivina fossor</i>	0.31 (0.07)	0.44 (0.11)	0.22 (0.07) a	0.00 (0.00) b	0.04 (0.03)	0.02 (0.02)
<i>Harpalus penn.</i>	0.00 (0.00) b	0.38 (0.11) a	0.00 (0.00)	0.00 (0.00)	0.18 (0.07) b	2.47 (0.28) a
<i>Poecilus chalcites</i>	0.00 (0.00)	0.00 (0.00)	0.38 (0.09) a	0.00 (0.00) b	6.42 (0.96) a	0.02 (0.02) b
<i>Anisodactylus sanct.</i>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.38 (0.24) a	0.00 (0.00) b
Formicidae	3.09 (0.48)	2.42 (0.34)	1.67 (0.22) a	0.71 (0.13) b	1.78 (0.24)	1.58 (0.21)
Opiliones	0.27 (0.10) b	3.98 (0.48) a	1.58 (0.22) a	0.16 (0.05) b	0.38 (0.11) a	0.11 (0.05) b
Staphylinidae	0.60 (0.12)	0.64 (0.15)	1.18 (0.16) a	0.16 (0.05) b	2.13 (0.26) a	0.16 (0.05) b
Lithobiidae	0.09 (0.04) b	1.78 (0.36) a	0.60 (0.15) a	0.09 (0.04) b	0.44 (0.10)	0.44 (0.14)
Anthicidae	0.00 (0.00)	0.00 (0.00)	0.64 (0.09) a	0.00 (0.00) b	0.20 (0.08) a	0.04 (0.03) b
Total Predators	9.60 (0.68) b	13.02 (0.97) a	9.93 (0.63) a	2.16 (0.23) b	30.73 (1.72) a	6.11 (0.49) b

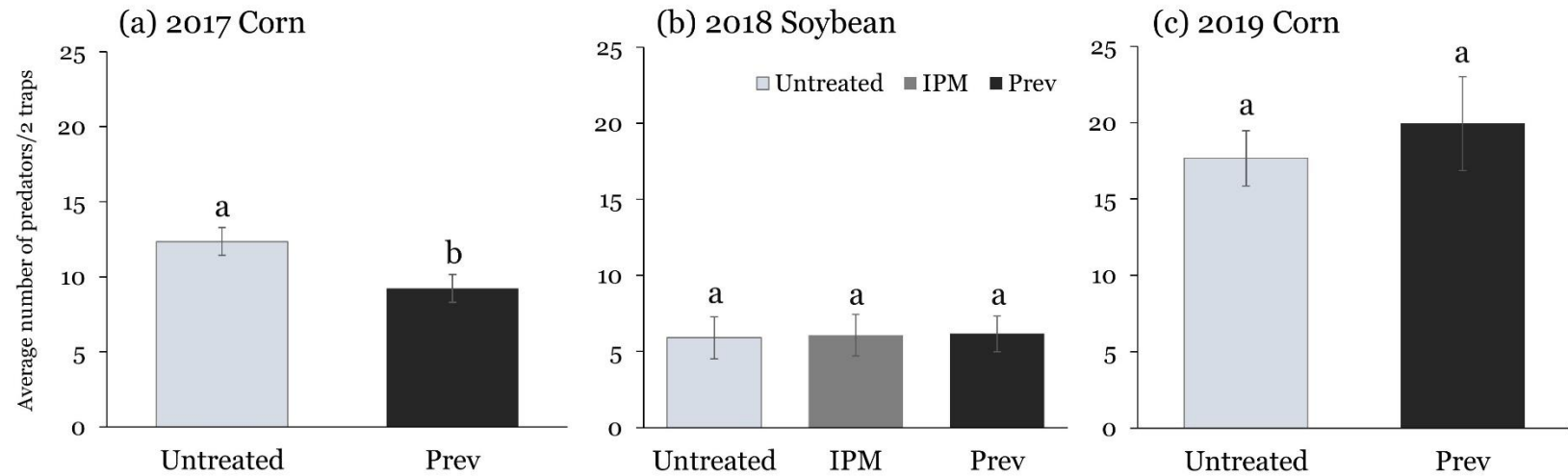


Figure 1.2. Effect of pesticidal seed treatment and foliar sprays (Prev – preventative treatment) on total predator activity-density (mean \pm SE) by year. In 2017 (a) and 2019 (c), Untreated and IPM plots received no pesticide inputs and therefore, were averaged together and listed as Untreated. In 2018 (b), IPM plots received a foliar spray application only. For each year, different letters indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$). Total predator activity-density was negatively impacted by seed treatments and sprays in the first year of the study.

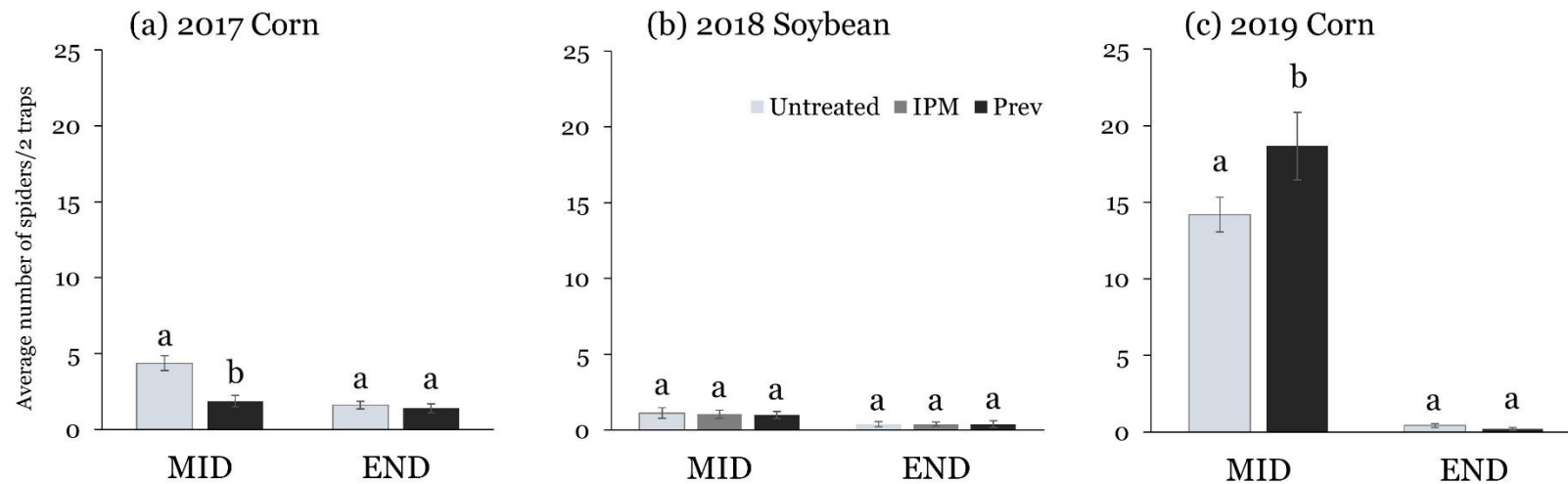


Figure 1.3. Effect of pesticidal seed treatment and foliar sprays (Prev – preventative treatment) on total spider activity-density (mean \pm SE) by season and year. In 2017 (a) and 2019 (c), Untreated and IPM plots received no pesticide inputs and therefore, were averaged together. In 2018 (b), IPM plots received a foliar spray application only. Different letters indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$). Total spider activity-density was negatively impacted by seed treatments and sprays midseason in the first year of the study.

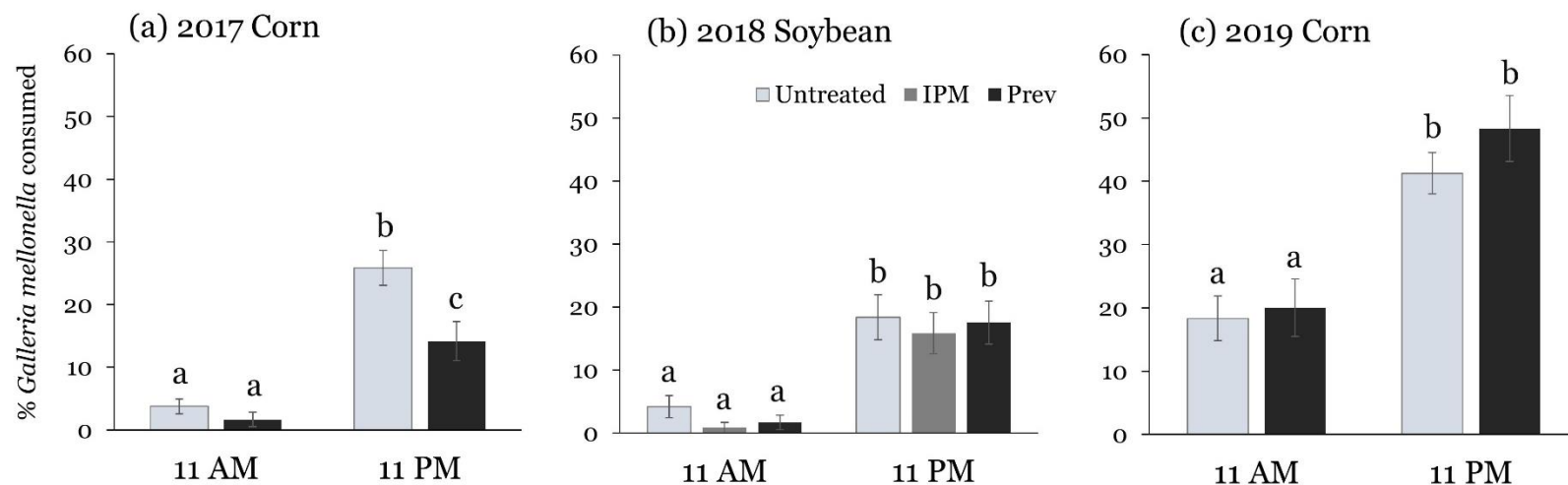


Figure 1.4. Effect of pesticidal seed treatment and foliar sprays (Prev – preventative treatment) on the predation of caged *G. mellonella* (mean \pm SE) during a 3hr observation window (8:00-11:00 = 11AM; 20:00-23:00 = 11PM) by year. In 2017 (a) and 2019 (c), Untreated and IPM plots received no pesticide inputs and therefore, were averaged together. In 2018 (b), IPM plots received a foliar spray application only. Different letters indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$). Regardless of pesticidal treatment, predation was significantly higher at 11PM than at 11AM in all three years.

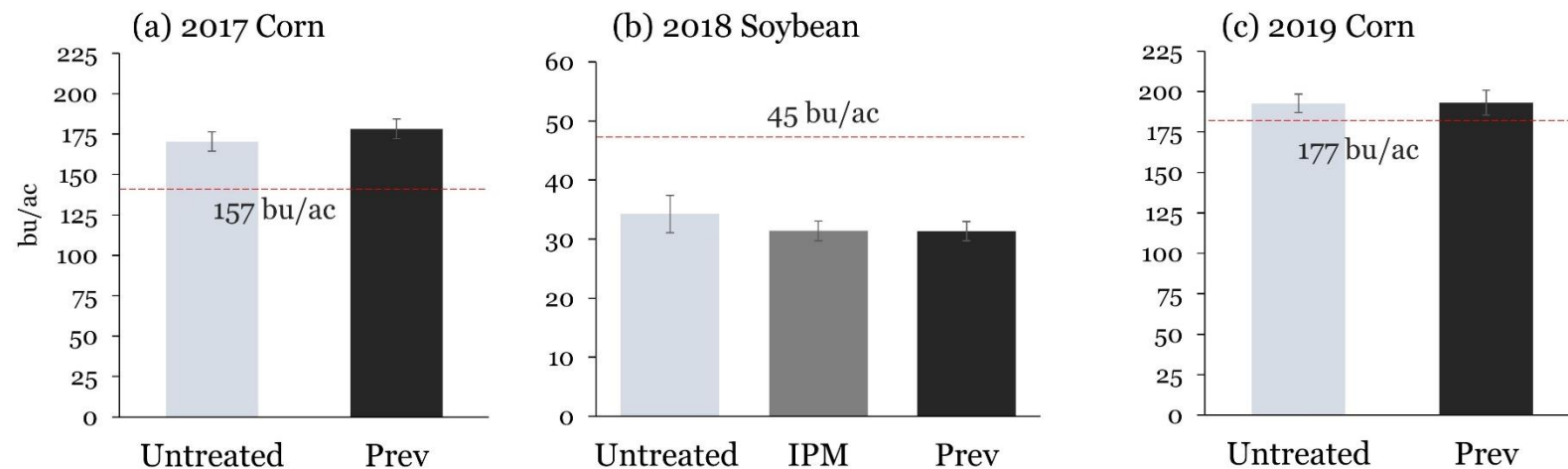


Figure 1.5. Effect of pesticidal seed treatment and foliar sprays (Prev – preventative treatment) on yield estimates for corn and soybean by year. In 2017 (a) and 2019 (c), Untreated and IPM plots received no pesticide inputs and therefore, were averaged together. In 2018 (b), IPM plots received a foliar spray application only. Different letters indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$). There was no effect of treatment on corn or soybean yield. Dashed lines indicate the average grain yield for corn or average soybean yield in Ontario County, New York State.

4. Discussion

The purpose of this study was to explore in a corn-soy cropping system the influence of a preventative pest management practice, i.e. seed treatments and foliar sprays, and a scouting-based IPM practice on soil biological control potential. A three-year field experiment (2017-2019) was conducted to compare the influence of these two approaches on aboveground soil surface predators and predation, and the belowground biological control potential of soil-borne entomopathogenic fungi.

With few exceptions, time of the season had the biggest influence on predator activity-density, predation, and entomopathogenic fungi. Over the course of the entire three-year study, total predator activity-density, morning predation, and *Metarhizium* infection was higher midseason compared to the end of the season, and *Beauveria* infection was higher at the end of season. The infectivity rates of EPF are known to be highly correlated with seasonal and yearly changes in temperature, moisture and relative humidity (Arthurs et al., 2001); all major factors affecting the ability of fungi to survive, propagate, infect and kill hosts (Hajek and Eilenberg, 2018). Within years, total predator activity-density was higher at the end of the season in 2017 and higher midseason in both 2018 and 2019. The seasonal fluctuation of predator activity-density in the first year of the study was most likely due to Opilionid and centipede (Lithobiidae) populations increasing from 12 midseason to 179 end of season, and 4 midseason to 80 end of season, respectively. Opilionid families that are commonly found in cropping systems, such as Phalangidae and Sclerosomatidae, typically have two to three generations per year with peaks in adult abundance occurring towards the end of August in both the Northeast and Southeast U.S. (Drummond et al., 2010; Newton and Yeagan, 2002). Seasonal variation found in pitfall captures may be influenced by tillage disturbance (Ellsbury et al., 1998), composition of predator

communities in surrounding landscapes and field margins (Öberg et al., 2007), as well as weed diversity (Pavuk et al., 1997). Given that important crop pests in corn and soybean usually inhabit fields between planting, when young seedlings are vulnerable, until approximately September in the Northeastern United States, it is important to consider the backdrop of natural enemy abundance when making management decisions as both seed treatments and sprays can influence biocontrol potential when it is most valuable for yield benefits.

Regardless of these seasonal patterns, there were treatment effects on the biological community that had important implications for biological control. While there were no cumulative effects of treatment on total predators, the overall activity-densities of spiders (both mid and end of season) and centipedes (end of the season) were significantly lower in preventatively managed plots compared to untreated controls. The cumulative result for spiders is most likely driven by the negative impact of foliar applications during midseason sampling in the first year of the study. In 2017, due to field conditions after a large rain event, I was unable to sample in July prior to the application of pyrethroid insecticides and strobilurin fungicides, and thus, unable to separate the effects of seed treatment and sprays. Midseason sampling took place in August one week after these sprays were applied and my results show significantly lower captures of spiders, specifically surface-active Lycosidae, in those preventatively treated plots. Previous studies show that cursorial hunting spiders are highly sensitive to pyrethroid insecticides (Baatrup and Bayley, 1993; Devotto et al., 2007) and that their biocontrol efficacy is greatest during months when temperatures range from 25 - 30°C, most suitable for breeding and when most pest populations are at their highest due to increased food availability (Saleem Khan et al., 2017). Lycosid spiders reduce populations of sucking herbivores, i.e. thrips (family: Thripidae) and aphids, and densities of Lepidoptera eggs and larvae through direct feeding

(Pfannenstiel, 2008) and non-consumptive effects, i.e. behavioral changes of pests in response to risk that either expose them to more predators or reduce foraging to lower detectability (Rypstra and Buddle, 2013). Considering that lycosids were the most abundant spider captured in pitfall traps in 2017, it is possible that the midseason preventative foliar sprays have the potential to suppress an important biological control agent during its most effective biocontrol window. Despite there being no cumulative or within year correlations between the activity-density of total predators and the predation of *G. mellonella*, in 2017, I found significantly higher predation in untreated plots at night, when lycosids are most active (Bradley et al., 2012).

In 2019, spiders were again significantly impacted by treatment, but with higher numbers in preventative plots compared to untreated plots, this response being driven again by lycosids with significantly higher captures in preventative plots midseason. I also found significantly higher predation in preventative plots midseason at night, although this effect only emerged in one field. A critical difference between the patterns observed in 2017 and 2019 was that foliar applications were not made prior to sampling in 2019, therefore this time point captures the effect of seed treatment only. This positive response to a pesticidal seed treatment across all fields may be the result of cascading trophic interactions. There is evidence to show that seed treatments may increase the surface activity of soil fauna, such as collembola, by either repelling them to the soil surface to avoid areas with insecticide-treated seed or through a reduction in predatory mites, common collembolan predators, at the soil surface (El-Naggar et al., 2013; Gan and Wickings, 2017). A recent study found that seeds coated only with fungicides increased collembola surface activity by 250% (Zaller et al., 2016). The potential of seed treatments to influence the movement and activity of collembola, a high value food source for lycosids, (Lawrence and Wise, 2000; Nyffeler and Benz, 1988) could explain their increased activity-

density and abundance in preventative plots. Further research is needed on the behavioral response of collembola to pesticidal seed coatings to elucidate potential trophic responses and their implications for biological control. Regardless of the mixed responses of spiders within individual years, my findings suggest that preventative pest management programs suppress spider activity-density at the soil surface.

The substantial decline in total predator-activity in 2018 could reflect a difference in crop type or an outcome of employing a fall tillage practice directly after corn harvest in November of 2017 at a depth of 27.9 cm. While meant to alleviate subsurface compaction that occurred from intensive heavy traffic on wet soil, it is possible that soil inversion buried crop residues and directly impacted species of Carabid beetles that are exclusive autumn breeders, such as *Pterostichus melanarius* and other overwintering populations (Purvis and Fadl, 2002). There was a considerable increase in total predator activity-density in 2019 that may have resulted from leaving all fields uncultivated at the end of 2018, allowing soybean residue to remain at the soil surface. Crop residues may have been conducive to the survival of overwintering predators leading to higher captures in the subsequent year (Wardle, 1995). Two carabid beetles, *Poecilus chalcites* and *Harpalus pennsylvanicus*, that were rarely captured in the first two years, dominated the carabid community in the third year. *Poecilus chalcites* totals were higher midseason and *H. pennsylvanicus* totals were higher at the end of season. These seasonal differences align with their breeding cycles as *P. chalcites* breeds in the spring with peak densities from late June to the end of July and *H. pennsylvanicus* has peak densities in August and September and is rarely seen before July (Leslie et al., 2009). While *P. chalcites* was absent from traps in the first two years, other species dominated such as *Poecilus lucublandus*, *Pterostichus melanarius*, and *Clivina fossor*. This massive shift in community composition could

likely be a response to mechanical disturbance when the fields were converted into a cropping system after being in alfalfa for four years. Conceivably, the differences I found in carabid community structure across years could reflect this environmental disturbance, which has been shown in previous work (Athayde Tonhasca, 1993; Scott and Anderson, 2003; Thiele, 1977) and suggests that individual species vary temporally and spatially in response to disturbance.

Season was a main driver in the recovery of soil-borne entomopathogenic fungi both across and within years. In 2017, I found significantly higher infection by *Metarhizium* spp. midseason compared to the end of the season and *Beauveria* spp. were significantly higher at the end of the season. *Beauveria* spp were rarely recovered from soils in years two and three. *Metarhizium* is known to have higher reservoirs of fungal densities in managed cropping systems (Rudeen et al., 2013; Scheepmaker and Butt, 2010).

Despite there being no cumulative response to treatment, *Metarhizium* spp. had a negative response to pesticides in 2017 midseason one week after foliar sprays were applied. Previous work has shown that fungicides can have mixed effects on fungal persistence under field and laboratory conditions (Clifton et al., 2015; Mietkiewski et al., 1997). In a laboratory study, da Silva et al. (2013) measured the effects of four fungicides, including strobilurins, and four insecticides, including thiamethoxam, at recommended field dosages and found that all fungicides inhibited the conidial germination of *Metarhizium*.; the first step in fungal infection. Thiamethoxam was found to be compatible with *Metarhizium* although this study was in vitro and does not allow for the numerous variables associated with field studies. Given that thiamethoxam has shown compatibility with EPF and I did not see an effect from seed treatments in the subsequent two years when sampling prior to pesticide sprays, this suppression is most likely a fungistatic response to pyraclostrobin applied in preventative plots in 2017. While this is

further evidence that foliar sprays have the potential to suppress beneficial soil organisms during a critical biocontrol window, my findings suggest this response is highly variable by year.

Metarhizium spores also have a highly patchy, clustered distribution throughout the environment (Jabbour and Barbercheck, 2009; Meyling and Eilenberg, 2006), which may have masked any potential effects in additional years, thus, future studies should explore heterogeneity in the field to improve our understanding of how pest management practices may be influencing native EPF.

Regardless of the pest management tactic employed, there were no differences in yield estimates for either corn or soybean. Depending on year, I found that preventative foliar sprays used in tandem with seed treatments suppressed important biological control agents and did not improve crop productivity. This study adds to the growing body of literature suggesting that prophylactic pesticidal seed treatments are not necessary to produce higher yields in fields with non-economically damaging levels of pests and have unexpected indirect effects on above and belowground biological control.

5. Conclusions

Pesticidal seed treatments and foliar sprays together in a corn-soy production system negatively suppressed total predator activity-density, spider activity-density, predation, and the infection potential of soil-borne entomopathogenic fungi, compared with no pesticide use in one out of three years of the field study. In the third year, I found an increase in the activity-density of lycosid spiders across all fields in preventative plots that included seed treatments, but prior to the application of foliar sprays, suggesting a behavioral shift in response to seed treatments and the potential of seed treatments to influence above ground predators in indirect and unexpected ways. Overall, the use of pesticides did not improve crop yield in any year and the yield estimates for corn in both 2017 and 2019 were considerably higher than the yield averages for

this region of NY State. Given that pest populations were low in this experiment, future studies should investigate varying levels of pest pressure in a corn-soy cropping system. Taken all together, these findings highlight that the use of seed treatments within an IPM practice may not always be necessary for maximum crop productivity and when used in conjunction with foliar sprays might have unintended consequences on above and below ground soil biota.

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Appendix A

Supplemental methods of the modified QuEChERS method for pesticide residue analysis conducted by the Cornell University Chemical Ecology Core Facility.

Sample preparation

Soil samples were extracted by Five grams of fresh soil were extracted with 10 mL of acetonitrile/water (1:1, v/v) and vortexed for 20 s. After complete homogenization, 4 g MgSO₄ and 1 g NaCl were added. Samples were then shaken and centrifuged at $7300 \times g$ for 5 minutes. After centrifugation, 1000 μ l of supernatant was collected and transferred into a d-SPE (dispersive solid phase extraction) tube containing 150 mg PSA, 900 mg MgSO₄. After the d-SPE step, 490 μ L of sample was collected and 10 μ L of internal standard solution (d₃-thiamethoxam 0.6 ng/ μ L) was added. Samples were filtered (0.22 μ m, PTFE) and analyzed immediately by LC-ESI-MS/MS.

Liquid Chromatography and Mass Spectrometry

Sample analysis was carried out with a Vanquish Flex UHPLC system (Dionex Softron GmbH, Germering, Germany) coupled with a TSQ Quantis mass spectrometer (Thermo Scientific, San Jose, CA). The UHPLC was equipped with a Kinetex EVO C18 column (150 mm \times 2.1 mm, 2.6 μ m particle size). The mobile phase consisted of (A) Water 0.1% formic acid and (B) Acetonitrile 0.1% formic acid. The temperature of the column was maintained at 35°C throughout the run and the flow rate was set at 500 μ L/min. The elution program was the following: 2 min equilibration (5% B) prior to injection, 0-3 min (5%-70% B, linear gradient), 3-3.2 min (70%-95% B, linear gradient), 3.2-4.5 min (95% B, column wash), 4.5-5 min (95%-5%

B, linear gradient), 5-5.5 min (5% B, re-equilibration). The flow from the LC was directed to the mass spectrometer through a Heated Electrospray probe (H-ESI). The settings of the H-ESI were: spray voltage 4000 V, Sheath gas 50 (arbitrary unit), Auxiliary gas 14 (arbitrary unit), Sweep gas 1 (arbitrary unit), Ion transfer tube temperature 325°C, Vaporizer temperature 35°C.

Chapter Two

Pest management practices in field crops can alter soil decomposer abundances and activities

Abstract

There is an increased interest amongst field crop growers towards building healthy, biologically active soils to improve crop productivity. Simultaneously, growers must also manage for crop pests and there is mounting evidence that pest management practices can inhibit soil biotic activity. A three-year field experiment (2017-2019) was conducted in a corn-soy-corn rotation to compare the influence of a preventative pest management practice, with pesticide-treated seed and foliar sprays, and a scouting-based IPM practice on soil fertility (pH, organic matter, macro- and micro- nutrients), soil decomposers (microarthropod abundance) and biological activity (microbial extracellular enzymes and substrate decomposition). The aim of the study was to explore feedbacks between soil health and pest management tactics, and to measure the influence of pest management on soil fertility and soil biological function, specifically decomposition. Overall, there were limited changes to soil fertility between pest management practices, although higher levels of copper were observed in preventative plots and higher levels of manganese in IPM plots at the end of the study, depending on field. I also found an increase in % aggregate stability over the course of the experiment. Preventative management increased microbial enzyme activity and feeding activity, and both practices increased the abundance of decomposer microarthropods, depending on taxa and pesticide input. These findings highlight that the type of pest management practices employed in field crops have diverse impacts on soil community composition and function, but that responses vary by biotic compartment, season, and the specific active ingredients used. These results emphasize the need for further research exploring

the mechanisms underlying the responses of soil biota to pesticidal seed treatments and foliar applications in field crops.

1. Introduction

Field crop growers in the United States (U.S.) are increasingly aware of the benefits of implementing practices to enhance soil health. The phrase ‘soil health’ is often used interchangeably with ‘soil quality’ and has been defined as the continued capacity of soil to function as a vital biologically active ecosystem (USDA NRCS 2001). Concurrently, growers are also faced with making decisions to manage crop pests, but these decisions may have unintended negative consequences for the soil biological communities and critical ecological functions, including decomposition. While there is much evidence to show the negative impact of pesticides on soil biota, the ecological feedbacks between managing for soil health and the influence of differing pest management strategies on soil biological function are less understood.

Soil health broadly portrays soil as a living, dynamic ecosystem whose functions depend on a diversity of organisms requiring conservation and management (Doran and Zeiss, 2000). Soil organisms, such as bacteria, fungi, nematodes, collembola, mites, and earthworms, directly and indirectly improve nutrient cycling, soil structure and stability, increase soil water infiltration, and regulate pathogen pressure, all leading to sustainable crop growth (Briones, 2018; Coleman et al., 2018; Holloway et al., 2008; Pretorius et al., 2018; Wilson-Rummenie et al., 1999). To harness these ecological services, growers employ practices such as conservation tillage and crop rotations, as well as the incorporation of perennials, such as alfalfa and hay. Conventional tillage practices, such as moldboard plowing, are well known to have detrimental impacts on physicochemical and biological properties of soil (Hobbs et al., 2008); however, conservation tillage involves maintaining a soil surface cover through the retention of crop residues achieved through minimal mechanical soil disturbance. Growers also employ crop rotations to offset pest life cycles and provide effective control of crops pests such as corn

rootworm (*Diabrotica* spp.) and soybean cyst nematode (*Heterodera glycines*) (Furlan and Kreutzweiser, 2015; van Es and Magdoff, 2009). Although some variants of northern corn rootworm have adapted to rotations of corn (*Zea mays* L.) and soybean (*Glycine max* L.) by extending diapause for two years until fields are rotated back to corn, it generally still remains a useful tool for managing soil-dwelling root pests (Furlan and Kreutzweiser, 2015; Shrestha et al., 2018). An additional tactic is to incorporate perennial grasses and legumes into rotations as they allow for periods of reduced disturbance and stabilization of biogeochemical properties of soils, i.e. improved carbon (C) and nitrogen (N) stocks and soil bulk density (Karlen et al., 2013). For example, alfalfa has been shown to improve biological and physical aspects of soil quality (Clark, 2007) and is one of the most frequently grown forage legumes and highest yielding perennial forage crop grown in New York State (USDA/NASS, 2018). Moreover, it has been demonstrated that perennial crops improve soil biological traits by increasing densities of soil predators, parasitoids, and decomposers (Cardoza et al., 2015). Collectively, these practices can enhance the functioning of biological processes in agricultural soils, leading to greater resistance and resilience in the face of biotic and abiotic stressors.

Simultaneously, while growers are carrying out soil health practices, they may also be utilizing pest management tactics that have non-target effects on beneficial soil biota i.e. microbes and soil fauna directly responsible for maintaining soil health. In 2019, 36.3 and 30.8 million hectares of corn and soybean respectively were grown in the U.S. (USDA/NASS, 2019), with a large portion, 79-100% of corn and 34-44% of soybean, planted with pesticide-coated seed (Douglas and Tooker, 2015). These prophylactic uses of pesticides are aimed at targeting early season pests such as white grubs (family: Scarabeidae), wireworm (*Melanotus communis* Gyllenhal), seedcorn maggot (*Delia platura* Meigen), and *Fusarium* spp. (Boerma et al., 2004;

Sappington et al., 2018), and typically include the neonicotinoid insecticides imidacloprid, clothianidin, and thiamethoxam (Elbert et al., 2008) in combination with fungicides, e.g. mefenoxam and fludioxonil. Despite the widespread adoption of seed treatments, the crop pests targeted have high spatio-temporal variability across most regions in the U.S. and are not necessarily experienced annually at economically damaging populations (Boerma et al., 2004; Mourtzinis et al., 2019), meaning that seed treatments may not always provide a substantial benefit. Moreover, they are often used outside of an IPM framework, a practice using scouting and economic thresholds to make pest control decisions.

There is increasing evidence that the insecticides used in seed treatments can contribute to declines in soil biota and the loss of soil biological functioning (Atwood et al., 2018; de Lima e Silva et al., 2017; Hussain et al., 2009; Pisa et al., 2014), potentially diminishing gains achieved from soil health management. For example, Nettles et al. (2016), showed that seed treatments can alter soil microbial community structure, specifically rhizosphere fungi and bacteria. Additionally, a recent study revealed that seed treatments influence the abundance, richness, and diversity of all soil fauna guilds, particularly predatory and detritivorous microarthropods (Atwood et al., 2018). In addition to community traits, seed treatments may also alter soil animal behavior (Zaller et al. 2016). The impact of foliar sprays that are commonly used in field crops, such as pyrethroid insecticides and strobilurin fungicides, are also known to influence decomposers typically at or just beneath the soil surface (Majeed et al., 2018; Rieff et al., 2020). There is emerging evidence that collembola can increase in density after foliar applications of thiamethoxam (El-Naggar et al., 2013) and cypermethrin (Frampton, 1999) due to ecological release from the mortality of predatory Acari.

It is widely recognized that biologically active soils, with a diversity of soil biota that contribute decomposition and improved nutrient cycling, can lead to higher yields (Doran and Zeiss, 2000; van Es and Magdoff, 2009; Welbaum et al., 2004), and the ubiquitous use of preventative pesticide applications may constrain growers' abilities to build healthy soil. In this study, I explore the impact of two pest management practices: (1) a scouting-based IPM practice and (2) a preventative pest management practice, involving pesticidal seed treatments and foliar sprays, on measures of soil quality and beneficial decomposers, i.e. microarthropods and microbes. My hypothesis was preventative management would degrade soil quality and limit the abundance of decomposers and their activity, while a scouting-based IPM approach, using pesticide applications only when warranted by adhering to standard action thresholds for pests and pathogens, would have an intermediate impact on soil quality measures compared to untreated controls, and have higher abundances and activities of decomposers compared to preventative management.

2. Materials and Methods

2.1. Study site and experimental design

The field experiment was located at the Cornell AgriTech Research Farm in Geneva, New York (42° 86' N, 77° 02' W). The climate of central NY is continental with a mean annual precipitation of 850 mm and mean monthly temperatures ranging from -0.3 C° (January) to 21.7°C (July). The dominant soil type at the research farm is Lima (Fine-loamy, mixed, semi-active, mesic Oxyaquic Hapludalfs) (Carr et al., 1910).

The experiment was conducted for three growing seasons (2017 – 2019) in a corn – soy – corn rotation. Three treatments were evaluated:

- (1) Preventative pest management (Prev) with pesticide-treated seed and calendar-based foliar pesticide applications
- (2) Integrated pest management (IPM) with untreated seed and foliar insecticide or fungicide applications based on scouting and economic thresholds
- (3) Untreated controls receiving untreated seed and no foliar pesticide applications.

The study was carried on three fields, each approximately 1 ha in area, that were in alfalfa for four years prior to conversion into the corn-soy rotation. All fields were managed using conservation tillage practices for the duration of the study to reflect a common soil health building tactic of leaving at least 30% or more of crop plant residue on the soil surface to reduce runoff and soil erosion (Magdoff and van Es, 2000). Field 1 was 2.4 km north of fields 2 and 3, which were separated by roughly 20 m. In field 1 and 2, plots were 30 m x 20 and in field 3, plots were 20 m x 20 m to accommodate the slightly smaller field size; each plot was comprised of 24 rows of either corn or soybean. In field 1, plots were surrounded by 4 m buffers and in fields 2 and 3 by 2 m buffers running east to west. Sampling occurred within a defined 10 m x 10 m area within the center of each plot to minimize edge effects. The identity of each plot was maintained over the course of the experiment to allow for the accumulation of treatments over time.

2.2. Crop management practices

2.2.1. Corn 2017

On June 6th of 2017 fields were chisel plowed, disked, and cultimulched. Corn (var: 552GR Doeblers, Agrisure® GT) was planted on June 13th at a density of 69,750 seeds per ha⁻¹

with 19 cm seed spacing and 76.2 cm spaced rows, along with an application of 56 kg ha⁻¹ starter fertilizer (15-15-10). Fertilizer rates were determined via soil sampling and following recommendations in the Cornell Guide for Integrated Field Crop Management (Cox et al., 2017). Glyphosate (Roundup WeatherMAX[®], Monsanto Company, St. Louis, MO, USA) was applied six weeks after planting at a rate of 0.65 L ha⁻¹ to suppress weeds; fields were side-dressed on July 31st with 98.63 kg ha⁻¹ of ammonium nitrate.

Preventative plots were planted with seeds pre-coated with a mixture of the systemic insecticide thiamethoxam (0.25 mg a.i.⁻¹; Cruiser[®] 5FS, Syngenta, Greensboro, NC, USA) and the contact fungicide fludioxonil and the systemic fungicides thiabendazole, mefenoxam, azoxystrobin (Maxim Quattro[®], Syngenta, Greensboro, NC, USA). On August 2nd plots received midseason foliar applications of a pyrethroid insecticide (lambda-cyhalothrin, Warrior II[®], 0.08 L ha⁻¹) and strobilurin fungicide (pyraclostrobin, Headline AMP[®], 0.65 L ha⁻¹).

Both IPM and control plots were planted with untreated seed. Additionally, IPM plots were scouted every 7-10 d throughout the entire growing season to determine if economic thresholds of insect pests and fungal pathogens had been met (Cornell Guide for Integrated Field Crop Management, 2017). These pests included wireworm (*M. communis* Gyllenhal), seed corn maggot (*D. platura* Meigen), white grubs (family: Scarabeidae), black cutworm (*Agrotis ipsilon* Hufnagel), fall armyworm (*Spodoptera frugiperda* J.E. Smith) and corn earworm (*Helicoverpa zea* Boddie); and fungal diseases such as common rust (*Puccinia sorghi* Schr.), northern leaf blight (*Exserohilum turcicum* Pass.), grey spot (*Cercospora zea-maydis* Tehon and Daniels) and anthracnose (*Colletotrichum graminicola* Wilson).

All plots were hand harvested on October 25th. Due to late planting and field conditions, only fields 1 and 2 were harvested at full physiological maturity. I followed a standard protocol:

number of plants and ears in two 4.04 m rows chosen at random within the defined sample area, fresh weight of total number of ears per 4.04 m , and a subset of 10 randomly chosen ears to determine moisture content (Lauer, 2002). Yield was calculated by the formula: [(pounds of harvested ear corn per plot) / (115)] x 1000 = estimated kilograms per hectare. After harvest, I employed a fall tillage practice at a depth of 27.9 cm to alleviate subsurface compaction.

2.2.2. Soybean 2018

On May 26th of 2018 fields were chisel plowed and disked twice. Soybean (var: SG 2125 Early Group II Seedway, Genuity® Roundup Ready 2 Yield®) was planted on May 31st at a planting density of 322,767 seeds per ha⁻¹ with 19 cm seed spacing and 76.2 cm spaced rows, along with an application of 302 kg ha⁻¹ starter fertilizer (0-20-20). Fertilizer rates were determined via soil sampling and following recommendations in the 2017 Cornell Guide for Integrated Field Crop Management (Cox et al., 2017). Glyphosate (Roundup WeatherMAX®, Monsanto Company, St. Louis, MO, USA) was applied a rate of 0.65 L ha⁻¹ to suppress weeds.

Preventative plots were planted with seeds precoated with CruiserMaxx®Vibrance® Beans (Syngenta, Greensboro, NC, USA), which includes the systemic insecticide thiamethoxam (class neonicotinoid, 0.25 mg a.i.⁻¹ seed), the fungicides mefenoxam 3.13%, fludioxonil 1.04%, sedaxane 1.04%, and a bacterial inoculant (*Bradyrhizobium japonicum* strain TA-11 Nod+, Urbana Laboratories, St. Joseph, MO, USA). On August 6th preventative plots received midseason foliar applications of a pyrethroid insecticide (lambda-cyhalothrin, Warrior II®, 0.08 L ha⁻¹) and strobilurin fungicide (pyraclostrobin, Headline AMP®, 0.65 L ha⁻¹).

Both IPM and control plots were planted with untreated seed and IPM plots were scouted every 7-10 d throughout the growing season for economic thresholds for insect pests and fungal

pathogens were followed based on recommendations in the 2017 Cornell Guide for Integrated Field Crop Management. These pests included wireworm (*M. communis* Gyllenhal), seed corn maggot (*D. platura* Meigen), white grubs (family: Scarabeidae), and bean leaf beetle (*Cerotoma trifurcate* Forster); and fungal diseases such as downy mildew (*Peronospora manshurica* Naumov), frogeye leaf spot (*Cercospora sojina*) and Phytophthora root and stem rot (*Phytophthora* spp.). Due to a Japanese beetle (*Popillia japonica*) outbreak (20% defoliation after bloom) (Hodgson et al., 2011), IPM plots received a midseason foliar application of pyrethroid insecticide (lambda-cyhalothrin, Warrior II®, 0.08 L ha⁻¹) at the same time as the application made in the Preventative treatment.

All plots were hand harvested on October 19th following a standard protocol for soybean yield estimates (Casteel, 2012). The number of pods were counted along a row length of 53.3 cm (3 to 4 plants) at 3 random locations chosen at random within the defined sample area, and the average number of seeds per pod was calculated from ten random pods. Yield was calculated by the formula: [(number of pods per plot x average number of seeds per pod)]/ (seed size factor of 18) = estimated kilograms per hectare. Soybean crop residue was left on the soil surface over winter to minimize soil disturbance as part of the conservation tillage practice (Shen et al., 2018; Turmel et al., 2015).

2.2.3. Corn 2019

On May 23rd fields were chisel plowed and on June 8th disked. Corn (var: 45T05, Organic Silage Hybrid) was planted on June 10th at a density of 69,750 seeds per ha⁻¹ with 19 cm seed spacing and 76.2 cm spaced rows, along with an application of 237 kg ha⁻¹ starter fertilizer (19-19-19). Fertilizer rates were determined via soil sampling and following recommendations in the

2017 Cornell Guide for Integrated Field Crop Management (Cox et al., 2017). A mixture of pre-emergent herbicides was applied on June 15th, Atrazine[®] (Drexel Chemical Company, Memphis, TN, USA), Prowl[®] H2O (BASF Corporation, NC, USA) and Dual Magnum[®] (Syngenta, Greensboro, NC, USA), at a rate of 1.2 L ha⁻¹ to suppress weeds.

Due to a lack of available untreated glyphosate tolerant seed, pesticide-treated seed was prepared by the Cornell University Seed Improvement Program and based on EPA pesticide labels to achieve the appropriate amount of active ingredient per kernel. Seeds were treated with a mixture of the insecticide thiamethoxam (0.25 mg a.i.⁻¹; Cruiser[®] 5FS, Syngenta, Greensboro, NC, USA) and the contact fungicide fludioxonil and the systemic fungicides thiabendazole, mefenoxam, azoxystrobin (Maxim Quattro[®], Syngenta, Greensboro, NC, USA). Preventative plots were planted on June 10th at the same time as IPM and control plots. On August 7th plots received midseason foliar applications of a pyrethroid insecticide (lambda-cyhalothrin, Warrior II[®], 0.08 L ha⁻¹) and strobilurin fungicide (pyraclostrobin, Headline AMP[®], 0.65 L ha⁻¹).

Both IPM and control plots were planted with untreated seed and IPM plots were scouted for pests as described above (2.2.1). All plots were hand harvested on October 23rd following a standard protocol: number of plants and ears in two 4.04 m rows chosen at random within the defined sample area, fresh weight of total number of ears per 4.04 m, and 10 randomly chosen ears to determine moisture content (Lauer, 2002). Yield was calculated by the formula [(pounds of harvested ear corn per plot) / (115)] x 1000 = estimated kilograms per hectare.

2.3. Soil Sampling

2.3.1. Soil Health

Whole field soil health samples were taken on May 17th in 2017, one month after the termination of alfalfa and prior to the establishment of experiment plots, to determine baseline values for physical, biological, and chemical soil traits. Five replicate cores (6 cm diameter, 6.3 cm depth) were taken at random locations within each field and then analyzed by the Cornell Soil Health Laboratory using the Cornell Assessment of Soil Health (CASH) protocol (Moebius-Clune et al., 2016). Measured physical indicators included wet aggregate stability (AgSt), available water capacity (AWC), surface hardness (SH) and subsurface hardness (SSH); biological indicators included total organic matter (OM), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial respiration; and chemical indicators measured were pH, extractable P, extractable K, Mg, Fe, Mn and Zn. All measurements were performed in the laboratory except for SH and SSH, which were assessed in the field using a soil penetrometer. Briefly, percent wet aggregate stability was measured using a rainfall simulation method, which delivers rain drops of a known kinetic force on a known weight of soil aggregates (0.25 – 2.0 mm in size) for five minutes (Moebius et al., 2007; Ogden et al., 1997); the fraction of soil that remains after simulation is used to calculate percent wet aggregate stability; AWC was determined using a soil moisture apparatus that equilibrates the soil to pressures of -10 and 1500kPa (field capacity and permanent wilting point, respectively), the gravimetric water content difference between the two was regarded as AWC (Reynolds and Topp, 2008) ; OM was determined by percent loss on ignition at 500 C° for 2 – hours (Storer, 1984); AC was determined by the permanganate oxidation method (Weil et al., 2003); PMN was measured using a seven – day anaerobic incubation technique as described by Drinkwater et al. (1996); and soil respiration was measured over a four – day incubation period using modified methods by Haney and Haney (2010) and Zibilske (1994). In addition, soil texture was measured using a rapid

quantitative method developed by Kettler et al. (2001). Chemical elements were determined according to the routine procedures of the Cornell Nutrient Analytical Laboratory outlined below. Scoring functions were used to interpret the measured physical and biological indicator values, standardized on a 0 to 100 scale (Fine et al., 2017).

To determine effects of treatment on a subset of soil health indicators over the duration of the experiment, soil samples were collected from each plot prior to planting in June 2017 and at the end of the experiment in October 2019. Five replicate cores were taken at plow depth with an Oakfield Apparatus soil probe and sent to the Cornell Nutrient Analytical Laboratory (Cornell University, 804 Bradfield Hall, Ithaca, NY 14853) for testing using standard procedures. Briefly, samples were analyzed for pH measured in a 1:1 slurry with water using a standard pH tester with refillable, double junction glass bulb pH electrodes (LIGNIN, LLC, Albuquerque, NM), organic matter content by percent weight loss on ignition at 500 C° for 2 hr (Storer, 1984); phosphorus (P) using the Modified Morgan method (1.25 M ammonium acetate (NH₄OAc), pH 4.8) (McIntosh, 1969); and plant available nutrients (K, Mg, Fe, Mn, Zn) measured on an inductively-coupled plasma (ICP) mass spectrometer (SPECTRO Analytical Instruments Inc., Mahwah, NJ). All nutrient values were expressed as milligrams kilogram⁻¹ dry soil. Additionally, measurements for AgSt were taken at the end of the experiment to elucidate changes in soil structure in response to the potential effects of pest management on soil biological function. Given that the sampling took place at the beginning of the experiment prior to the establishment of treatments, only soil fertility at end of the experiment was analyzed for treatment effects.

2.4. Decomposer activity and abundance

2.4.1. Soil Sampling

To measure microbial enzyme activity and characterize soil microarthropod abundance, twelve soil cores (6 cm diameter, 6.3 cm depth) were collected from each plot using a turf plugger (Turf-Tec International®, Tallahassee, FL, USA) at random locations in 2 rows within the 10 m² sampling area of each plot. Samples were collected at two time points in the season: Midseason (MID) and End of Season (END), corresponding to August 6th and October 16th in 2017, June 25th and September 14th in 2018, and July 18th and September 17th in 2019. Six soil cores for microbial enzymes and six cores for microarthropods from each plot were placed into separate bags that were stored in coolers in the field until they were brought back to the laboratory. A subset of the bulked soil, approximately 20g, was sieved at 4 mm and frozen at -20°C for extracellular enzyme analyses.

2.4.2. Enzyme activity

In years two and three, I measured the potential activity of 5 microbial extracellular enzymes using protocols outlined by Saiya-Cork (2002) and Wickings and Grandy (2011). I assessed three hydrolytic enzymes, N-acetyl-βD-glucosaminidase (NAG), β-glucosidase (BG) and acid phosphatase (PHOS) and two oxidative enzymes, phenol oxidase (POX) and peroxidase (PER). Soil slurries were created by mixing a 1 g soil subsample (taken from bulked and sieved soil cores collected from each plot and stored at -20°C) with 120 mL sodium acetate buffer using a blender for 30 s. Hydrolytic enzyme activities were measured on black 96 well plates with substrates containing the fluorescent compound methylumbelliferone (MUB) and oxidative enzymes were measured using clear 96 well plates, with the substrate L-3,4-dihydroxyphenylalanine (L-DOPA) alone for POX and L-DOPA plus 0.3% hydrogen peroxide for PER. Hydrolytic enzyme plates were incubated for 3–4 h and oxidative enzyme plates were

incubated for 22–24 h. All hydrolytic enzyme plates were analyzed via fluorometry at 360 nm excitation and 460 nm emission wavelengths and oxidative enzyme plates were analyzed via spectroscopy at a 450 nm absorbance wavelength using a microplate reader (Synergy, BioTek Instruments, Winooski, VT, United States). Potential enzyme activity for each substrate was calculated as nmol of substrate $\text{h}^{-1} \text{g}^{-1}$ dry soil.

2.4.2. Microarthropods

Immediately after sampling, soil cores were brought back to the laboratory and placed onto modified Berlese funnels for heat extraction of microarthropods. The temperature of extraction began at 30°C and was increased by 10°C over a 3-day period. Specimens were stored in 95% ethanol until further identification. Mites were identified to order or cohort following taxonomy in the Key to Major Mite Taxa (Walter, 2005) and only adult stages of mites were tallied with the exception of phoretic immature astigmatid mites (hypopi). Collembola were identified to family using Borror and DeLong Key for Insects (Triplehorn et al., 2005). After samples were removed from funnels, soil dry mass was determined each sample. The abundance of microarthropods is reported as number of individuals kg^{-1} dry soil.

2.4.3. Bait lamina strips

The bait lamina strip method was developed in 1990 by Von Törne to quantify feeding activity of soil meso- and macro- fauna (Von Törne, 1990). More recent studies have concluded that the primarily contributors to lamina strip consumption include mites, collembolans, enchytraeids, and earthworms (Hamel et al., 2007; Rozen et al., 2010; Simpson et al., 2012). Lamina strips were composed of plastic plant labeling stakes (Pylon® sourced from Growers

Solution, 16 cm long and 0.5 cm wide) with 16 holes, 5 mm apart, drilled vertically starting 100 mm from the top of each strip. A homogenous substrate was prepared by hand mixing 6.5 g of cellulose fiber (Vital Nutrients® Middletown, CT), 1.5 g agar-agar powder (Alfa Aesar, Thermo Scientific, Tewksbury, MA), 1.0 g bentonite (Fulkerson Winery Supplies, Dundee, NY) and 1.0 g unprocessed wheat bran (Bob's Red Mill Natural Foods®, Milwaukie, OR) with distilled water until the mixture formed a paste. The holes within each lamina strip were then filled manually and allowed to fully dry before additional applications due to shrinkage.

Twelve bait lamina strips were placed in each plot roughly 6 inches apart in 2 separate rows, collectively constituting a single experimental unit (Fig. 1). In total, 1,080 strips were used each year, 540 midseason (MID) and end of season (END). The timing of this assay was aligned with microarthropod sampling to best link soil mesofauna captured with feeding activity and occurred on August 17th and October 12th in 2017, June 26th and September 13th in 2018, and July 15th and September 16th in 2019. Lamina strips were installed by first inserting a steel knife into the soil to avoid damage to the substrate during insertion, and then placed vertically into the preformed slit until the uppermost substrate hole sat just beneath the surface. This placement allowed me to measure feeding activity within the top 8 cm of soil. Because lamina strip exposure time is strongly correlated with soil moisture content, additional test strips were inserted in each field and checked every 2 to 3 days until approximately 25% (4 holes) of the bait was removed: 12- 14 days after exposure in all three years (Kratz, 1998). Once lamina strips were collected and transported to the lab, bait status was recorded as either consumed (empty or partially consumed) or not consumed (full) for each strip and % overall feeding activity per plot was determined by the average of the percentage consumed from each of the 12 strips. Feeding activity by depth was determined by summing the total bait consumed (empty or half empty) per

hole out of 12 strips; the first 10 holes were averaged to determine % consumption at 0-5 cm and the last 6 holes were averaged to determine % consumption at 5-8 cm. Soil moisture content was calculated by subtracting the dry weight of a 5g subsample of soil from the wet weight, and then dividing by the weight of dry soil.

3. Statistical Analyses

The overall experiment was laid out in a randomized block design with five replicates per treatment: 15 plots per field and 45 plots total. Data from each year was analyzed separately. In both 2017 and 2019, due to low pest pressure there were no inputs to the IPM plots, thus they were identical to the untreated controls. In these two years, decomposer feeding activity and enzyme activity data were combined as the ‘Untreated’ plots and microarthropod data were analyzed from Untreated and Preventative plots only.

Linear mixed effects models were used to test the effects of treatment on soil fertility, extracellular enzyme activity, microarthropod abundances, and decomposer feeding activity. For soil fertility models, pesticide use treatment and field served as fixed effects and block was treated as a random effect. For all other models, fixed effects were treatment, field, and season with block as a random effect. I assumed normally distributed residuals and data were log transformed if assumptions of normality were unmet. To identify significant effects, type III analysis of variance (anova) was used with Satterwaite’s method to calculate degrees of freedom. All statistical analyses were performed in R 3.6.1 (R Core Team, 2019) using the lme4 and lmerTest packages for linear mixed effects models (Bates et al., 2015; Kuznetsova et al., 2017).

4. Results

4.1. Soil Fertility

Soil health baseline values at the start of the experiment for physical, biological, and chemical indicators were averaged across fields and are shown in Table A2.1, Appendix A. Over the course of the study, there were slight decreases in pH, % organic matter, calcium, potassium, and magnesium, and for several other nutrients we found minor increases: aluminum, copper, iron, manganese, sodium, phosphorus, sulfur and zinc (Table 2.1). Most notably, there was a 51% increase in soil wet aggregate stability over the course of the experiment (Table 2.1). There were no main effects of treatment for any soil metric (Table 2.2), although I found a treatment*field interaction for manganese in field 1 with lower amounts in IPM plots compared to untreated and preventative plots ($F_{4,36} = 3.09$, $P = 0.02$) and a treatment*field interaction for copper with lower amounts in untreated plots compared to preventative in field 3. ($F_{4,24} = 5.24$, $P < 0.01$).

4.2 Decomposer activity and abundance

4.2.1. Enzyme Activity

In both 2018 and 2019, all extracellular enzymes showed considerable seasonal variation (Table 2.3). In 2018, all measured enzymes, N-acetyl- β -D-glucosaminidase (NAG), β -glucosidase (BG), acid phosphatase (PHOS), phenol oxidase (POX) and peroxidase (PER), showed significantly higher activity midseason (NAG: $F_{1,71} = 35.34$, $P < 0.001$; BG: $F_{1,71} = 4.11$, $P = 0.04$; PHOS: $F_{1,71} = 20.83$, $P < 0.001$; POX: $F_{1,71} = 12.01$, $P < 0.001$; PER: $F_{1,71} = 9.88$, $P < 0.001$). In 2019, NAG, BG, and PHOS activities were higher midseason (NAG: $F_{1,77} = 26.71$, $P < 0.001$; BG: $F_{1,77} = 205.66$, $P < 0.001$; PHOS: $F_{1,77} = 6.06$, $P = 0.01$), whereas POX and PER

activities were higher at the end of the season (POX: $F_{1,77} = 58.29$, $P < 0.001$; PER: $F_{1,77} = 20.86$, $P < 0.001$) (full statistics for season, field, and treatment by year reported in Table A2.2, Appendix A). In addition to seasonal effects, acid phosphatase (PHOS) activity was also higher in preventative plots compared to IPM and untreated controls in both years two and three (2018: $F_{2,59} = 3.78$, $P = 0.02$; 2019: $F_{1,65} = 6.47$, $P = 0.01$, Fig. 2.2).

4.2.2. Microarthropods

Soil microarthropods were comprised of mites (Acari) from the orders Oribatida, Mesostigmata and Prostigmata and the cohort Astigmatina, and the collembolan families Entomobryidae, Isotomidae, Sminthuridae, and Onychiuridae. There were significant seasonal differences for all microarthropods, except for predatory mesostigmatid mites which had similar abundances midseason compared to the end of the season in all three years (Table 2.4). All collembolan taxa were more abundant at the end of the season than midseason (Table 2.4). In contrast, seasonal differences for mites varied by taxon, where astigmatids tended to be higher midseason and oribatids higher at the end of the season (Table 2.4) (full statistical output for season and field by year reported in Tables A2.3, Appendix A).

In 2017, there were significant but opposing treatment effects for the collembolan family Isotomidae ($F_{1,35} = 5.43$, $P = 0.03$) and prostigmatid mites ($F_{1,46} = 6.28$, $P = 0.01$) with isotomid abundance being higher in preventative plots compared to untreated controls (Fig. 2.3a), and prostigmatid abundances being lower in preventative (2.60 ± 0.61) compared to untreated plots (5.29 ± 1.24) ($F_{1,46} = 6.28$, $P = 0.01$). I observed a main effect of treatment for oribatid mites in 2018 with higher numbers in IPM plots compared to preventative ($F_{2,70} = 3.68$, $P = 0.03$, Fig.

2.3b), as well as for astigmatid mites in 2019 with higher abundances in preventative plots compared to untreated controls ($F_{1,47} = 5.15$, $P = 0.03$, Fig. 2.3c). In 2019, prostigmatid mites were again significantly lower in preventative plots (1.68 ± 0.42) compared to untreated controls (3.27 ± 0.52) ($F_{1,47} = 5.52$, $P = 0.02$).

I also found a significant treatment*season interaction in 2017 for mesostigmatid mites with higher abundances in preventative plots than in untreated plots ($F_{1,35} = 5.47$, $P = 0.02$, Fig. 2.4a), and a treatment*season interaction in 2018 for both prostigmatids ($F_{2,70} = 3.96$, $P = 0.02$, Fig. 2.-) with lower numbers in preventative plots midseason and entomobryids ($F_{2,70} = 3.55$, $P = 0.03$, Fig. 2.4c) with higher numbers in IPM plots compared to untreated controls at the end of the season. I also found a treatment*season*field interaction for the collembolan family Onychiuridae with higher abundances in IPM plots (12.29 ± 5.49) compared to both preventative plots (9.32 ± 3.22) and untreated controls (5.00 ± 0.98) in field 3 at the end of the season ($F_{4,70} = 7.00$, $P < 0.001$).

4.2.3. Bait Lamina Strips

By strip

In all three years of the study, overall substrate removal was significantly higher at the end of the season (2017: $69.38\% \pm 3.03$; 2018: $81.01\% \pm 1.54$; 2019: $40.57\% \pm 1.85$) compared to midseason (2017: $30.15\% \pm 2.29$; 2018: $47.58\% \pm 2.49$; 2019: $10.37\% \pm 1.07$) (2017: $F_{1,71} = 142.45$, $P < 0.001$; 2018: $F_{1,71} = 33.05$, $P < 0.001$; 2019: $F_{1,77} = 114.52$, $P < 0.001$). There was no effect of treatment in either 2017 ($F_{1,66} < 0.01$, $P = 0.98$) or 2019 ($F_{1,77} = 0.65$, $P = 0.98$) in corn, although in 2018 in soybean, I found a significant treatment*season interaction with higher predation in preventive plots compared to untreated controls midseason ($F_{2,59} = 3.32$, $P = 0.04$,

Fig. 2.5a). There was also a significant effect of field in 2018 with lower feeding activity in field 3 (57.81 ± 4.61) compared to fields 1 ($65.27\% \pm 3.92$) and 2 ($69.81\% \pm 3.05$), ($F_{2,16} = 6.86$, $P < 0.01$).

By depth

As with feeding activity by strip, in all three years the feeding activity by depth followed a similar trend with higher at the end of the season in the top 0-5 cm of the soil profile compared to midseason (2017: $F_{1,72} = 151.77$, $P < 0.001$; 2018: $F_{1,71} = 47.08$, $P < 0.001$; 2019: $F_{1,74} = 136.70$, $P < 0.001$), and higher at the depth of 5-8 cm end of season compared to midseason (2017: $F_{1,71} = 91.18$, $P < 0.001$; 2018: $F_{1,68} = 9.19$, $P < 0.01$; 2019: $F_{1,77} = 43.39$, $P < 0.001$). In 2017, there was no effect of treatment at 0-5 cm ($F_{1,66} = 0.19$, $P = 0.66$) or 5-8 cm ($F_{1,66} = 0.62$, $P = 0.43$). In 2018, there was a significant treatment*season interaction for 0-5 cm ($F_{2,60} = 3.50$, $P = 0.03$, Fig. 2.5b) with lower feeding activity in IPM plots compared to untreated controls at the end of the season, and for 5-8 cm ($F_{2,59} = 3.61$, $P = 0.03$, Fig. 2.5c) with higher activity in preventative plots compared to untreated controls. In 2019, I also found a significant treatment*field interaction at 0-5 cm with higher feeding activity in preventative plots ($31.19\% \pm 6.94$) compared to untreated plots ($22.37\% \pm 4.37$) in field 3 ($F_{2,65} = 3.15$, $P = 0.04$).

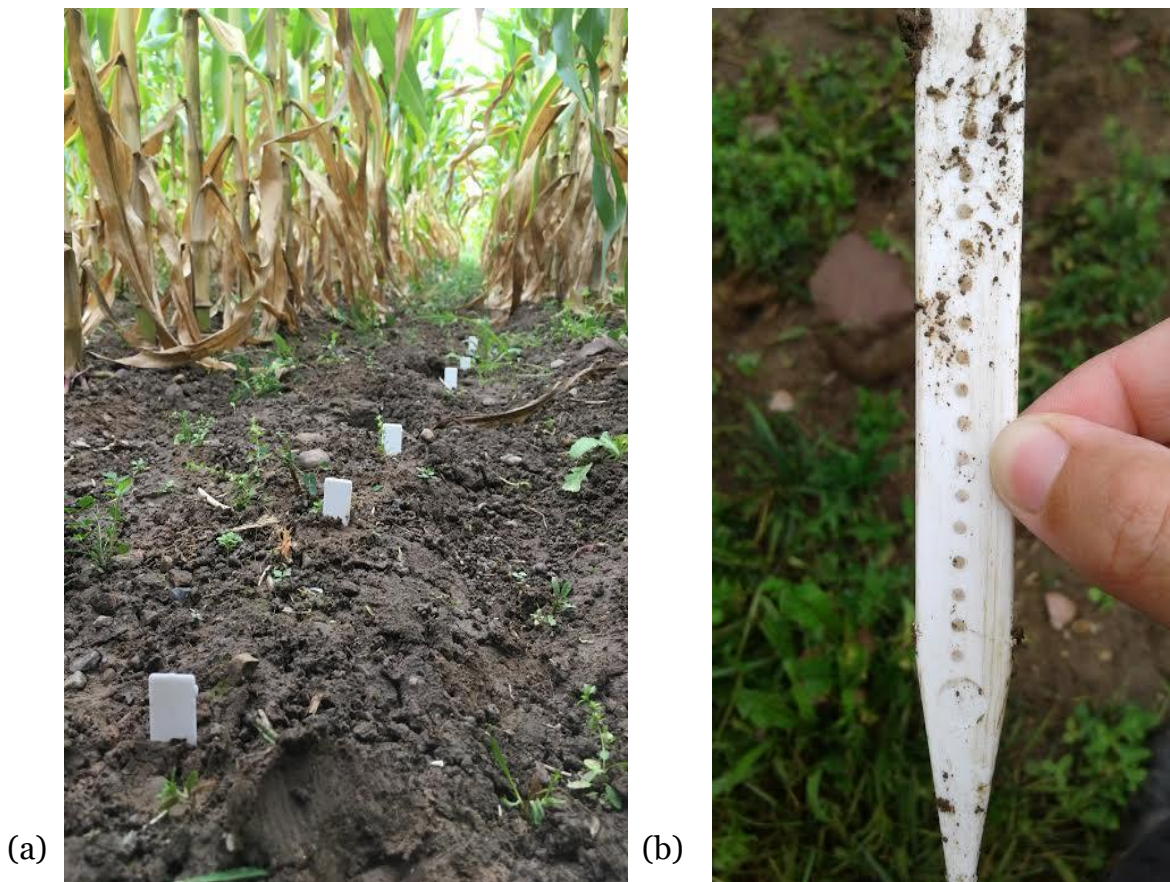


Figure 2.1. (a) Placement of bait lamina strips within each crop row. Six lamina strips were inserted approximately 15 cm apart in 2 cropping rows 6 m apart. (b) Lamina strip containing fully-intact substrate.

Table 2.1. Average (\pm SE) values of soil health metrics from the Cornell Nutrient Analysis Laboratory. Samples were taken at the start of the study, prior to pesticide inputs, and at end of the experiment. Values are averaged across fields and include 45 replicate plots, except for % wet aggregate stability at the start which was taken at the field level and includes 3 replicates.

Soil Metric	Start	End	% Change
pH	6.97 (0.04)	6.79 (0.06)	- 2.67
Aluminum (mg/kg)	10.45 (0.37)	11.41 (0.60)	+ 8.40
Calcium (g/kg)	1.92 (0.04)	1.86 (0.05)	- 3.25
Copper (mg/kg)	0.09 (0.00)	0.11 (0.00)	+ 18.85
Iron (mg/kg)	0.96 (0.04)	2.02 (0.09)	+ 52.52
Potassium (mg/kg)	78.57 (1.57)	65.34 (1.61)	- 20.25
Magnesium (g/kg)	0.22 (.005)	0.20 (.006)	- 11.34
Manganese (mg/kg)	9.30 (0.34)	17.68 (0.67)	+ 47.44
Molybdenum (mg/kg)	0.01 (0.001)	0.00 (0.00)	0.00
Sodium (mg/kg)	22.56 (0.51)	23.44 (0.24)	+ 3.74
Phosphorus (mg/kg)	6.92 (0.60)	11.68 (0.71)	+ 40.73
Sulfur (mg/kg)	5.53 (0.10)	15.49 (2.61)	+ 64.29
Zinc (mg/kg)	0.33 (0.01)	0.35 (0.02)	+ 4.28
Organic matter %	2.52 (0.05)	2.44 (0.07)	- 3.61
Wet Aggregate Stability %	8.13 (1.46)	16.86 (0.74)	+ 51.78

Table 2.2. Average (\pm SE) values of soil health metrics from the Cornell Nutrient Analysis Laboratory by treatment and field at the end of the experiment. Values marked with different letters within a metric and within each field are significantly different based on Tukey's post-hoc tests ($p < 0.05$).

Soil Metric	Field 1			Field 2			Field 3		
	Untreated	IPM	Prev	Untreated	IPM	Prev	Untreated	IPM	Prev
pH	6.87 (0.11)	7.21 (0.17)	6.83 (0.22)	6.69 (0.17)	6.88 (0.17)	6.69 (0.12)	6.80 (0.16)	6.58 (0.21)	6.52 (0.21)
Al (mg/kg)	11.63 (0.91)	9.28 (1.47)	12.92 (2.34)	12.32 (1.94)	12.69 (2.11)	13.76 (1.38)	8.91 (1.73)	9.96 (1.96)	11.21 (2.04)
Ca (g/kg)	2.08 (0.08)	2.18 (0.13)	1.89 (0.19)	1.76 (0.09)	1.89 (0.10)	1.73 (0.08)	1.76(0.18)	1.88 (0.17)	1.64 (0.05)
Cu (mg/kg)	0.12 (0.01)	0.10 (0.01)	0.09 (0.00)	0.10 (0.01)	0.10 (0.00)	0.12 (0.01)	0.10 (0.01) a	0.11 (0.01) ab	0.13 (0.01) b
Fe (mg/kg)	1.98 (0.12)	1.49 (0.11)	2.08 (0.22)	1.99 (0.29)	2.00 (0.18)	2.14 (0.12)	1.98 (0.48)	2.17 (0.38)	2.37 (0.37)
K (mg/kg)	66.50 (3.98)	68.53 (3.63)	59.89 (3.91)	55.20 (1.36)	59.95 (2.36)	60.79 (2.35)	70.43 (8.15)	72.48 (5.44)	74.29 (4.04)
Mg (g/kg)	0.18 (0.007)	0.21 (0.02)	0.18 (0.02)	0.183 (0.02)	0.19 (0.01)	0.17 (0.008)	0.23 (0.02)	0.24 (0.02)	0.23 (0.01)
Mn (mg/kg)	17.77 (1.05) b	9.57 (0.83) a	14.45 (2.02) b	19.63 (1.11)	19.69 (1.28)	21.27 (1.71)	17.96 (1.84)	21.19 (1.38)	17.63 (0.76)
Mo (mg/kg)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Na (mg/kg)	22.75 (0.51)	22.53 (0.48)	23.69 (0.54)	23.12 (0.56)	23.27 (0.08)	22.94 (0.65)	23.87 (1.33)	25.19 (0.84)	23.60 (0.73)
P (mg/kg)	14.98 (0.86)	15.13 (1.46)	13.27 (2.11)	13.95 (1.74)	15.18 (1.54)	13.63 (1.14)	6.37 (0.86)	5.83 (0.41)	6.78 (1.35)
S (mg/kg)	18.69 (7.96)	5.47 (0.27)	32.24 (19.03)	20.80 (5.70)	18.35 (7.20)	16.45 (4.83)	8.32 (0.54)	9.65 (0.87)	9.43 (1.10)
Zn (mg/kg)	0.28 (0.03)	0.24 (0.03)	0.24 (0.04)	0.26 (0.01)	0.25 (0.02)	0.34 (0.03)	0.50 (0.04)	0.47 (0.08)	0.55 (0.01)
OM%	2.63 (0.23)	2.38 (0.24)	2.17 (0.15)	2.09 (0.07)	2.15 (0.13)	2.44 (0.19)	2.60 (0.18)	2.55 (0.23)	2.91 (0.24)
%Ag Stability	12.47 (2.03)	12.31 (0.67)	11.45 (1.17)	16.29 (0.73)	16.09 (1.11)	15.44 (1.06)	21.56 (1.77)	21.61 (1.30)	23.10 (1.24)

Table 2.3. Seasonal averages (\pm SE) of the extracellular enzyme activities ($\text{nmol h}^{-1} \text{g}^{-1} \text{soil}$) of N-acetyl- β D-glucosaminidase (NAG), β -glucosidase (BG), acid phosphatase (PHOS), phenol oxidase (POX) and peroxidase (PER) by year at midseason (MID) and end of season (END) sampling dates. Values marked with different letters within a year are significantly different based on Tukey's post-hoc tests ($p < 0.05$).

	2018		2019	
	MID	END	MID	END
NAG	154.72 (7.54) a	60.24 (3.26) b	40.12 (3.28) a	21.97 (1.32) b
BG	1065.85 (147.14) a	316.02 (12.49) b	209.79 (8.16) a	116.71 (5.61) b
PHOS	1725.99 (94.37) a	636.13 (37.66) b	383.26 (22.79) a	318.07 (21.18) b
POX	4.82 (0.19) a	2.91 (0.11) b	0.79 (0.04) b	1.61 (0.08) a
PER	9.33 (0.39) a	5.96 (0.22) b	1.64 (0.10) b	2.27 (0.07) a

Table 2.4. Seasonal averages (\pm SE) of soil invertebrates captured via Berlese funnel extraction by year at midseason (MID) and end of season (END) sampling dates. Values marked with different letters within a taxon and within each year are significantly different based on Tukey's post-hoc tests ($p < 0.05$).

2017			2018		2019	
Taxa	MID	END	MID	END	MID	END
Oribatida	10.03 (1.91)	15.37 (1.72)	13.59 (1.37) b	6.59 (1.33) a	2.46 (0.33) a	14.56 (2.56) b
Mestigmata	9.10 (0.97)	6.93 (0.92)	12.30 (2.06)	13.83 (1.06)	7.88 (1.25)	9.56 (1.07)
Prostigmata	1.35 (0.31) a	6.55 (0.96) b	6.68 (0.75) b	2.67 (0.33) a	1.89 (0.34)	3.08 (0.43)
Astigmatina	25.45 (4.41) b	6.21 (1.17) a	3.99 (0.88) b	1.43 (0.29) a	8.02 (1.64)	3.50 (1.31)
hypopi	32.41 (7.49) a	72.24 (14.07) b	1.12 (0.31)	2.00 (0.92)	2.59 (0.91)	11.02 (4.00)
Entomobryidae	20.09 (4.19)	21.70 (2.85)	3.57 (0.57) a	5.66 (0.77) b	3.27 (0.49)	4.62 (0.59)
Isotomidae	26.76 (3.93) a	41.02 (4.34) b	2.40 (0.55) a	21.64 (3.51) b	8.38 (1.52)	12.71 (2.09)
Sminthuridae	1.57 (0.25) a	8.30 (0.93) b	0.30 (0.08) a	5.17 (0.69) b	0.22 (0.08) a	9.31 (1.40) b
Onychiuridae	3.67 (0.70) a	12.26 (1.69) b	0.97 (0.23) a	13.57 (2.32) b	1.56 (0.41) a	6.57 (0.89) b

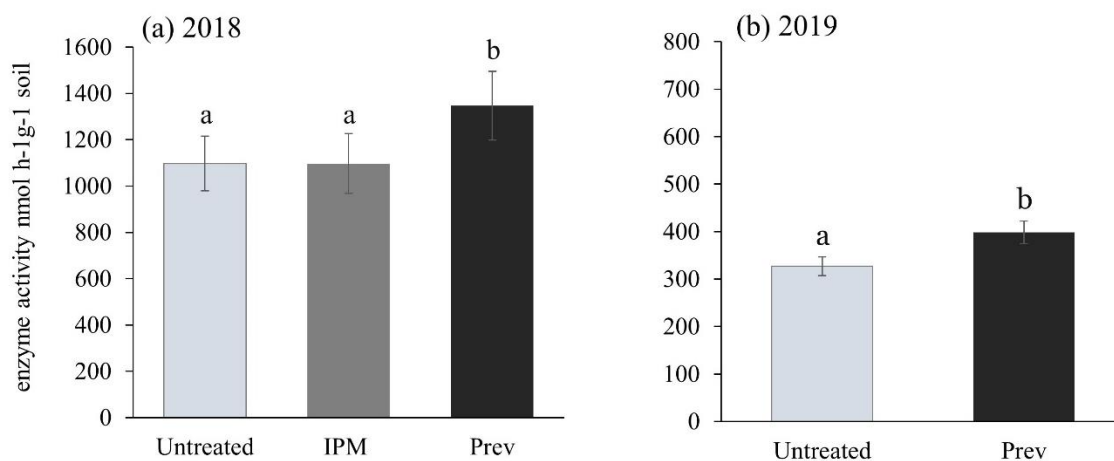


Figure 2.2. Average (\pm SE) enzyme activity ($\text{nmol h}^{-1} \text{g}^{-1} \text{soil}$) of acid phosphatase (PHOS) by year. In 2018 (a), IPM plots received a foliar spray application only and Preventative (Prev) plots included pesticidal seed treatments and foliar sprays. In 2019 (b), Untreated and IPM plots received no pesticide inputs and therefore, were averaged together and listed as Untreated. For each year, different letters indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$).

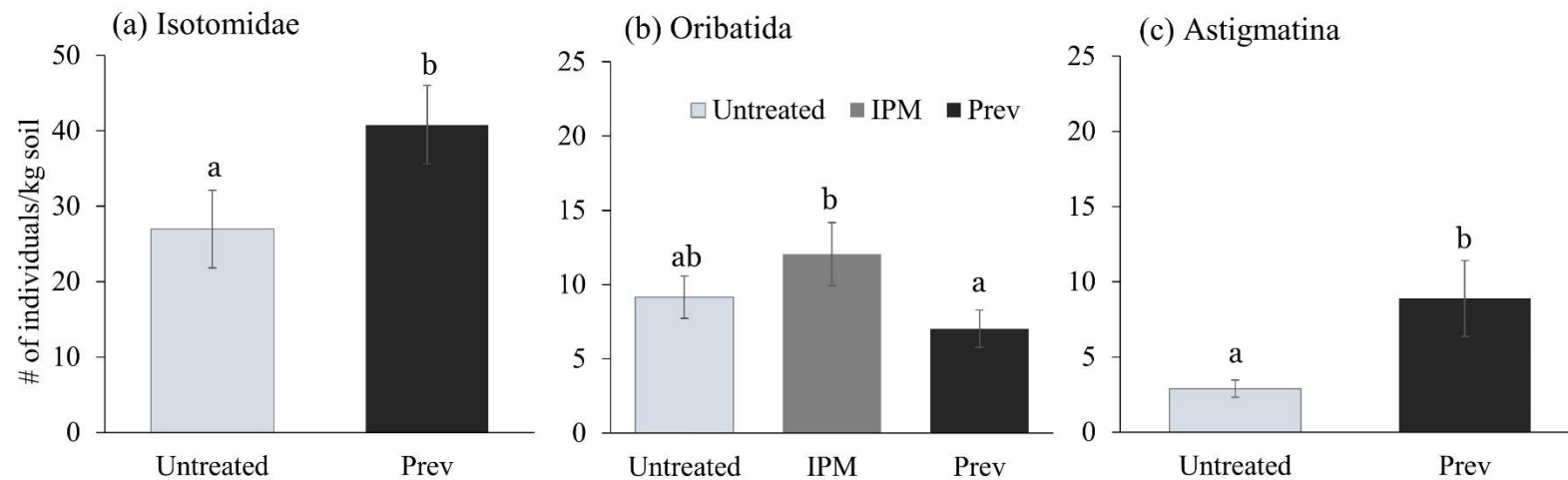


Figure 2.3. Average (\pm SE) number of individuals kg^{-1} soil of Isotomidae in 2017 (a), Oribatida in 2018 (b), and Astigmatina in 2019 (c). For Isotomidae and Astigmatina, Untreated and IPM plots received no pesticide inputs and therefore, were averaged together and listed as Untreated. For Oribatida, IPM plots received a foliar spray application only. Different letters within a taxa indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$).

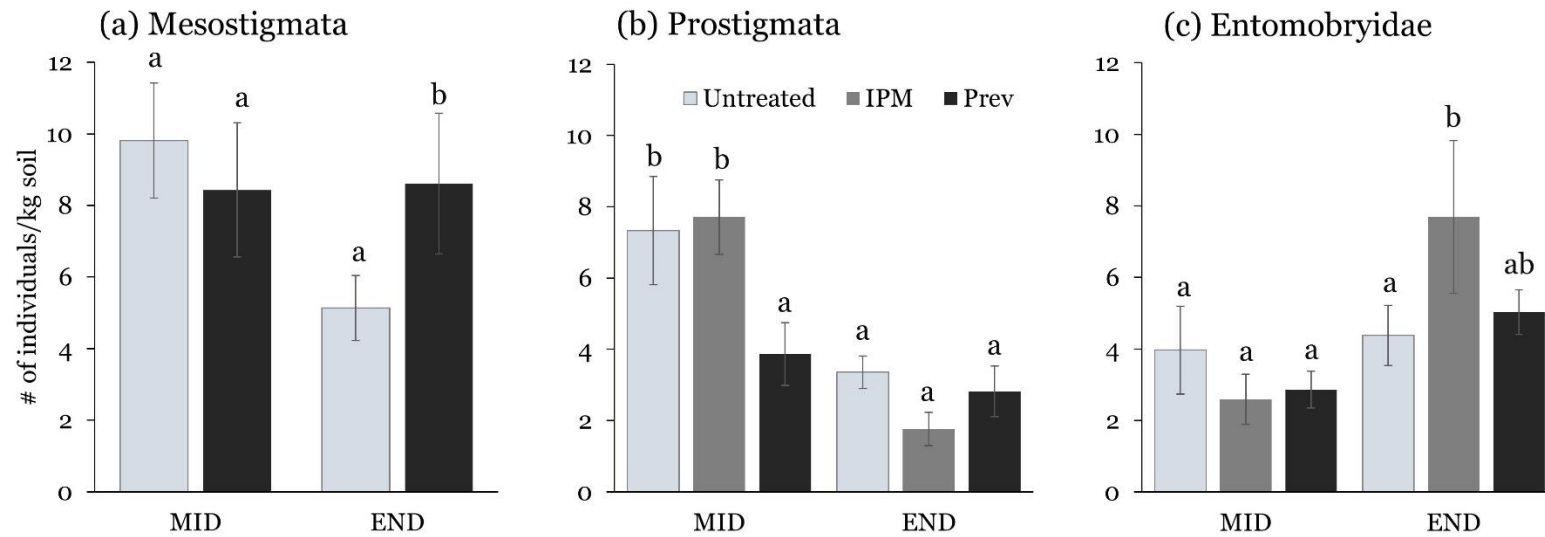


Figure 2.4. Average (\pm SE) number of individuals kg^{-1} soil of Mesostigmata in 2017 (a), and Prostigmata (b) and Entomobryidae (c) in 2018, by season. For Mesostigmata, Untreated and IPM plots received no pesticide inputs and therefore, were averaged together and listed as Untreated. For Prostigmata and Entomobryidae, IPM plots received a foliar spray application only. Different letters within either mid or end of season indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$).

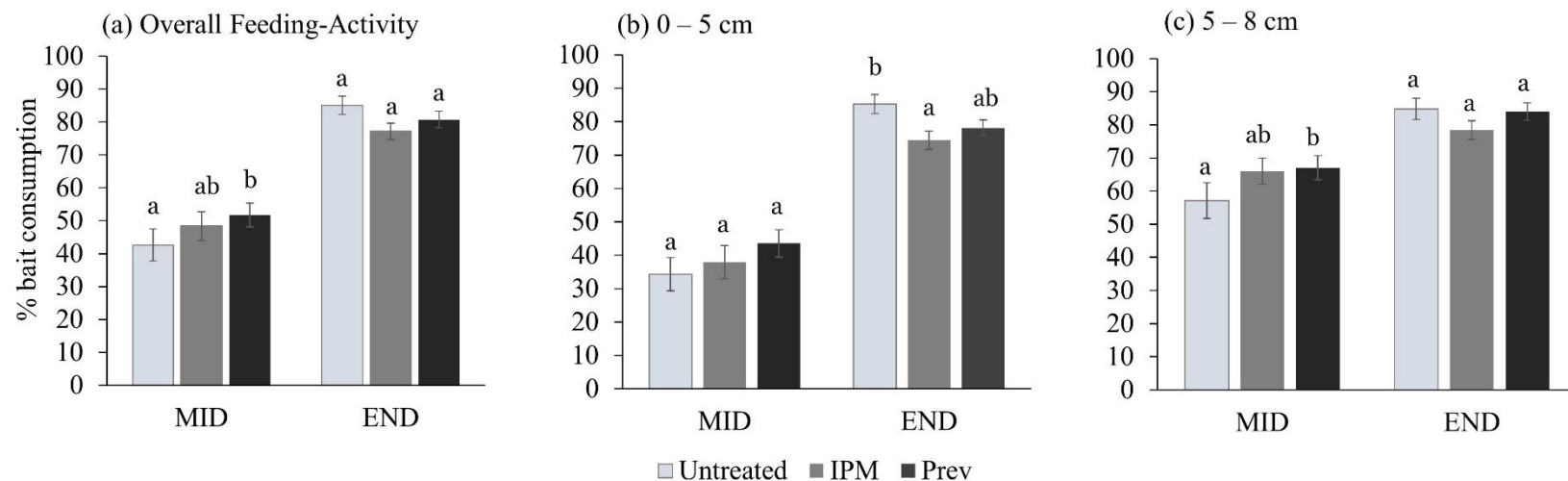


Figure 2.5. Average percentage (\pm SE) of bait removal for entire bait lamina strips (a), 0-5cm (b) and 5-8cm (c) in 2018. The IPM treatment in 2018 consisted of a foliar pyrethroid application only while the Preventative (Prev) treatment in all years consisted of pesticidal seed coatings and foliar sprays. Different letters within either mid or end of the season indicate significant differences between the means based on a Tukey HSD test ($p < 0.05$).

5. Discussion

The main objective of this study was to measure and contrast the impacts of a scouting-based IPM practice and a preventative pest management practice, i.e. seed treatments and foliar sprays, on soil decomposers and their activities. Another objective was to determine the influence of these practices on measurements of soil fertility. My findings revealed that microbial activity was stimulated by pesticide inputs and that soil fauna can have varying responses to pesticides, depending on the taxa or timing and form of pesticide input, having implications for belowground functioning. Despite mixed responses at the taxon level, preventative management stimulated overall decomposer feeding activity. These results illustrated that biological soil factors were more sensitive to pest management than physiochemical traits.

Impact of management practices on soil fertility

Soil health values at the start of the experiment indicated suboptimal soil functioning; however, some of these metrics generally improved over the course of the three-year experiment. There was a slight decrease in organic matter %, from 2.5 % to 2.4%, although a considerable increase in % wet aggregate stability, from 8.1% to 16.8%. Greater aggregate stability percentages are indicative of a greater proportion of total aggregates that are resistant to degradation after a precipitation event and a marker of good soil structure (Gugino et al., 2009). Both manganese and copper had a field dependent response to treatment. In field 1, manganese was significantly lower in IPM plots compared to untreated controls and preventative plots; IPM plots only received a pyrethroid insecticide spray in 2018. In field 3, copper was significantly higher in preventative plots compared to untreated controls. Considering that I found increased

enzyme activity and abundances of microarthropods in preventative plots, it is possible that this translated into higher levels of nutrient availability in field 3, where I also found trends of more aluminum, iron, potassium, phosphorus, zinc, % organic matter and % wet aggregate stability in preventative plots. Taken together, commonly used soil fertility metrics revealed that physical and chemical soil properties were less sensitive to pest management practices than soil biological factors.

Response of decomposer community to pest management

Microbial enzyme activity

Soil microbes synthesize and release diverse extracellular enzymes into the soil matrix which transform organic molecules such as chitin, cellulose, and lignin into less complex mineral nutrients. This process forms the basic foundation of biological nutrient cycling in soil and results in a reservoir of mineral nutrients accessible to microbes, plants and animals (Paul, 2015). The hydrolytic enzymes measured in the present study mineralize organic forms of phosphorus, nitrogen, and carbon, while oxidative enzymes such as phenol oxidase and peroxidase are responsible for the breakdown of complex aromatic molecules including lignin.

I found enzyme activity to be strongly influenced by season in both years of the study. All hydrolytic activities, NAG, BG, and PHOS, were higher midseason in both 2018 and 2019. Previous studies have shown that hydrolase activity frequently increases following synthetic fertilizer application (Frey et al., 2004; Grandy et al., 2013; Tiemann and Billings, 2011). In 2018, soybean fields were planted with a starter fertilizer of ammonium phosphate and potassium sulfate (302 kg ha⁻¹), and in 2019, all three corn fields were planted with starter fertilizer of ammonium nitrate, ammonium phosphate and potassium sulfate (56 kg ha⁻¹) and then side-

dressed with ammonium nitrate (30 kg ha^{-1}) one week before midseason sampling occurred, therefore, the input of chemical fertilizers likely led to the stimulation of NAG, BG, and PHOS. Additionally, in 2018 midseason sampling occurred directly following a 1.27 cm rain event (NCDC - NOAA), and while short-term weather changes impact soil enzymes in complex ways that are difficult to predict, extracellular enzymes generally respond positively to increased soil moisture (Burns et al., 2013). Therefore, it is plausible that the pulse of hydrolase activity in 2018 was also influenced by seasonal precipitation patterns.

I found mixed seasonal responses for oxidase activity with a stimulation midseason in 2018 and a suppression midseason in 2019. The midseason suppression in 2019, could also be explained by the application of ammonium nitrate, as N fertilization decreases lignin-degrading enzyme activity (Freedman et al., 2016; Hobbie et al., 2012). Waldrop and Zak (2006), found that, unlike hydrolytic enzymes, oxidative enzyme activity in systems with highly lignified litter responded negatively to nitrate deposition (Waldrop and Zak, 2006). This is based on the “microbial N mining” hypothesis that postulates decomposers may degrade lignin primarily to acquire N, thus, oxidative enzymes and lignin decay could be suppressed at high soil N concentrations (Carreiro et al., 2000; Fog, 1988). In contrast, the greater activity of POX and PER midseason compared to end of season in 2018 may be explained by the deep tillage event that occurred in all fields at the end of the season in 2017 to alleviate subsurface compaction. Previous studies have shown that tillage disturbances commonly increase oxidative activities (Wickings et al., 2011).

Regardless of any seasonal variation, there was significantly higher PHOS activity both years in preventative plots receiving pesticide-treated seed and foliar pesticide applications. Previous work has shown that soil dwelling bacteria, fungi, and algae, can degrade pesticides

through direct mineralization and co-metabolism, i.e. many synthetic chemicals are analogs of natural compounds, (Jaiswal et al., 2017; Mai et al., 2001). Bacteria and fungi are among the most important degraders of pesticides, and are present in high numbers in soil and water (Bollag, 1974). My findings suggest that the active ingredients introduced with our pesticidal seed treatments and foliar sprays, had a stimulatory effect on microbial activity, specifically phosphatase activity. I am unable to determine from this study whether this response is attributed to the insecticidal/fungicidal seed dressings or insecticide/fungicide foliar sprays, or their combination, although strobilurin fungicides have been shown to inhibit PHOS activity (Baćmaga et al., 2018; Chen et al., 2001). Conversely, an experiment on the bioremediation of soils contaminated with pyrethroid insecticides demonstrated that both bacteria and fungi are capable of degrading various pyrethroids, including lambda-cyhalothrin (Cycon and Piotrowska-Seget, 2016), and a recent review by Hussain et al., (2016) found that some bacterial strains are capable of biodegrading neonicotinoids, such as thiamethoxam. Previous work also demonstrates that the systemic, soil-applied insecticides imidacloprid and cyantraniliprole, can stimulate microbial enzyme activities in soil (Bray and Wickings, in prep). Singh and Singh (2005) also found imidacloprid in soils after seed treatment had stimulatory effects on microbial enzyme activity for up to 60 days. While the exact mechanism driving the stimulation of acid phosphatase activity by the insecticides used in this study is unclear, this finding suggests that the use of extracellular enzymes in assessing soil health may still be a valuable tool, yet requires good judgement. Soil microbial activities are not only highly sensitive to short- and long-term soil management practices, such as tillage, residue management and fertilization, but to pest management practices as well. Hence, microbial enzymes as soil health indicators should be

measured alongside additional physicochemical and biological measurements, and taken into consideration when making pest management decisions.

Microarthropods

Microarthropods are a vital component of terrestrial soil food webs. Their feeding habits range from fungivorous and detritivorous, to predatory (Coleman et al., 2018) and, collectively, their feeding activities contribute substantially to decomposition and nutrient cycling (Hopkin 1997, Filser 2002, Nico 2010). This study highlights that pesticide use practices can have widespread effects on soil microarthropods, however, the direction and magnitude of their responses varied by taxon, treatment, and season. Among the most consistent responses observed were the stimulation of isotomids, astigmatids and mesostigmatids, and the suppression of prostigmatid mites under preventative pesticide applications. Also consistent among my findings was a stimulation of select microarthropod taxa under IPM compared to untreated controls and/or preventative pesticides use.

The increased abundances of isotomids in 2017 in preventative plots compared to untreated controls may be explained by top-down ecological release due to the impact of insecticides on aboveground predators such as spiders and ground beetles (Pekár, 2013; Pfiffner and Luka, 2003; Tahir et al., 2019). Studies show that collembola are a high value food source for surface-active hunting spiders (Lawrence and Wise, 2000; Nyffeler and Benz, 1988) and I observed lower abundances of predatory macroinvertebrates, specifically lycosid spiders, in preventatively managed plots in 2017. The decreased spider activity-density in response to pesticides could have potentially liberated collembola from predation. There is also evidence that seed treatments alter collembolan behavior in soils. Zaller et al. (2016) observed increased

collembolan activity at the soil surface in soils planted with fungicide-treated seeds (e.g. fluoxastrobin, fluopyram, tebuconazole, and prothioconazole), a possible avoidance of soils contaminated with chemical stressors.

The seasonal variation exhibited in microarthropod responses to pesticide use practices may offer additional insight into other mechanisms driving positive versus negative responses. In both 2017 and 2019, I found a suppression of prostigmatid mites in preventative plots, and in 2018, a suppression in preventative plots midseason only, at which point, fields had received seed treatments and not foliar sprays, suggesting the suppression is most likely due to sensitivity to seed treatments. This could have been a result of direct toxicity (Peck, 2009) or a possible indirect response resulting from competition for resources with mesostigmatid mites, a top predator of soil food webs (Neher and Barbercheck, 2019). In contrast, the stimulation of entomobryid abundances in 2018 in IPM plots and mesostigmatid abundances in preventative plots 2019 both occurred only at the end of the season, following foliar pyrethroid applications. Together, these results suggest that the pyrethroid insecticides, regardless of the pest management program they fall under, can increase abundances of collembolan and mite taxa.

Again, this finding aligns with recent studies showing collembola and mite abundances, e.g. oribatids, are either unaffected or increase following pyrethroid insecticide sprays (Badieritakis et al., 2020; Frampton and van den Brink, 2007; Knoepp et al., 2012; Peck, 2009), most likely as a result of the direct toxicity of pyrethroids to predators (Palmquist et al., 2012) or indirectly, as a feeding repellent, temporarily reducing prey consumption rates in wolf spiders (Shaw et al., 2006). Given this response tendency, collembola may be useful as an indicator tool for tracking the effects of foliar sprays on beneficial predatory arthropods, such as surface-active spiders, a group not routinely monitored in field crops (Frampton and van den Brink, 2007;

Hardin et al., 1995). Additionally, the increased abundance of predatory mesostigmatids at the end of the season in preventative plots could be a multitrophic response to increased prey, with higher abundances of isotomids in preventative plots. Mesostigmatids are important predators in agricultural systems where they regulate populations of pests, such as pathogenic nematodes, and have shown tolerance to pyrethroid insecticides (Duso et al., 2020; El Adouzi et al., 2017; Hoy et al., 1980).

These differential responses of soil biota to seed treatments and foliar sprays suggest that soil taxa vary in their susceptibility and response to pesticide inputs. These data provide compelling evidence that pesticide-treated seed used in tandem with sprays can cause shifts in the densities of soil microarthropods, and the timing of pesticide inputs can play an important role in the unintended downstream consequences on the soil food web.

Decomposer feeding activity

Bait lamina strips are a tool meant to capture decomposition through the feeding activity of soil invertebrates, mainly mites, collembolans, enchytraeids, and earthworms. In all three years of our study, I observed higher feeding activity at the end of the season than midseason. I also found higher microarthropod abundances at the end of season in all three years, which could have contributed to increased substrate removal. The general activity pattern of anecic and endogeic earthworms is also known to increase over the growing season (Schorpp and Schrader, 2016) and although I did not measure macrofauna in this experiment, I did observe high earthworm densities in my fields and can infer that they may have played a role in the seasonal variation in substrate decomposition.

In 2018, I found significantly higher overall feeding activity in preventative plots midseason compared to IPM and untreated plots prior to foliar sprays and most likely a response to seed treatments. Yet, not all microarthropods responded the same to treatment. For instance, I observed a decrease in the abundance of prostigmatid mites in preventative plots midseason. I also found a disconnect between substrate removal and microarthropod abundance with depth, with lower feeding activity at 0-5cm in IPM plots at the end of the season and higher abundances of entomobryids, a common surface-active decomposer, in IPM plots at the end of season.

Despite that lamina strips are not best suited to capture the feeding activity of soil microbes (Gongalsky et al., 2004; Helling et al., 1998), my results suggest that substrate removal was correlated to microbial activity. I found increased substrate removal in preventative plots at the 5-8 cm depth, corresponding to the planting depth of 6.5 cm. This could be an indirect effect of microbes, which had higher activities in preventative plots in 2018, modifying soil fauna in the general area where strips were placed. Regardless, feeding activity reflected a stimulation of soil fauna in preventatively managed plots in one out of three years of the study. Yet, the inconsistencies identified between lamina strips and microarthropods suggest that microarthropods were not the only driver of substrate decay, and that other soil fauna, such as macroinvertebrates, played an equally important role. Other factors contributing to this disconnect may include the naturally patchy distribution of soil animals throughout the soil profile which can hinder the ability to identify clear linkages between soil animal composition and function at the field scale. My findings highlight that lamina strips are a valuable tool for measuring the feeding activity of soil animals, although additional laboratory and field studies are needed to investigate the contribution of specific fauna to substrate removal and to better link

the use of lamina strips to soil animal community data, and ultimately to soil health monitoring efforts.

6. Conclusions

My hypothesis that preventative pesticide use would degrade soil quality and limit the abundance of decomposers and their activity was not entirely supported. Overall, I found that pesticides had little impact on soil fertility, stimulated microbial extracellular activity, and had both stimulatory and suppressive effects on soil fauna and their activity, depending on the timing of application and input. Although the pyrethroid spray was applied in both preventative and IPM plots, preventative management, which included pesticidal seed treatments and fungicides sprays, also stimulated the soil microbial community and overall decomposer feeding activity, and altered the abundance of belowground soil fauna in unintended ways. These results suggest that pest management practices commonly used in field crops can have unexpected consequences on metrics of soil biological health, e.g. nutrient cycling and decomposition. My findings emphasize the need for further research on the effects of both pesticidal seed treatments and foliar applications on soil microbes and soil fauna to elucidate their influence on belowground soil biological functioning.

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Appendix A

Supplemental results for the whole-field soil health samples taken at the beginning of the experiment, one month after the termination of alfalfa and prior to the establishment of experiment plots, to determine baseline soil health. Data analyzed by the Cornell Soil Health Lab.

Table A2.1. Average values (\pm SE) of soil health indicators from the Cornell Assessment of Soil Health analysis. Values are averaged across fields.

Soil Health Indicator		Value
Physical	Available water capacity (g/g)	0.24 (0.00)
	Surface Hardness (psi)	97.00 (19.08)
	Subsurface Hardness (psi)	318.33 (7.26)
	Aggregate Stability (%)	8.13 (1.46)
Biological	Organic Matter (%)	2.50 (0.10)
	Soil Protein Index	4.83 (0.28)
	Soil Respiration (mg)	0.57 (0.07)
	Active Carbon (mg/kg)	414.00 (45.08)
Chemical	pH	7.00 (0.00)
	Extractable Phosphorus (mg/kg)	7.03 (2.41)
	Extractable Potassium (mg/kg)	63.47 (3.96)
	Magnesium (mg/kg)	211.77 (16.92)
	Iron (mg/kg)	0.97 (0.22)
	Manganese (mg/kg)	12.40 (1.27)
	Zinc (mg/kg)	0.20 (0.00)

Supplemental results for extracellular enzyme activity.

Table A2.2. *F* statistics and *P* values for linear mixed model analysis of the extracellular enzyme activities (nmol h⁻¹ g⁻¹ soil) of N-acetyl-βD-glucosaminidase (NAG), β-glucosidase (BG), acid phosphatase (PHOS), phenol oxidase (POX) and peroxidase (PER) by season, field, and treatment in 2018 and 2019. Values in bold denote p<0.05 for the treatment factor (pesticidal seed treatments and foliar sprays).

Enzymes	2018						2019					
	Season		Field		Treatment		Season		Field		Treatment	
	F _{1,71}	<i>P</i>	F _{2,70}	<i>P</i>	F _{2,71}	<i>P</i>	F _{1,77}	<i>p</i>	F _{2,77}	p	F _{1,77}	<i>P</i>
NAG	35.34	< 0.001	9.95	< 0.001	0.31	0.73	26.71	< 0.001	1.54	0.21	0.01	0.91
BG	4.11	0.04	1.77	0.17	0.77	0.46	205.66	< 0.001	12.06	< 0.001	0.33	0.56
PHOS	20.83	< 0.001	16.80	< 0.001	3.78	0.02	6.06	0.01	2.75	0.09	6.47	0.01
POX	12.01	< 0.001	2.08	0.13	0.40	0.66	58.29	< 0.001	0.72	0.49	0.79	0.37
PER	9.88	< 0.001	9.92	< 0.001	0.17	0.83	20.86	< 0.001	1.00	0.36	0.57	0.45

Supplemental results for soil microarthropod abundances.

Table A2.3. *F* statistics and *P* values for linear mixed model analysis of soil microarthropod taxa (number of individuals kg⁻¹) by season and field in 2017. Numerator and denominator degrees of freedom are listed as subscripts following F-values. Values in bold denote significant differences between the means based on a Tukey HSD test (*p*<0.05).

	2017				2018				2019			
	Season		Field		Season		Field		Season		Field	
Taxa	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Oribatida	0.47 _{1,33}	0.49	0.33 _{2,10}	0.72	9.91 _{1,70}	<0.01	0.81 _{2,70}	0.44	15.82 _{1,47}	<0.01	2.33 _{2,47}	0.10
Mestigmata	1.93 _{1,35}	0.17	3.82 _{2,12}	0.05	2.53 _{1,70}	0.11	3.18 _{2,70}	0.04	0.84 _{1,47}	0.36	6.46 _{2,47}	<0.01
Prostigmata	25.00 _{1,46}	<0.01	6.38 _{2,46}	<0.01	25.88 _{1,70}	<0.01	0.42 _{2,70}	0.65	3.12 _{1,47}	0.08	3.78 _{2,47}	0.02
Astigmatina	10.30 _{1,46}	<0.01	2.18 _{2,46}	0.12	13.81 _{1,70}	<0.01	2.96 _{2,70}	0.06	3.28 _{1,47}	0.07	1.03 _{2,47}	0.36
hypopi	4.77 _{1,35}	0.03	1.66 _{2,12}	0.22	0.19 _{1,70}	0.65	1.47 _{2,70}	0.23	2.48 _{1,47}	0.12	0.62 _{2,47}	0.54
Entomobryidae	0.25 _{1,34}	0.61	0.62 _{2,12}	0.55	11.44 _{1,70}	<0.01	3.88 _{2,70}	0.02	3.16 _{1,47}	0.08	1.16 _{2,47}	0.32
Isotomidae	5.74 _{1,34}	0.02	4.07 _{2,12}	0.04	30.81 _{1,59}	<0.01	1.26 _{2,12}	0.31	2.08 _{1,47}	0.15	2.38 _{2,47}	0.10
Sminthuridae	33.35 _{1,34}	<0.01	1.23 _{2,12}	0.32	41.14 _{1,70}	<0.01	4.22 _{2,70}	0.01	54.04 _{1,47}	<0.01	11.54 _{2,47}	<0.01
Onychiuridae	15.99 _{1,46}	<0.01	0.85 _{2,46}	0.43	45.72 _{1,70}	<0.01	1.90 _{2,70}	0.15	18.08 _{1,35}	<0.01	1.69 _{2,12}	0.22

SUMMARY

The broad aim of this research was to better understand how pest management practices influence components of soil biological health in field crops, i.e. biological control and decomposition. My hypothesis that the use of pesticides in a preventative manner would limit biological activity, compared to a scouting-based IPM practice was partially supported. Over the course of the experiment, I found that the use of seed treatments and sprays together negatively suppressed total predator activity-density, spider activity-density, predation, and the infection potential of soil-borne entomopathogenic fungi, compared with no pesticide use in one out of three years of the field study. Yet, I also observed increased microbial enzyme activity and feeding activity in preventatively managed fields, as well as a suppression and stimulation of soil fauna, depending on taxa and pesticide inputs. Additionally, I found that the introduction of a pyrethroid spray in IPM plots increased the abundance of decomposer microarthropods, specifically one collembola taxon, Isotomidae, and one mite taxon, Oribatida.

In 2017, I found preventative management negatively impacted spider activity-density, specifically surface-active lycosid spiders, midseason one week after foliar sprays were applied. This finding adds to the body of knowledge that pyrethroid insecticides have negative effects on above ground epigeal spiders. I also observed higher abundances of collembola in 2017 in preventatively managed plots, as well as in 2018, in IPM plots that received a foliar application. This result could be a response to the top-down ecological release due to the impact of pyrethroid insecticides on aboveground spiders, as collembola are a high value food source for lycosid spiders. Studies have documented that the fungicides used in seed treatments can result in increased collembolan densities at the soil surface, a response to a chemical stressor in the environment. In 2019, I found a significant increase in spider activity-density in preventatively

managed plots, midseason prior to the foliar spray, and therefore, most likely an indirect response to the pesticidal seed treatment. This could potentially be explained by increased collembola at the soil surface, although I did not find higher abundances of collembola midseason in preventative plots in 2019. Yet, these responses of spiders and collembola to seed treatments and sprays, across seasons and years, are exciting and novel. Future researchers interested in the multitrophic interactions between above and belowground soil food webs should focus on these taxa as model organisms. My observations on the biological control potential of entomopathogenic fungi contribute to the existing literature on the seasonal distributions of soil-borne EPF in the field. The infection potential of *Metarhizium spp.* was higher midseason while infection by *Beauveria spp.* was higher at the end of the season. Previous work has found similar seasonality in the pathogenicity of EPF.

A valuable takeaway from this research is the importance of pesticide input and timing of application. In 2017, the use of seed treatments and foliar sprays suppressed predators, potentially during their most effective biocontrol window. In subsequent years, the use of a pyrethroid insecticide alone in IPM plots stimulated microarthropods, and in preventative plots, combined with seed treatments and fungicides, stimulated microbial activity and decomposer feeding activity, and both stimulated and suppressed decomposer abundance. Additionally, overall feeding activity was stimulated midseason in preventative plots prior to a foliar application. While the bait lamina method is not meant to capture the feeding activity of soil microbes, my findings suggest that substrate decay was correlated to increased microbial activity in preventative plots. I found lower feeding activity in IPM plots at the end of season in 2018 and higher abundances of collembola in IPM plots at the end of the season in 2018. These inconsistencies I identified between lamina strips and microarthropods suggest that

microarthropods were not the only driver of substrate removal, and that other soil biota, such as microbes or macroinvertebrates, which I did not measure in this study, played an equally important role. Future laboratory and field studies should investigate the contribution of specific fauna to substrate removal to better connect the use of lamina strips to soil animal community data. The increased extracellular enzyme activity is in line with previous work that microbes are capable of degrading synthetic pesticides, although I am unable to link this response to soil animal community composition in this study. Future research is needed on the effect of seed treatments on soil microbes and soil fauna to elucidate their influence on the soil food web and belowground soil biological function.

My results also illustrated that physiochemical traits were less sensitive to management than biological soil factors, aside from a slight increase in % wet aggregate stability over the course of the study. Considering the aforementioned, a key takeaway from this research is that overall, the use of pesticides, specifically prophylactic seed treatments, did not improve crop yield in either corn or soybean. My findings highlight that the use of seed treatments within an IPM practice may not always be necessary for maximum crop productivity and when used in conjunction with foliar sprays might have unintended and unforeseen consequences on above and below ground soil biota and their function. My research provides valuable insight into the consequence of timing and pesticide input on soil biological communities and contributes to our understanding of how to best integrate soil and pest management practices to advance both the effectiveness and sustainability of productivity in field crops.