EFFECTS OF AGRICULTURAL PRACTICES ON SOIL COMMUNITIES AND THEIR ASSOCIATED ECOSYSTEM SERVICES

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EFFECTS OF AGRICULTURAL PRACTICES ON SOIL COMMUNITIES AND THEIR ASSOCIATED ECOSYSTEM SERVICES

BY

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DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Earth and Environmental Sciences

September, 2017
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On 13 July 2017

Original approval signatures are on file with the University of New Hampshire Graduate School.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS .................................................................................................................. viii  
FUNDING SOURCES ................................................................................................................... x  
ABSTRACT .................................................................................................................................. xi  

CHAPTER 1: INTRODUCTION ........................................................................................................ 1  
  Organization of dissertation ........................................................................................................ 5  
LITERATURE CITED ..................................................................................................................... 6  

CHAPTER 2: EFFECTS OF SOIL FUNCTIONAL ZONE MANAGEMENT ON SOIL ARTHROPOD COMMUNITIES .......................................................................................................................... 10  
ABSTRACT .................................................................................................................................. 10  
INTRODUCTION ........................................................................................................................... 11  
METHODS ..................................................................................................................................... 16  
  Site description and experimental design ...................................................................................... 16  
    Figure 1 ..................................................................................................................................... 17  
  Field management ...................................................................................................................... 18  
  Soil arthropod sampling ............................................................................................................ 19  
  Statistical analyses .................................................................................................................... 20  
RESULTS ........................................................................................................................................ 21  
  Soil arthropod community structure .......................................................................................... 21  
  Responses of soil arthropod communities inhabiting crop rows ............................................... 22  
    Figure 2 ..................................................................................................................................... 24  
    Table 1 ..................................................................................................................................... 25  
  Responses of soil arthropod communities inhabiting crop inter-rows ....................................... 26  
    Figure 3 ..................................................................................................................................... 27  
    Figure 4 ..................................................................................................................................... 28  
  Effects of tillage and cover crops on community composition in crop rows and inter-rows... 29  
    Figure 5 ..................................................................................................................................... 30  
    Table 2 ..................................................................................................................................... 31  
DISCUSSION ................................................................................................................................ 32
CHAPTER 4: DIMINISHED RETURNS? EFFECTS OF NEONICOTINOID SEED TREATMENTS ON SOIL ARTHROPOD COMMUNITIES VARY ALONG A PESTICIDE USE GRADIENT

ABSTRACT

INTRODUCTION

METHODS

Experimental design

Site histories

Collection of soil communities

Pot setup and growth conditions in greenhouse

Figure 1

Destructive harvest

Maize response to PST

Soil faunal community

Root fungal pathogens

Statistical analyses

RESULTS

Soil arthropod taxa at each site

Figure 2

Effects of PST on soil arthropod communities

Figure 3

Effects of PST on total soil arthropod abundance

Effects of PST on abundance of taxa groups

Figure 4

Figure 5

Effects of PST on soil arthropod diversity

Figure 6

DISCUSSION

LITERATURE CITED

Figure 7

Figure 8

Effects of seed treatment on yields

Figure 9

Effects of PST on soil arthropod diversity
Dedicated to my parents, Judy and Butch Atwood, with love.
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ABSTRACT

EFFECTS OF AGRICULTURAL PRACTICES ON SOIL COMMUNITIES AND THEIR ASSOCIATED ECOSYSTEM SERVICES

by

Lesley Wren Atwood

University of New Hampshire, September, 2017

To maximize crop yields, commodity crop production systems typically rely on inputs of fertilizers, pesticides, and irrigation; simplification of crop rotations (e.g., monocultures); and strategic use of soil disturbance (e.g., tillage, cultivation, etc.). While these practices are intended to optimize the soil conditions for crop development and reduce spatial and temporal variability in crop yield, they also impact soil biological diversity and the important agroecosystem services soil communities provide. Identification of management practices that are less prone to causing undesirable changes in the soil food web community are central to improving the sustainability of our agricultural systems. In this dissertation, I examined the effects of two agricultural management practices – conservation tillage and pesticide seed treatments – on the soil food web and soil-derived ecosystem services. The objective of my first study (Chapter 2) was to quantify the effects of zonal and uniform conservation tillage (ridge tillage vs. chisel plow) and strategic crop residue management (or soil functional zone management) on the abundance and diversity of the soil arthropod food web community inhabiting the crop row and inter-row zones in a maize-soybean system. In this two-year field experiment, I demonstrated that by using soil functional zone management, we can create unique zonation of the row and inter-row soil
arthropod communities compared to uniform tillage. However, there were tradeoffs associated with this strategy, as the higher abundance of soil arthropods and more non-pest taxa associated with the crop rows under ridge tillage were offset by a more depauperate community inhabiting the crop inter-row compared to the uniformly tilled system. The objectives of Chapters 3 and 4 of this dissertation were to improve our understanding of how pesticide seed treatments (PST) with neonicotinoids affect soil food web communities and the soil functions they regulate. PST with neonicotinoids are widely used in commodity row cropping systems managed with conservation tillage to preemptively protect crop seeds and seedlings from soil-borne diseases and soil-dwelling insects. There is emerging evidence, however, that PST can negatively affect non-targeted organisms, yet its effects on soil arthropod communities are poorly understood. In Chapter 3, I demonstrate with a field experiment that PST with neonicotinoids can alter the abundance and diversity of non-targeted soil fauna spanning multiple trophic-levels with no detectable effect on herbivores – the guild that is the intended target of PST. Lastly, in Chapter 4, I demonstrate for the first time that the initial introduction of PST into a soil community results in dramatic changes in soil community abundance and diversity, while communities with prolonged histories of seed treatment exposure appear to be relatively unaffected by subsequent exposure. Together this research provides insight into how a specific set of conservation tillage strategies, and their often-associated pesticide technologies, impact the community of soil arthropods that are critical to the sustainability of agriculture.
CHAPTER 1
INTRODUCTION

There have been substantial advancements in the global production of food and fibers; however, these advancements have often come at the expense of the environment (Hunter et al., 2017). Commodity crop production systems have typically attempted to maximize crop yields through inputs of fertilizer, pesticides, and irrigation; simplification of crop rotations (e.g., monocultures) and strategic use of soil disturbance (e.g., tillage, cultivation, etc.) (Giller et al., 1997; Mäder et al., 2002; de Vries et al., 2013; Tsiafouli et al., 2015). While these practices are intended to create optimum soil conditions for crop development and reduce spatial and temporal variability in crop yield, they also impact soil biological diversity (Wardle et al., 1999) and the important agroecosystem services soil communities provide (Bardgett and van der Putten, 2014; Wagg et al., 2014; Bender and Heijden, 2015; Lundgren and Fausti, 2015). Some of the services soil communities contribute to agricultural production include nutrient cycling, soil development, and biological control of pests (Coleman et al., 2004; Bardgett et al., 2005). Recognition of the important role that soils and soil biodiversity play in sustaining agriculture has driven adoption of alternative management practices that strive to reduce the negative environmental impacts of agricultural production on soil health and functioning.

Conservation agricultural practices aim to reduce soil erosion, build soil biodiversity, and limit nutrient losses compared to conventional agricultural practices through the implementation of three general crop management principles: (1) reduced tillage, (2) retention of crop residues, and (3) crop rotation (Hobbs et al., 2008; Pittelkow et al., 2015). Reduced tillage practices, also known as “conservation tillage”, are particularly important for building soil communities, as
tillage strongly affects both the biophysical and biochemical attributes of the soil (Roger-Estrade et al., 2010). Conservation tillage practices vary in intensity and depth of soil disturbance relative to moldboard plowing (the most intense and disruptive form of tillage), from moderately intense (e.g., chisel plow), intermediate (e.g., zonal tillage), and minimal disturbance (no tillage) (Reicosky, 2015). As the intensity of tillage decreases, the quantity of plant residues retained on the soil surface increases. These two features of conservation tillage (reduced disturbance and increased surface residues) result in increased water retention and cooler soils compared to fully inverted soils (Williams et al., 2016a). However, these changes in the soil environment can also promote populations of less desirable organisms, including pathogens, insect pests, and weeds, which can ultimately harm crop yields (Bockus and Shroyer, 1998; Govaerts et al., 2007; Nichols et al., 2015).

Thus, conservation tillage has created something of a management paradox. Reducing tillage for the purpose of erosion control and environmental improvement often results in soil conditions that promote pest populations (invertebrate soil pests and soil borne pathogens). This, in turn, has increased farmer reliance on pesticides to address the pest management challenges that arise from reducing tillage (e.g., Bockus and Shroyer, 1998; Shaw et al., 2012). The pesticides and associated technologies that are now often “bundled” with conservation tillage practices (e.g., pesticide seed treatments, stacked herbicide traits, etc.) have both monetary and environmental costs, the latter of which are largely externalized (Robertson and Swinton, 2005; Douglas and Tooker, 2015).

Historically, crop rotation and mechanical disturbance have been used to disrupt pest cycles; however, as crop diversity has declined (Howard, 2009; Khoury et al., 2014) and incentives to reduce mechanical disturbance (e.g., federal programs that incentivize erosion
control) have increased (Knowler and Brashaw, 2007) pesticide technologies have enabled farmers to replace mechanical pest management practices with chemical management practices, especially in large-scale agroecosystems. Such technologies include herbicides, insecticides, fungicides, and pesticide seed treatments, and it is not uncommon for multiple pesticides to be applied in a single field multiple times per season (Douglas and Tooker, 2015; Kniss, 2017). This widespread use of pesticides raises new environmental issues including pesticide resistance in targeted species and negative effects on non-targeted beneficial organisms (Wolfenbarger et al., 2008; Mortensen et al., 2012; Egan et al., 2014; Douglas et al., 2015; Gibbons et al., 2015), highlighting some of the tradeoffs associated with the implementation of conservation tillage today.

Given that conservation tillage practices vary in how intensively the soil is disturbed (Reicosky, 2015), some practices may be less prone to causing undesirable changes in the soil food web community. Tillage practices that integrate aspects of both no- or reduced-tillage (for the soil quality and biodiversity benefits) with more intensive tillage (for pest management benefits) may be expected to have fewer tradeoffs with regard to soil communities compared to more uniform forms of tillage. Zonal tillage practices (e.g., strip tillage and ridge tillage) are conservation tillage strategies that integrate varying levels of disturbance, as they create distinct permanent zones of disturbed and less- or un-disturbed areas within a single field. This spatial zonation in both disturbance, and subsequently residue placement, should have important implications for the soil faunal community—and therefore the soil-based regulating ecosystem services provided within row and inter-row zones within crop fields; however, no studies to date have examined entire soil faunal communities within these zones (Williams et al., 2016b). The objective of the first study in this dissertation (Chapter 2) was to quantify the effects of zonal and
uniform conservation tillage (ridge tillage vs. chisel plow) and strategic crop residue management (or soil function zonal management) on the abundance and diversity of the soil arthropod food web community inhabiting the crop row and inter-row zones in a maize-soybean system.

During the early stages of my work on the zonal tillage experiment, I became increasingly intrigued by the pesticide seed treatments (PST) that came pre-coated on the maize and soybean used in the study. I was particularly concerned whether the insecticide-fungicide mixtures might be interacting with the faunal communities I was examining. Pesticide seed treatments are intended to prophylactically protect the crop against soil borne fungal pathogens and soil-inhabiting insect pests during the early stages of plant development (Taylor and Harman, 1990), and are nearly ubiquitous in commodity cropping systems (Jeschke et al., 2011; Douglas and Tooker, 2015; Simon-Delso et al., 2015). In fact, finding commodity crop seeds without PST, or “raw seeds”, can be exceedingly difficult (personal experience and communication with farmers).

The use of PST has come under scrutiny because the toxins in these mixtures, particularly the neonicotinoids, have been linked to negative impacts on populations of some non-target organisms (Hallmann et al., 2014; Pecenka and Lundgren, 2015; Gibbons et al., 2015; Rundlöf et al., 2015), particularly bees (Girolami et al., 2009; Krupke et al., 2012; Goulson, 2013; Godfray et al., 2014; Godfray et al., 2015; Rundlöf et al., 2015; Mogren and Lundgren, 2016). While the non-target effects of PST with neonicotinoids on terrestrial fauna (e.g., bees, (Woodcock et al. 2017)) have received significant recent attention, much less attention has been paid to quantifying their effects on soil communities and the ecosystem functions they regulate (Pisa et al. 2015). Recent studies suggest that a diversity of non-target soil organisms can be negatively
affected by PST (Seagraves and Lundgren, 2012; El-Naggar and Zidan, 2013; Nettles et al., 2016), that these effects may extend across trophic levels (Douglas et al., 2015), and that important soil functions may be altered as a result (Smith et al., 2016). These studies illustrate that the effects of PST on soil-inhabiting organisms can occur at multiple trophic positions and thus may be capable of restructuring the soil food web, thereby potentially disrupting the provisioning of soil community-driven ecosystem services. The objectives of Chapters 3 and 4 of this dissertation were to improve our understanding of how PST with neonicotinoids affect soil food web communities and the soil functions they regulate because this information is critical for designing pest management strategies that support, rather than disrupt, the important services that soil food webs provide to agricultural production systems.

Organization of dissertation

The dissertation includes three empirical chapters formatted as manuscripts for journal submission. In Chapter 2, I report the results of a field experiment conducted at Rock Springs, PA investigating the response of the soil faunal community to soil functional zone management (SFZM) which integrates zonal tillage with strategic management of crop residues. Specifically, I was interested in examining if the SFZM system (ridge tillage) creates distinct communities of soil fauna in the crop row and inter-row that complement the broader goal of SFZM of creating distinct ‘soil building’ and ‘nutrient provisioning’ zones in close proximity to the cash crop. In Chapters 3 and 4, I report the results of two experiments examining the effects of PST on the soil arthropod community. In Chapter 3, I report the results from a replicated three-year field experiment (2013-2015) conducted at Rock Springs, PA in which we quantified soil arthropod communities and agroecosystems services in treatments that included corn and soybean planted with either PST with neonicotinoids or no PST. In Chapter 4, I report the results of a study that
addresses questions that emerged from the field experiment concerning how the successive use of PST with neonicotinoids may impact soil arthropod communities. In this greenhouse study, I collected soils (and their associated soil arthropod food web communities) from four sites near Durham, New Hampshire with varying histories of PST use. I sowed maize seeds either with or without PST into these soils and quantified the response of the soil communities. Together these chapters provide insight into how a specific set of conservation tillage strategies, and their often-associated pesticide technologies, impact the community of soil arthropods that are critical to the sustainability of agriculture.

LITERATURE CITED


CHAPTER 2
EFFECTS OF SOIL FUNCTIONAL ZONE MANAGEMENT ON SOIL ARTHROPOD COMMUNITIES

ABSTRACT

Soil disturbances such as tillage can negatively affect soil arthropod communities and the critical agroecosystems services they regulate. Soil functional zone management (SFZM), where soil disturbance is confined to distinct sub-meter zones, coupled with cover crops may promote the conservation of soil arthropod communities and concentrate them in closer proximity to the crop rhizosphere relative to conventional uniform tillage. To quantify the effects SFZM has on the soil arthropod community, we assessed the abundance, diversity, and species composition of the soil arthropod community in crop rows and interrows in an SFZM (ridge tillage) and uniformly managed system (chisel plow) with and without cover crops. In this two-year field experiment, a higher abundance of soil arthropods and more non-pest taxa were associated with crop rows under ridge tillage compared to chisel plow. We also found evidence of unique zonation (i.e., differences in row or inter-row communities between the two tillage systems) in the ridge tillage system in one year, but not the other. In this case, the inter-row position under ridge tillage appeared to become less hospitable for the soil arthropod community. Cover crops resulted in only moderate increases in the abundance and richness of the soil arthropod community inhabiting the inter-row position. It is becoming more widely recognized that soil fauna are critical to the ecosystem processes that underpin crop productivity; therefore, additional research examining how novel agricultural management strategies, such as SFZM,
INTRODUCTION

Soil biodiversity can be negatively impacted by the intense disturbances (e.g., tillage and agrochemicals) and low crop plant resource diversity (e.g. monocultures and minimal crop residues) that are characteristic of conventional annual row crop systems (Giller et al., 1997; Mäder et al., 2002; de Vries et al., 2013; Tsiafouli et al., 2015). Losses in species and functional diversity in soil communities can lead to reductions in regulating services, including nutrient turnover, soil carbon storage, and pest suppression, which can ultimately impact provisioning services (i.e., crop yield) (Bardgett and van der Putten, 2014; Wagg et al., 2014; Bender and Heijden, 2015; Lundgren and Fausti, 2015). For this reason, there is much interest in identifying tillage systems and cropping practices that integrate different types of soil disturbance within the same field to promote within-field species and functional diversity in soil communities, while at the same time facilitating crop planting and weed management (Williams et al. 2016). Zonal tillage systems, which create distinct alternating zones of no or reduced soil disturbance, coupled with the use of cover crops, may help conserve soil community abundance and diversity by maintaining soil refugia (undisturbed areas of soil) and concentrating plant-derived soil resources in space and time (Benton et al., 2003). However, whether or not zonal tillage systems foster more abundant and diverse soil communities relative to non-zonal conservation tillage systems is unclear.

Soils are inhabited by a diverse assemblage of microbes, arthropods, and other organisms, each with its own resource and habitat requirements, and all of which interact to form
what is known as the soil food web (Coleman et al., 2004; Bardgett et al., 2005). The micro- and macro-arthropod members of the soil food web are particularly functionally diverse, occupying nearly all trophic positions within the food web, including the detritivore and decomposer, herbivore, predator, and omnivore feeding guilds (Hendrix et al., 1986). Plant-derived resources form the base of the soil arthropod food web, and members occupy a variety of habitats within the soil, ranging from primarily soil-dwelling (euedaphic) to surface inhabiting (epigeal) (Digel et al., 2014; Scheunemann et al., 2015). Euedaphic arthropods (i.e., Acari and Collembola) inhabit primarily air-filled pore spaces in the soil, while epigeal arthropods may spend significant portions of their life on the soil surface under permanent or semi-permanent vegetative cover (Coleman et al., 2004; Bardgett et al., 2005). This diversity in resource and habitat requirements among members contributes to the sensitivity of the soil arthropod food web to agricultural practices that disturb the soil or alter plant litter inputs to the soil (Brussaard et al., 2007).

Tillage may be the agricultural practice that most strongly affects the soil arthropod food web community, as it quickly changes both the biophysical and biochemical attributes of the soil (Roger-Estrade et al., 2010). By redistributing litter and other organic materials throughout the soil profile, tillage changes the availability of habitats (e.g. provision of shelter and favorable microclimates) and resources (e.g. animal and floral food sources) which affects the activity-density, species richness, and community composition of soil arthropods (Reeleder et al., 2006; Diehl et al., 2012; Jabbour et al., 2016). Moreover, soil arthropods and other soil biota can be physically injured, killed, or exposed to predation during a tillage event (Roger-Estrade et al., 2010), with larger-bodied and species-poor organisms being the most sensitive (Postma-Blaauw et al., 2010). Conversely, pest problems can be exacerbated when the soils are minimally disturbed. For example, populations of slugs have increased in grain and forage systems.
managed with conservation tillage practices in the Mid-Atlantic region of the US (Douglas and Tooker, 2012).

Changes in plant litter inputs to the soil can also strongly affect soil arthropod communities and the services they provide to agriculture (Wardle et al., 1999a). Bare fallow periods have been shown to be associated with reduced soil biodiversity (Wardle et al., 1999b) and reductions in the provisioning of soil-derived ecosystem services (Bardgett and van der Putten, 2014; Wagg et al., 2014; Bender and Heijden, 2015; Lundgren and Fausti, 2015). Conversely, inclusion of a winter cover in annual row crop production systems has been shown to lead to shifts in soil food web composition and improved pest regulation (Tillman et al., 2004; Lundgren and Fergen, 2011). Adoption of cover crops remains limited, however, as evidenced by the fact that in 2012 only 10.3 million acres out of 915 million acres of U.S. farmland were planted with a cover crop (USDA-NASS, 2012).

Despite the positive effects that reducing soil disturbance and growing cover crops can have on soil food webs and the agroecosystems services they provide (Hobbs et al., 2008; Palm et al., 2014; Finney and Kaye, 2017), reducing or eliminating tillage (i.e., conservation tillage) can also be associated with negative impacts on agronomic performance. This is especially the case in regions with short growing seasons, where soil disturbance is necessary to speed up soil warming or humid temperate regions where excess crop residue and cool, wet soils can impede crop performance (Carter, 1994). Tillage is also an important tool for managing weeds and terminating cover crops; hence, reductions in tillage are often facilitated through increased reliance on herbicides, which can incur additional environmental and economic costs (Mortensen et al., 2012; Nichols et al., 2015).
Soil functional zonal management (SFZM) is a conservation tillage strategy that aims to integrate the soil quality improvement benefits associated with no- and reduced-tillage with the agronomic and weed management benefits associated with more intensive soil disturbance. This strategy aims to increase the heterogeneity of within-field microhabitats and plant inputs through non-uniform management of soil and crop residues (Williams et al., 2016d). Two of the most common types of SFZM are strip tillage and ridge tillage. In contrast to approaches that uniformly disturb (or not) the soil across a field (e.g., chisel plow, no tillage, etc.), SFZM creates distinct sub-meter zones of soil that vary in disturbance. With strip-tillage, zones alternate between disturbed (crop row) and undisturbed (crop inter-row). Under ridge-tillage, in contrast, both zones are disturbed to some degree, with the crop row zone being relatively less disturbed compared to the inter-row zone. Moreover, ridge tillage involves strategic spatial and temporal management of crop residues. Specifically, during the planting process, established ridges are truncated with coulters during which the top layer of accumulated soil and plant residues in this zone are moved and concentrated in the inter-rows, which then function as ‘soil building’ zones. Later in the growing season after the planted crops have emerged from the ridges, the soil and partially decomposed residues in the inter-rows are moved (re-ridged) into the crop rows, which then serve as ‘nutrient provisioning’ zones. When the spatial location of rows and inter-rows are maintained from year to year, higher concentrations of organic materials accumulate in the rows compared to the inter-rows, as both aboveground and belowground plant residues are ultimately moved to this zone (Shi et al., 2012).

Previous research suggests SFZM can have variable effects on soil communities (DuPont et al., 2009; Marahatta et al., 2010; Wang et al., 2011); however, these studies were primarily conducted in strip tillage SFZM systems and all involved collection of composite plot-level
samples that did not account for the within-field zones created under SFZM. In other words, samples collected from the crop row and inter-row zones were pooled together. This methodological issue is particularly important given that SFZM systems are expected to lead to functionally distinct zones due to spatially-distributed variation in soil disturbance and crop residues. This spatial zonation in both disturbance and residue placement should have important implications for the soil arthropod food web community—and therefore the soil-based regulating ecosystem services provided within row and inter-row zones; however, no studies to date have examined entire soil arthropod food web communities at the zone level under ridge tillage SFZM (Williams et al., 2016d).

The objectives of this study were to quantify the effects of tillage system (ridge tillage SFZM versus uniform chisel plow) and cover crops (winter annual small grain planted after corn or soybean harvest versus no winter cover crop) on the abundance and diversity of the soil arthropod food web community inhabiting the crop row and inter-row zones. We hypothesized that soil arthropod abundance, richness, and diversity would be higher in crops rows managed with ridge tillage and cover crops compared to in crop rows managed with uniform tillage (chisel plow) without cover crops because SFZM reduces soil disturbance deeper in the soil profile and concentrates plant residues in the row while chisel plowing results in uniformly distributed disturbance (Fig. 1a). In contrast, we expected that soil arthropod abundance, taxa richness, and diversity in the inter-rows would be similar between the SFZM (ridge tillage) and uniformly managed (chisel plow) treatments because both management systems result in similar levels of disturbance in this zone. Lastly, we hypothesized that soil arthropod communities in the row and inter-row zones would be more dissimilar in the ridge tillage treatment with cover crops.
compared to in the uniformly managed treatment (chisel plow) because SFZM creates spatial and temporal heterogeneity in disturbance and crop residue inputs to the soil (Fig. 1b).

METHODS

Site description and experimental design

The field experiment was conducted over a two-year period at the Pennsylvania State University Russell A. Larson Agricultural Research Center in Rock Springs, Pennsylvania, USA (40°-43’ N, 77°55’ W, 350 m elevation). The experiment was part of a larger multi-state project aimed at determining if SFZM creates distinct and complimentary functional zones that enhance soil-derived ecosystem services and climate resilience within row crop agroecosystems, details of which are reported elsewhere (Kane et al., 2015; Williams et al., 2016b, a; Williams et al., 2016c; Williams et al., 2016d; Williams et al., 2017). Soils at the field site are shallow, well-drained lithic Hapludalf formed from limestone residuum, and the dominant soil type is a Hagerstown silt loam (fine, mixed, semi-active, mesic Typic Hapludalf), (Braker, 1981). The soil is characterized by a silt loam surface texture and subsurface textures of silty clay loam and silty clay.

The experiment was established in 2012 and data on soil arthropod communities were collected in 2013 and 2014. The soil communities under ridge tillage management are likely “in transition” similarly to the transitional period previously observed at sites being converted from conventional to organic management (Lundgren et al., 2006). The design of the field experiment was a randomized complete block with eight plots per block and four blocks. Four of the eight plots within each block were planted with maize and the other four with soybean (Glycine max (L.) Merr.). Maize and soybean were rotated annually. Treatments were tillage system (chisel
Figure 1. Conceptual model illustrating hypothesized response of the soil arthropod community to tillage and cover crop treatments. In (a) crop rows in SFZM (ridge tillage) with cover crops will have higher soil arthropod abundance, richness, and diversity compared to crop rows in uniform tillage (chisel plow) without cover crops, because SFZM reduces soil disturbance deeper in the soil profile and concentrates plant residues in the crop row, while communities in the inter-row will be similar among treatments. In (b) crop row and inter-row community composition will be more dissimilar under SFZM with cover crops compared to uniformly tilled systems because SFZM creates in-field spatial heterogeneity in disturbance and plant-based resource inputs to the soil.
plow or ridge tillage) and cover crops (winter rye (*Secale cereal* L.) or winter fallow). Individual plots measured between 15 m and 9 m by 9 m. While both maize and soybean were present each year, we restricted soil arthropod sampling (described below) to the plots that were planted to the maize phase of the rotation (16 plots sampled per year).

The uniform tillage system included in this study was chisel plowing, which results in spatially uniform disturbance of the soil and intermediate conservation of soil residues; however, as a conservation tillage practice it results in relatively less intense soil disturbance compared to other types of tillage such as moldboard plowing which fully inverts the soil (Reicosky, 2015). The SFZM system was ridge tillage. Ridge tillage is similar in intensity and residue retention to chisel plowing (Reicosky 2015) and is characterized by the formation and skimming of soil ridges (crop rows) and furrows (inter-rows) via cultivation conducted several times during the growing season; the tops of ridges are truncated with coulters during the planting process and re-ridging typically occurs when maize reaches the six-leaf stage (V6) or soybean is at the three-leaf stage (V3). In this system, rows and inter-rows were maintained in the same position from year to year, resulting in a permanent zone of relatively undisturbed soil approximately 5 cm below the ridge (crop row) that received partially decomposed residue and soil from the inter-row zone at the time of re-ridging. In contrast, inter-row zones in this system experience relatively intense disturbance, comparable to that experienced under chisel plowing or skim plowing.

Field management

Prior to planting, plots under uniform tillage were chisel plowed and disked (2 May 2013 and 15 May 2014). On 6 May 2013 and 19 May 2014, maize (hybrid TA510-31, Agrisure Viptera 3111 with CruiserMaxx© 250 seed treatment) was planted at a seed density of 78,300
seeds ha\(^{-1}\). Urea was applied at a rate of 358 kg ha\(^{-1}\) two weeks after planting, and a post-emergence application of 1,390 g ha\(^{-1}\) glyphosate (potassium salt form) was applied 20 June 2013 and 21 June 2014 for weed control. When the maize reached approximately the six-leaf stage (V6) and soil moisture was optimal, plots under SFZM were ridge tilled (i.e., re-ridged, 27 June 2013 and 27 June 2014). Maize was harvested for grain on 30 October 2013 and 24 October 2014. Following maize harvest, treatments receiving a cover crop were planted to winter rye (seeds drilled at a rate of 123 kg ha\(^{-1}\)) on 9 November 2012 and 3 November 2013.

*Soil arthropod sampling*

We collected soil samples from the crop row and inter-row positions in all treatments at the time of maize anthesis (12 August 2013 and 1 August 2014). These dates corresponded to 46 and 35 days post-riding in the ridge tillage plots in 2013 and 2014, respectively. Soil cores measuring 5 cm diameter x 17 cm depth were collected from the center of the row between corn plants and the center of each inter-row location in each plot. Locations were randomly selected within each plot with a minimum distance of one meter from plot borders and other subsamples. Each sample was immediately placed in sealed plastic bags and stored in a cooler with ice. Once in the laboratory, all soil samples were stored in a 4°C refrigerator.

In the laboratory, fauna were extracted from each soil core over a period of 48 hours using collapsible Berlese funnels (Bioquip, Rancho Dominguez, CA). During the extraction temperatures slowly increased from room temperature (22°C) to a maximum of 50°C. This method extracts live and active fauna by slowly desiccating the soil encouraging organisms to burrow downward into a collection vial. Extracted organisms were stored in 90% ethyl alcohol at room temperature for later identification.
All soil arthropods were identified using distinguishable morphological characteristics based on Triplehorn and Johnson (2005) and cross checked with the University of New Hampshire Insect Collection. The level of taxonomic resolution varied by organism (e.g. species, morphospecies, genus, family, order). Hereafter, “taxon” refers to a single biological type determined as the finest taxonomic resolution to which each organism was identified. A comprehensive list of taxa found in this study are presented in Appendix A. Taxa were then organized into functional guilds based on Wolfenbarger et al. (2008) and Douglas and Tooker (2016). Arthropod abundance is reported as the number of individuals per square meter.

Statistical analyses

A combination of univariate and multivariate analyses was used to compare the communities inhabiting the row and inter-row positions between tillage systems. Taxon-level data were used to calculate community richness, evenness, and diversity (Shannon-Weiner Index (H)), (Lichtenberg et al., 2017). A list of these taxa is provided in Appendix A. Soil arthropod total abundances and taxon-level richness, evenness, and diversity (Shannon-Weiner Index (H)) data were analyzed with a three-factor ANOVA in SAS (Version 9.4, SAS Institute, Cary, NC) using the PROC MIXED procedure (significance level at $p < 0.05$). For these models, tillage system (ridge tillage vs. chisel plow), cover crop (winter rye vs. winter fallow), and year (2013 vs. 2014) and their interactions were treated as fixed effects, and block was treated as a random effect. Crop row and inter-row data were analyzed separately. Prior to all univariate analyses, Shapiro Wilkes and Levene’s tests were used to determine if these data were normally distributed with homogeneous variances. To meet the assumptions of ANOVA, soil arthropod abundance data were log (x+1) transformed prior to analysis. Soil arthropod abundances are reported as the number of individuals per m$^2$ ± standard error of the mean (SEM).
An indicator species analysis (Dufrene and Legendre, 1997) was performed to determine which taxa within the soil arthropod community inhabiting each row position were most strongly associated with ridge tillage or chisel plow. These analyses were conducted using a matrix of non-transformed taxa abundance values in PCord (McCune and Mefford, 1999). Only species with significant indicator values (p < 0.05) are reported.

Several multivariate analyses were used to test for differences in row and inter-row soil arthropod communities among tillage systems. Within each row position, differences in community composition between tillage systems were assessed using a permutation-based multivariate analysis of variance (PerMANOVA, Anderson, 2001) on a distance matrix of Bray-Curtis dissimilarity coefficients calculated from taxa-level abundances. The analysis was conducted using the adonis2 command in the ‘Vegan’ package in R (Oksanen et al., 2007; R Core Team, 2014). For individual response variables, data were analyzed separately by year if initial analyses indicated a significant interaction with year. All pairwise perMANOVA comparisons were made using the package “RVAideMemoire” pairwise.perm.manova command in R (Hervé, 2015). Differences in community composition and dimensionality among individual sample units within and across tillage systems and row and inter-row positions were assessed and visualized using a non-metric multidimensional scaling ordination using PCord (McCune and Mefford, 1999).

RESULTS

Soil arthropod community structure

Over the course of the experiment, we identified a total of 50 arthropod taxa across all treatments and sampling positions. In the ridge tillage system, we identified a total of 27 taxa in
the row position and 25 taxa in the interrow position. In the crop rows in the ridge tillage system there were an average of 9,848 ± 1,361 individuals per m², with Acari, Collembola, Myriapoda (including Chilopoda, Diplopooda, Pauropoda, and Symphyla), and Diplura accounting for 39.8 ± 3.2%, 33.9 ± 3.2%, 15.3 ± 4.4%, and 6.3 ± 1.1% of the total abundance, respectively. In the inter-rows in the ridge tillage system there were an average of 3,187 ± 399 individuals per m², approximately two-thirds fewer individuals than in the ridge tillage crop row. The inter-row community consisted of primarily of Collembola (39.4 ± 4%), Acari (34.0 ± 2.8%), Diplura (12.5 ± 2.5%), and Myriapoda (2.7 ± 0.7%).

In the chisel plow system, we identified a total of 28 taxa in both the row and inter-row positions. There was an average of 7,998 ± 1,158 and 4,722 ± 651 individuals per m² in the crop row and inter-row positions, respectively, or roughly double the amount of soil arthropods in the crop row compared to the crop interrow. In the crop rows, Acari (40.8 ± 4.2%), Collembola (34.2 ± 3.4%), Myriapoda (7.0 ± 1.7%), Coleoptera (adults and immatures, 4.4 ± 1.1%), and Diplura (3.0 ± 0.7%) were the most abundant taxa. The interrow community consisted primarily of Collembola (44.7 ± 2.9%), Acari (34.7 ± 2.9%), Diplura (6.3 ± 1.0%), Myriapoda (5.8 ± 1.1%), and Coleoptera (2.3 ± 0.5%).

Responses of soil arthropod communities inhabiting crop rows

Soil arthropod abundance in the crop row position differed by tillage system and year. Specifically, soil arthropods were more abundant in crop rows under ridge tillage than chisel plow (Fig. 2a), and more abundant overall in 2014 compared to 2013 (P < 0.0001); however, there was no effect of cover crops and no interaction between cover crop and tillage or between year and the other treatment factors (ANOVA: Tillage: F₁,₂₀ = 5.54, P = 0.0289, Cover crops: F₁,₂₀ = 0.15, P = 0.6999, Tillage x Cover crop: F₁,₂₀ = 0.85, P = 0.3686).
There were no effects of tillage system or cover crops on soil arthropod taxa richness and evenness in the crop row position; however, richness and evenness differed among years (ANOVA: Richness: Tillage: $P = 0.3121$, Cover crops: $P = 0.6008$, Year: $P = 0.0029$, Interactions all $P > 0.05$; Evenness: Tillage: $P = 0.4893$, Cover crops: $P = 0.7633$, Year: $P = 0.0247$, Interactions all $P > 0.05$).

The effect of tillage system on soil arthropod diversity (Shannon’s diversity) in the crop row position depended on the cover crop treatment (ANOVA: Tillage x Cover crop: $F_{1,20} = 5.06$, $P = 0.0360$; Fig. 2b). Specifically, diversity in the crop row position was lower in the ridge tillage system compared to the chisel plow system in the presence of a cover crop ($P = 0.0985$), but did not differ between tillage systems in the absence of a cover crop ($P = 0.1808$). There was no year effect on diversity or interactions between year and any of the treatment factors (all $P > 0.05$).

We conducted indicator species analysis across cover crop treatments to determine which taxa in the crop row position were associated with each tillage strategy (Table 1). A total of five taxa in the row position demonstrated significant associations with one of the two tillage systems. In 2013, Diplura Campodeidae, a predaceous omnivore taxon ranging from 3-5 mm in length, was associated with the ridge tillage treatment and Myriapoda Pauropoda, a small (0.5-2 mm in length), soft-bodied detritivorous taxon was associated with the chisel plow treatment. In 2014, two detritivorous taxa (Oribatida and Diplopoda) and the predaceous omnivore (Diplura Campodeidae) were associated with the ridge tillage treatment, while seed corn maggot (*Delia platura* (Meigen)), an agronomic pest, was associated with the chisel plow treatment.
Figure 2. Effects of tillage (ridge tillage vs chisel plow) and cover crops (winter rye vs winter fallow) on (a) total soil arthropod abundance and (b) Shannon-Weiner diversity (H) in the crop row position in Rock Springs, PA. Data are means ± SEM; n =16 (a) and 8 (b); data are averaged across years. Data in (b) are broken out by cover crop type because ANOVA indicated a significant tillage*cover crop interaction (p < 0.05).
Table 1. Indicator species analysis results for tillage type (ridge tillage or chisel plow) on the soil arthropod communities for each row position and year (n = 8). All indicator taxa with \( P < 0.06 \) are reported with their indicator value. Each taxon’s trophic level is also included.

<table>
<thead>
<tr>
<th>Position</th>
<th>Year</th>
<th>Tillage</th>
<th>Taxa</th>
<th>Trophic level</th>
<th>Indicator value</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Row</td>
<td>2013</td>
<td>Chisel plow</td>
<td>Myriapoda Pauropoda</td>
<td>Detritivore</td>
<td>74.8</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Ridge tillage</td>
<td>Diplura Campodeidae</td>
<td>Omnivore</td>
<td>74.7</td>
<td>0.0244</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Chisel plow</td>
<td>Delia platura (Meigen)</td>
<td>Herbivore</td>
<td>73.0</td>
<td>0.0316</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ridge tillage</td>
<td>Acari Oribatida</td>
<td>Detritivore</td>
<td>65.1</td>
<td>0.0220</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diplura Campodeidae</td>
<td>Omnivore</td>
<td>97.4</td>
<td>0.0020</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diplopoda</td>
<td>Detritivore</td>
<td>72.1</td>
<td>0.0534</td>
</tr>
<tr>
<td>Interrow</td>
<td>2013</td>
<td>Chisel plow</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ridge tillage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Chisel plow</td>
<td>Acari Mesostigmata</td>
<td>Predator</td>
<td>69.6</td>
<td>0.0033</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ridge tillage</td>
<td>Psocoptera</td>
<td>Detritivore</td>
<td>57.1</td>
<td>0.0394</td>
</tr>
</tbody>
</table>
Responses of soil arthropod communities inhabiting crop inter-rows

We analyzed the soil arthropod communities inhabiting the inter-row position to determine if tillage and cover crop treatments affected these communities in a manner similar to those in the crop row position. In contrast to what we observed in the crop row position, total abundance of soil arthropods in the inter-row position was 31% lower in the ridge tillage (3,302 ± 525) compared to the chisel plow treatment (4,784 ± 511) (ANOVA: Tillage: F_{1,20} = 6.26, P = 0.0212, Fig. 3). The cover crop treatment also affected total arthropod abundance in the inter-row position; however, this effect depended on year (Cover crop*Year: F_{1,20} = 4.45, P = 0.0478; Fig. 4a). There was no interaction between tillage and cover crop or between year and any other treatment factors (all P > 0.05).

The cover crop treatment also affected taxa richness (number of taxa) in the inter-row position and these effects depended on year (ANOVA: Cover crop*Year: F_{1,20} = 4.07, P = 0.0578 Fig. 4b). Specifically, taxa richness was lower in the inter-row in the presence of a cover crop in 2014 (P = 0.036), but not in 2013. There was no effect of tillage treatment on taxa richness in the inter-row or interactions between tillage and cover crop or year (all P > 0.05).

Soil arthropod community evenness differed between tillage systems and was more even in the ridge tillage system (evenness = 0.8229 ± 0.00159) compared to chisel plow system (0.7685 ± 0.01527) (ANOVA: Tillage: F_{1,20} = 6.08, P = 0.0228). There was no effect of cover crop on arthropod community evenness in the inter-row (Cover crop: F_{1,20} = 2.74, P = 0.1136) and no year or interaction effects (all P > 0.05). Similarly, there were no tillage or cover crop treatment effects or interactions on soil arthropod diversity (Shannon diversity) (ANOVA: Tillage: F_{1,20} = 1.59, P = 0.2219; Cover crops: F_{1,20} = 0.85, P = 0.3687; Interactions all P > 0.05).
Figure 3. Effects of tillage (ridge tillage vs chisel plow) on total abundance of the soil arthropod community in the inter-row in Rock Springs, PA. Data are means ± SEM; n = 16.
Figure 4. Effects of cover crops (winter rye vs. winter fallow) on (a) total abundance and (b) taxa richness (S) of the soil arthropod community in the inter-row in 2013 and 2014 in Rock Springs, PA. Data are broken out by Year because ANOVA indicated a year*cover crop interaction (p < 0.05). Data are means ± SEM; n = 8. Asterisk (*) denotes significance of one way ANOVA at p < 0.05.
The indicator species analysis conducted across cover crop treatments indicated that fewer taxa in the inter-row position were associated with one of the two tillage treatments compared to the crop row analysis (Table 1). In 2013, no inter-row taxa were associated with either tillage treatment. In 2014, Acari Mesostigmata (predaceous mites) were associated with the chisel plow treatment; whereas Psocoptera (booklice), soft-bodied detritivores ranging from 1-3 mm in length, were more closely associated with the ridge tillage treatment.

**Effects of tillage and cover crops on community composition in crop rows and inter-rows**

PerMANOVA was used to quantify differences in arthropod community composition between the two sampling positions (crop row and inter-row) within and between the two tillage systems. Soil arthropod communities differed between the row and inter-row positions in both years; however, these differences were affected by tillage system only in 2013 (PerMANOVA: Position: $F_{1,21} = 4.7994, P = 0.001$, Tillage*Position: $F_{1,21} = 1.9775, P = 0.040$; 2014: Position: $F_{1,20} = 13.7612, P = 0.001$; Tillage*Position: $F_{1,20} = 2.2182, P = 0.064$; Fig. 5). Specifically, in 2013 communities in the inter-row position differed between the ridge tillage and chisel plow treatments, while row communities were similar between the two tillage treatments (Table 2, Fig. 5a). Several taxa were strongly correlated ($R^2 > 0.3$) with row position each year. In 2013, two detritivorous groups, Collembola Onychiuridae and Diplopoda (millipedes), and one predatory group, Acari Mesostigmata, were positively correlated with the crop row position. The following year, two detritivorous taxa (Acari Oribatida and Collembola Entomobryidae) and two predaceous taxa (Acari Mesostigmata and Acari Prostigmata) were positively correlated with crop rows. Cover crops affected the composition of the soil arthropod community in 2013 (PerMANOVA: Cover crop: $F_{1,21} = 2.0826, P = 0.043$), but had no effect in 2014 (Cover crop:...
Figure 5. Non-metric multidimensional scaling (NMS) ordinations of soil arthropod communities for (a) 2013 and (b) 2014 using total abundance of each taxon. Filled symbols are crop rows; open (white) symbols are inter-rows; triangles are SFZM; circles are uniform tillage. Statistically similar groups identified with perMANOVA (p < 0.05) are circled. Strong correlations (R² > 0.3) between ordination axis scores and specific taxa are indicated with joint plot arrows.
Table 2. Pairwise perMANOVA table showing p-values for tests of the effect of tillage and row position on soil arthropod community composition.

<table>
<thead>
<tr>
<th></th>
<th>Chisel plow, Interrow</th>
<th>Chisel plow, Row</th>
<th>Ridge-tillage, Interrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chisel plow, Row</td>
<td>0.018</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ridge-tillage, Interrow</td>
<td>0.032</td>
<td>0.019</td>
<td>-</td>
</tr>
<tr>
<td>Ridge-tillage, Row</td>
<td>0.003</td>
<td>0.377</td>
<td>0.002</td>
</tr>
</tbody>
</table>
There were no interactions between tillage and cover crop or year and any of the treatment factors (all $P > 0.05$).

DISCUSSION

Our results provide partial support for the hypothesis that ridge tillage in combination with cover crops promotes higher soil arthropod abundance and diversity in crop rows compared to uniform tillage. Our prediction was based on the assumption that SFZM disturbs soils primarily at the surface and crop residues are concentrated in the crop row position relative to uniform tillage systems, thereby creating a more hospitable and resource-rich environment deeper in the soil profile for soil arthropods. Our observation that there was higher abundance of soil arthropods (Fig. 2a) and more non-pest taxa (Fig. 2a, Table 1) associated with crop rows under ridge tillage compared to chisel plow seems to support our hypothesis. Counter to our expectations, however, the addition of a winter cover crop did not lead to increased community diversity in the crop rows in the ridge tillage system, only in the chisel plow treatment (Fig. 2b). Furthermore, we found evidence of unique zonation (i.e., differences in row or inter-row communities between the two tillage systems) in the ridge tillage system in one year, but not the other (Fig. 5a and b). In this case, the inter-row position under ridge tillage appeared to become less conducive to the soil arthropod community, as the abundance of soil arthropods in this position was 31% lower compared to the uniformly tilled treatment (i.e., chisel plow) (Fig. 3). Cover crops resulted in only moderate increases in the abundance and richness of the soil arthropod community inhabiting the inter-row position in 2013, and resulted in even less benefit in the second year of the experiment (Fig. 4a and b). While we did not measure changes in the soil physical environment directly, and are therefore unable to determine the mechanism
responsible for the observed effects on the soil arthropod community, our observations and previous research suggests that maintaining permanent ridge tillage row and inter-row positions can create strong physical and organic matter gradients (Shi et al., 2012), which may have limited the abundance and diversity of soil arthropods inhabiting the crop interrows in our experiment.

Under ridge tillage, organic residues are purposefully concentrated in crop rows leaving little interannual accumulations in crop inter-rows. In a 29-year field experiment with clay loam soils, ridge tillage resulted in 10% lower organic carbon content and 8% higher compaction at 5-10 cm depth in the inter-rows compared to the row position (Shi et al., 2012). Moreover, soil compaction from wheel traffic in permanent inter-rows can persist up to 30 cm below the soil surface, stifling even the growth of crop roots (Liebig et al., 1993). Over the study period, we observed that the silty loam soils in our experiment became more compacted in the inter-rows under ridge tillage compared to under uniform tillage. Compacted soils can severely limit the abundance of soil mesofauna due to loss of suitable habitat, particularly coarse pore spaces (> 120 µm) because many soil mesofauna are dependent on air-filled pores and are incapable of making their own pore spaces (Dittmer and Schrader, 2000; Larsen et al., 2004).

Furthermore, soil arthropods inhabiting the inter-row positions managed with ridge tillage were also likely constrained by the reduction of surface residues following re-ridging (Carmona and Landis, 1999; Pullaro et al., 2006; Diehl et al., 2012). One of the intentions of the re-riding event, which occurs approximately 35-45 days after planting, is to concentrate accumulated surface residues into the crop row for nutrient provisioning. While this appeared to benefit soil arthropods inhabiting the crop rows, communities in the inter-rows experienced a loss of surface residues and the protective shelter, food resources, and favorable microclimates
these materials provided. In fact, the removal of surface litter may have been more detrimental to the soil arthropod community than the apparent increase in soil compaction, as has been observed with collembolan populations inhabiting soils in loblolly pine plantations (Eaton et al., 2004).

Counter to our hypotheses, we did not see a consistent relationship between soil arthropods and the addition of winter cover crops in either of the tillage treatments. While this result was unanticipated, particularly under the ridge tillage treatment, it is important to note that because the rows are permanent, crop root balls and other plant residues remain in this area. Therefore, it is possible that the additional biomass from the cover crops was negligible relative to the quantity of organic matter and residues already in the soil.

The dynamic nature of the soil food web in both space and time (Berg and Bengtsson, 2007), in addition to surface disruption that occurs in the ridge tillage inter-row position, likely influenced what we observed in that position. Our sampling time likely underestimates the relative importance of the inter-row position for soil arthropods, given that it does not capture community dynamics occurring early in the growing season when plant residues are concentrated in the inter-row. Our decision to sample the soil arthropod community when the cash crops reached maturity was based on the desire to provide the soil communities in the ridge tillage treatment enough time to “recover” from the re-ridging operation and because soils exist in the re-ridged state for the majority of the time (approx. 10.5 months). Future studies should consider examining inter-row communities prior to the re-ridging event to determine if there is a sustained loss in inter-row soil biota throughout the growing season or if it is primarily a consequence of the midseason tillage event.
These data provide evidence that ridge tillage supports an increased abundance of non-pest soil arthropods in the crop row compared to uniform chisel plowing; however, the simultaneous reduction in soil arthropods in the inter-rows highlights a potential tradeoff associated with this SFZM strategy. Whether or not this increase in soil arthropod abundance in row and reduction in soil arthropod abundance in the inter-row has substantive consequences for agroecosystem biodiversity is unknown. What is known is that soil fauna are critical to soil organic matter and nutrient dynamics (Osler and Sommerkorn, 2007; Grandy et al., 2016) and the biocontrol of pests (Jonsson et al., 2008); therefore, additional research examining how novel agricultural management strategies, such as SFZM, affect these communities and the services they provide at sub meter scales (Benton et al., 2003) will be essential to developing sustainable systems of agriculture.

LITERATURE CITED


CHAPTER 3

RESTRUCTURING THE SOIL FOOD WEB? EVIDENCE FOR MULTI-TROPHIC EFFECTS OF PESTICIDE SEED TREATMENTS ON NON-TARGETED FUNCTIONAL GUILDS IN THE SOIL FAUNAL COMMUNITY

ABSTRACT

The use of pesticide seed treatments (PST) with neonicotinoids is widespread in large-scale row crop agriculture. Recently, PST use has come under scrutiny due to its adverse effects on non-targeted organisms. Amidst these growing concerns, however, few studies have examined how this practice may impact the community of organisms in the soil that contribute to the regulation of important soil processes including decomposition and nutrient cycling. In this three-year field experiment, we found the effect of seed treatments on the soil faunal community to be greatest directly after planting and driven by non-uniform changes at the functional guild-level, with no effect on herbivores – the guild that is the intended target of PST. We found no evidence that PST affected nitrogen mineralization, surface litter decomposition or grain yields. Collectively, these data suggest that PST with neonicotinoids affect all non-targeted trophic levels of the soil faunal community soon after planting and that these effects can persist in higher trophic levels throughout the growing season. Additional research will be necessary to determine if the provisioning of other soil driven-processes are disrupted with PST use.
INTRODUCTION

Most commodity crops seeds in the US are pre-coated with pesticides. These pesticide seed treatments (PST) commonly include a mixture of systemic and contact fungicide and systemic neonicotinoid insecticide active ingredients intended to prophylactically protect the crop against soil borne fungal pathogens and soil-inhabiting insect pests during the early stages of plant development (Taylor and Harman, 1990). World-wide, adoption of PST with neonicotinoids in row crops such as maize, soybean, wheat and cotton has grown rapidly, resulting in nearly ubiquitous use of PST in some regions (Jeschke et al., 2011; Simon-Delso et al., 2015). In the US, it was estimated that in 2011 up to 44% of soybean and more than 79% of maize acres planted were pre-coated in PST with neonicotinoid insecticides, almost triple their usage in maize since 2003 (Douglas and Tooker, 2015). This surge in the use of PST with neonicotinoids is due, in part, to their purported effectiveness in providing broad-spectrum and systemic control of serious crop pests such as aphids and wireworms, that they provide prophylactic suppression as an insurance treatment, and the perception that PST use reduces overall pesticide use and has lower environmental impacts compared to other forms of application of these pesticides (Tomizawa and Casida, 2005; Bonmatin et al., 2015).

Recently, however, use of PST has come under scrutiny because the toxins in these mixtures, particularly the neonicotinoids, have been linked to negative impacts on populations of some non-target organisms (Hallmann et al., 2014; Pecenka and Lundgren, 2015; Gibbons et al., 2015; Rundlöf et al., 2015), particularly bees (Girolami et al., 2009; Krupke et al., 2012; Goulson, 2013; Godfray et al., 2014; Godfray et al., 2015; Rundlöf et al., 2015; Mogren and Lundgren, 2016). Neonicotinoids from PST have also increasingly been detected in off-target locations, including water ways (Hladik et al., 2014; Gibbons et al., 2015; Rundlöf et al., 2015).
Consequently, their use has been restricted or prohibited in a number of countries (European Commission, 2013; U.S. EPA, 2013; Ministry of the Environment and Climate Ministry of the Environment and Climate Change, 2015).

While the non-target effects of PST with neonicotinoids on terrestrial fauna (e.g., bees) and aquatic ecosystems have received significant recent attention, much less attention has been paid to quantifying their effects on soil communities and the ecosystem functions they regulate (Pisa et al. 2015). Soil communities regulate the decomposition of organic matter and the cycling of nutrients (Coleman et al., 2004; Bardgett et al., 2005) and changes in their community composition, total abundance, and diversity can alter these processes (Wagg et al., 2014). Soil community exposure to PST components can occur via direct exposure to the pesticides in the seed coating itself, but also through consumption of treated plant tissues, contact with seed treatment dust particles, and through contact with sap (Godfray et al., 2014; Godfray et al., 2015). Furthermore, the neonicotinoid components of PST are highly water soluble and moderately persistent in soil (UOH, 2013; Hladik et al., 2014). Thus, there are compelling reasons to suspect that non-target soil organisms may come into frequent contact with PST components where PST is used.

Indeed, recent studies suggest that a diversity of non-target soil organisms can be negatively affected by PST (Seagraves and Lundgren, 2012; El-Naggar and Zidan, 2013; Nettles et al., 2016), that these effects may extend across trophic levels (Douglas et al., 2015), and that important soil functions may be altered as a result (Smith et al., 2016). For example, PST use has been shown to alter soil microbial community structure (Nettles et al., 2016) and negatively impact populations of predatory arthropods (Moser and Obrycki, 2009; Seagraves and Lundgren, 2012; Douglas et al., 2015; Douglas and Tooker, 2016). Some taxa, such as collembolan, which
are agriculturally important fungivores (Crossley et al., 1992), increase in density (El-Naggar and Zidan, 2013) and surface activity (Zaller et al., 2016) with PST usage. While populations of natural enemies in agronomic fields have been demonstrated to decrease in density where PST with neonicotinoids are applied (Seagraves and Lundgren, 2012; Douglas et al., 2015; Douglas and Tooker, 2016); this result has been observed in populations of *Chlaenius tricolor* (Coleoptera: Carabidae), *Nabis americoferus* (Hemiptera: Nabidae), and *Chrysoperla* (Neuroptera: Chrysopidae). In the field, PST induced reductions in soil predator abundance and changes in the soil microbial community likely underpin the observed reductions in the biocontrol of invertebrate pests (Douglas et al., 2015) and weed seeds (Smith et al., 2016). Collectively, these studies illustrate that the effects of PST on soil-inhabiting organisms can occur at multiple trophic positions and thus may be capable of restructuring the soil food web, thereby potentially disrupting the provisioning of soil community-driven ecosystem services.

An improved understanding of how PST with neonicotinoids affect soil food web communities and the soil functions they regulate will be critical to designing pest management strategies that support, rather than disrupt, the important services that soil food webs provide to agricultural production systems. Here we report the results of a three-year field experiment in which we grew maize and soybean in rotation with and without PST with neonicotinoids and measured the response of the soil arthropod community, surface litter decomposition, plant-available soil nitrogen, and crop yields. We hypothesized that PST use would alter the composition, diversity, and total abundance of the soil arthropod community compared to control plots in which PST was not used. Additionally, we hypothesized that changes in rhizosphere fungal communities induced by the fungicides in PST (Nettles et al., 2016), combined with reductions in total arthropod abundance from the neonicotinoids in PST, would decrease rates of
surface litter decomposition and the availability of plant-available nitrogen compared to those in the control plots.

MATERIALS AND METHODS

Site description

The field experiment was conducted at the Pennsylvania State University Russell A. Larson Agricultural Research Center in Rock Springs, PA. USA (40°43´ N, 77°55´ W, 350 m elevation). Soils at the field site are shallow, well-drained lithic Hapludalf formed from limestone residuum, and the dominant soil type is a Hagerstown silt loam (fine, mixed, semi active, mesic Typic Hapludalf) (Braker, 1981). The soil is characterized by a silt loam surface texture and subsurface textures of silty clay loam and silty clay. In the five years preceding this study, the field was planted and managed conventionally as no-till maize for grain (2008 and 2009), no-till soybean (2010), no-till spring oats (2011), and barley and wheat crops (2012).

Experimental design

The experiment was established in May 2013 and continued for three years. Each year the same genotype of a glyphosate resistant cash crop (maize in 2013, soybean in 2014, and maize in 2015) was planted either with or without PST in a completely randomized design with five replications. Each plot was 6 m by 3 m, encompassing four experimental crop rows (76 cm-spaced rows). Treatments were maintained in their respective plots throughout the duration of the experiment. Planting densities and crop management depended on the crop and were based on standard agronomic practices for the region (described below).

Maize
Maize was planted in 2013 and 2015. Prior to planting in 2013, 1,520 g ha\(^{-1}\) glyphosate (potassium salt form) and 1,400 g ha\(^{-1}\) dichlorophenoxyacetic acid (2,4-D) was applied for weed control (26 April). In preparation for planting, the field was then S-tined, disked, and cultimulched (14-15 May 2013). No-till planting practices were implemented for all subsequent planting periods. On 16 May 2013, maize (hybrid TA510-18, TA Seeds, Jersey Shore, PA, USA) was planted at a density of 78,300 seeds ha\(^{-1}\). Urea was applied at a rate of 358 kg ha\(^{-1}\) on 31 May 2013, and a post-emergence application of glyphosate (1,390 g ha\(^{-1}\)) was applied on 20 June 2013. In 2015, a tank mix of 1,529 g ha\(^{-1}\) glyphosate (potassium salt form) and 1,400 g ha\(^{-1}\) 2,4-D was applied for weed control on 7 May. Maize (hybrid FC 397 3122, 1st Choice Seeds, Milton, IN, USA) was no-till planted into soybean residue at a density of 78,300 seeds ha\(^{-1}\) on 13 May. Urea was applied at a rate of 312 kg ha\(^{-1}\) on 28 May 2015. For both years, maize seeds included the genetic code for Bacillus thuringiensis (Bt) proteins. In both 2013 and 2015, maize seeds planted in the treated treatment (hereafter ‘PST’) were pre-coated with a mixture of the systemic insecticide thiamethoxam (class neonicotinoid), the contact fungicide fludioxonil, and the systemic fungicides mefenoxam, azoxystrobin, and thiabendazole (CruiserMaxx® Corn 250, Syngenta, Greensboro, NC, USA). Maize seeds planted in the control did not contain the coating.

Soybean

Soybean was planted in 2014. Prior to planting, 1,520 g ha\(^{-1}\) glyphosate (in the form of the potassium salt) and 1,400 g ha\(^{-1}\) 2,4-D was applied for weed control (27 May). On 30 May 2014, soybean (TS2849R2S, TA Seeds, Jersey Shore, PA, USA) was no-till planted into the maize residue at a seed density of 432,250 seeds ha\(^{-1}\). For this year, soybean seeds included the genetic code for Bacillus thuringiensis (Bt) proteins. The soybean seed planted in the PST treatment was coated with a pesticide mixture that included the systemic insecticide
thiamethoxam (class neonicotinoid), and the contact fungicide fludioxonil, and the systemic fungicides mefenoxam and sedaxane (CruiserMaxx® Beans with Vibrance®, Syngenta). The soybean seeds planted in the control did not contain the coating. On 16 June 2014, a post-emergence application of glyphosate (1,390 g ha⁻¹) was applied to control emerged weeds.

**Litter decomposition**

Litter bags were used to quantify the decomposition dynamics of surface residue in the PST and control treatments. Each bag measured 18 cm x 18 cm and was constructed from nylon mesh with 1.5 mm square openings to allow entry by soil mesofauna. Each litter bag contained approximately 9 g dry wt. of cereal rye (*Secale cereal*). The cereal rye litter was harvested as living shoots and stems from a nearby field each spring and then oven dried at 60°C until constant mass was maintained. Dried litter was then cut into 3-5 cm pieces and homogenized prior to being placed into the bags. Litter bags were weighed and placed in the plots each year on the same day as crop planting and removed at regular intervals throughout the growing season, with the last removal occurring approximately two weeks prior to crop harvest. Litter bags were secured to the soil surface with galvanized nails and effort was made to ensure uniform contact with the soil by gently clearing the soil of any living and dead plant biomass where necessary. In each plot, six litter bags were tacked to the soil in-line with the crop rows such that there was at least one meter between litter bags. In addition to the bags placed in the field, ten “handling bags” were also constructed and transported to and from the field each year to better account for mass lost during transportation (Harmon et al., 1999). In 2013, litter bags were placed in the field on 31 May and two bags in each plot were collected after 32, 61, and 130 days of decomposition. In 2014, litter bags were placed in the field on 30 May and two bags in each plot collected after 24, 61, and 136 days. In 2015, litter bags were placed in the field on 13 May and two bags were
collected after 39, 75, and 145 days. Litter bags remained in the field during management activities.

Upon retrieval, litter bags were immediately placed in sealed plastic bags, and stored in a cooler with ice. Once in the laboratory, bags were weighed and then placed into Berlese funnels for soil arthropod extraction (described below) followed by at least 48 hours in an oven at 60°C so constant mass was maintained. Intact dried litter bags were then weighed and all remaining litter in the bag was removed, weighed, and incinerated at 500°C for 8 hours to correct for mineral accumulation while in the field (Wider and Lang, 1982). To determine mass remaining in each bag, the mass of each ashed sample was subtracted from the total mass of remaining litter and soil accumulated in each respective bag. The average mass lost during transport was subtracted from the initial mass of litter (adjusted initial mass of litter). Percent of ash free dry mass remaining was calculated by subtracting the mass remaining in the bag from the adjusted initial mass of litter; this value was then divided by the adjusted initial mass of litter.

Soil fauna sampling

We assessed the response of soil fauna communities to seed treatments by extracting the community that colonized the litter bags (described above) and by collecting soil cores directly below the litter bags at the time of litter bag removal. Soil cores measured 5 cm width x 17 cm depth. Hence, in each plot, we collected two subsamples (one subsample includes both a litter bag and its respective soil core) three times during the growing season. Upon collection, each subsample was immediately placed in sealed plastic bags, and stored in a cooler with ice.

In the laboratory, all litter bag and soil samples were stored at 4°C prior to fauna extraction. Fauna were extracted with collapsible Berlese funnels (Bioquip, Rancho Dominquez, CA) over a period of 48 hours, during which extraction temperatures slowly increased from
room temperature (22°C) to a maximum of 50°C. This method extracts live and active fauna by slowly desiccating the soil/litter encouraging organisms to burrow downward into a collection vial. Organisms were stored in 90% ethyl alcohol at room temperature for later identification.

All soil arthropods were identified using distinguishable morphological characteristics based on Triplehorn and Johnson (2005) and the UNH Insect Collection and then organized into functional guilds primarily based on Wolfenbarger et al. (2008). The level of taxonomic resolution varied by organism (e.g. species, genus, family, order). Hereafter, “taxon” refers to a single biological type determined as the finest taxonomic resolution to which each organism was identified. A comprehensive list of taxa found in this study are presented in Appendix B. Taxa were then organized into functional guilds. Functional guilds included detritivore, herbivore, mixed, and predator groups which were “based on a crop production perspective when ecological function of an organisms varied with life stage” (Wolfenbarger et al., 2008). The “mixed” group included taxa that were identified at higher taxonomic groupings (e.g. order) where the groups’ members (e.g. family, genus) represented multiple feeding guilds. The only deviation from the functional guilds described by Wolfenbarger et al. (2008) was the classification of Acari Oribatida. In a separate experiment conducted on an adjacent agricultural site, we found that Oribatida at our site were primarily secondary decomposers (Atwood, unpublished data). Because of this, we classified Oribatida as detritivores, rather than mixed, for all analyses.

Arthropod abundances are reported as the combined number of individuals collected from the soil cores and the respective litter bags.

*Plant available soil nitrate*

Buried anion exchange resins were used to capture turnover rates of plant available nitrate (N-NO₃⁻) in the soil in 2013 and 2014. Ion exchange resins are strips of an organic
polymer that adsorb ions from soil solutions. We used 2.5 cm x 10 cm strips cut from sheets of anion-absorbing resins. These strips were buried vertically 15 cm deep (spanning 5-15 cm below the soil surface). To account for the spatial variation in inorganic nitrogen (Nyiraneza et al., 2011), we buried three ion exchange resin strips in each plot. Strips were located in-line with the crop row within the plot and were deployed approximately two weeks prior to each litter bag collection time (i.e., June 5, July 12, September 26, 2013; and May 30, June 13, and July 23, 2014). Thus, there were a total of three N-NO$_3^-$ sampling periods each year, with sampling duration ranging from 9 to 27 days.

At each removal date, all three strips in each plot were collected and then rinsed with distilled water to remove any adhering soil. The clean strips were then transferred to clean 75 mL vials with 70 mL of 2 M KCl and shaken at 40 rpm for one hour to extract adsorbed NO$_3^-$. NO$_3^-$ was analyzed colorimetrically using a single solution containing vanadium (III) sulfanilamide, and N-(1-naphthyl)-ethylenediamine (NED) (adapted from Doane and Horwáth (2003)). Dilutions of the samples were made using nano-pure water if the NO$_3^-$ concentration exceeded the detectable range, as in the case of the strips placed in the field following fertilizer applications in 2013.

**Crop grain yield**

At the end of the growing season, all harvestable grains from the four experimental rows in each plot were harvested with a two-row combine. Grain yields are reported as kg/ha at the standardized moisture for each crop (e.g. 15.5% for maize, 13.0% for soybean).

**Statistical analyses**

A combination of univariate and multivariate analyses was used to compare the effects of PST with neonicotinoids on soil arthropod communities. Taxon-level data were used to calculate
community richness and diversity (Shannon-Weiner Index (H) and Simpson’s Index (D’)), (Lichtenberg et al., 2017). A list of these taxa is provided in Appendix B. Soil arthropod total abundances, taxa richness, and diversity as well as surface litter decomposition and plant available soil nitrogen data were analyzed with a three-factor ANOVA in SAS (Version 9.4, SAS Institute, Cary, NC) using the PROC MIXED procedure (significance level at \( p < 0.05 \)). For these models, seed treatment (pesticide seed treatment (PST) vs. no seed treatment (Control)), sampling event (post-planting, crop maturity, and crop harvest), and year (2013, 2014, and 2015) and their interactions were treated as fixed effects. Because initial analyses indicated significant interactions between seed treatment and sampling period, each sampling period was analyzed separately. Yield data were analyzed with a two-factor ANOVA in SAS (Version 9.4, SAS Institute, Cary, NC) using the PROC MIXED procedure (significance level at \( p < 0.05 \)). For this model, seed treatment (pesticide seed treatment (PST) vs. no seed treatment (Control)) and year (2013, 2014, and 2015) and their interaction were treated as fixed effects. Prior to all univariate analyses, Shapiro Wilkes and Levene’s tests were used to determine if these data were normally distributed with homogeneous variances. To meet the assumptions of ANOVA, soil arthropod abundance data were log \((x+1)\) transformed prior to analysis. Soil arthropod abundances are reported as the number of individuals per m\(^2\) ± standard error of the mean (SEM). An indicator species analysis (Dufrene and Legendre, 1997) was performed to determine which taxa within the soil arthropod community were most strongly associated with seed treatment (PST or Control). These analyses were conducted using a matrix of non-transformed taxa abundance values in PC-ORD (McCune and Mefford, 1999). Only species with significant indicator values (\( p < 0.05 \)) are reported.
Within each seed treatment, differences in community were assessed using a permutation-based multivariate analysis of variance (PerMANOVA, Anderson, 2001) on a distance matrix of Bray-Curtis dissimilarity coefficients calculated from taxa-level abundances. The analysis was conducted using the adonis2 command in the ‘Vegan’ package in R (Oksanen et al., 2007; R Core Team, 2014). Data were analyzed separately by year because initial analyses indicated a significant interaction with year. Differences in community composition and dimensionality among individual sample units within and across seed treatments were assessed and visualized using a non-metric multidimensional scaling ordination using PC-ORD (McCune and Mefford, 1999).

RESULTS

*Soil arthropod community composition*

Over the course of the experiment, we identified a total of 54 taxa, including 35 taxa of mesofauna and 19 taxa of macrofauna. In the combined soil and litter samples, mesofauna consisted of Oribatida, Entomobryidae, Mesostigmata, Onychiuridae, Coleoptera larva, and Sminthuridae contributing 67.0 ± 2.0%, 10.3 ± 0.8%, 10.3 ± 0.8%, 4.1 ± 0.5%, 1.7 ± 0.4%, and 1.7 ± 0.3% of total mesofauna abundance across all sampling events, respectively. The most abundant taxonomic macrofauna groups were Diplopoda (33.0 ± 3.6%), Symphyla (24.9 ± 3.2%), Diplura (13.8 ± 1.8%), adult Coleoptera (10.4 ± 1.4%), Annelida (4.9 ± 0.8%), and Chilopoda (4.4 ± 1.1%). The average contribution of each functional guild to the total abundance of soil arthropods varied, with detritive comprising 85.2 ± 1.0 %, herbivores contributing 1.0 ± 0.1 %, mixed contributing 13.4 ± 0.9 %, and predators comprising 0.34 ± 0.05 % (Fig. 1).
**Figure 1.** Relative abundances of functional guilds at each sampling event (Post-plant, Crop maturity, and Harvest) averaged across three years. Data are mean proportions (n = 30) as a percentage of the total sum of all fauna at each sampling event.
Community-level responses to seed treatment

At the aggregate community-level, there were no significant effects of seed treatment on soil arthropod abundance, richness, or diversity (Table 1). Variation observed in these responses was best explained by time of sampling within a season and year (Fig. 2). In maize (2013 and 2015), the abundance of soil arthropods decreased as the growing season progressed, with the largest abundances occurring at the first sampling event in both years (Fig. 2a & c).

In soybean (2014), the highest abundance of soil arthropods occurred at crop maturity (Fig. 2b). Within each year, the most abundant communities also had the greatest taxa richness (Fig. 2d, e, and f). Among all sampling events and years, the greatest abundance (8752.0 ± 682.0) and richness (20.8 ± 0.6) of soil fauna was observed at the post-planting sampling period in 2013. The most diverse communities each year, measured by Simpson’s (H) and Shannon’s (D’) indices, were observed at the harvest time sampling period in the two maize years and at the post-planting period in the soybean year (Fig. 2g, h, i, j, and k).

Soil functional guild responses to seed treatment

Effects of seed treatments on soil arthropod taxa richness and diversity at each sampling period varied by functional guild. Two weeks after planting, the richness of the detritivore guild was higher in the PST compared to the control (ANOVA, Richness: Treatment: F_{1,23} = 9.10, P = 0.0062); however, total abundance and diversity (Shannon’s H and Simpson’s D’) of the detritivore guild were unaffected by seed treatment (Fig. 3a, b, c, and d). Similarly, the diversity of the mixed guild was significantly lower two weeks after planting in the PST compared to the control treatment (ANOVA, Shannon: Treatment: F_{1,7,3} = 5.16, P = 0.0559; Year: P = 0.0463; Treatment*Year: P = 0.4276, Simpson: Treatment: F_{1,7,74} = 4.83, P = 0.0604; Year: P = 0.0027; Treatment*Year: P = 0.5544); however, abundance and species richness of the mixed guild did
Table 1. ANOVA table showing p-values for tests of the effects of pesticide seed treatment (PST) on the total abundance, richness, and diversity of the soil faunal community during the 2013, 2014, and 2015 growing seasons. Abundance, richness, and diversity data are averaged across sampling events. Values are means ± SEM, n = 45.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Abundance (# individuals)</th>
<th>Richness (S)</th>
<th>Shannon's (H)</th>
<th>Simpson's (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1151.9 + 110.9, -101.2</td>
<td>14.9 ± 0.4</td>
<td>1.1 ± 0.03</td>
<td>0.5 ± 0.01</td>
</tr>
<tr>
<td>PST</td>
<td>1124.4 +108.9, -98.4</td>
<td>15.3 ± 0.4</td>
<td>1.1 ± 0.03</td>
<td>0.5 ± 0.01</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>F</th>
<th>p-value</th>
<th>F</th>
<th>p-value</th>
<th>F</th>
<th>p-value</th>
<th>F</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Treatment (T)</td>
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<td>0.4833</td>
<td>0.8721</td>
<td>0.9457</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Sampling Event (S)</td>
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<td>0.0118</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year (Y)</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T * S</td>
<td>0.8854</td>
<td>0.1317</td>
<td>0.1528</td>
<td>0.2162</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>T * Y</td>
<td>0.4651</td>
<td>0.2192</td>
<td>0.8516</td>
<td>0.8235</td>
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</tr>
<tr>
<td>Y * S</td>
<td>&lt; 0.0001</td>
<td>0.0003</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Y * T * S</td>
<td>0.5147</td>
<td>0.6779</td>
<td>0.2601</td>
<td>0.1743</td>
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</table>
Figure 2. Seasonal and annual variation in soil faunal community (a-c) total abundance, (d-f) richness, (g-i) Shannon’s diversity (H), and (j-l) Simpson’s index (D’). Data are broken out by year (2013, 2014, and 2015) and averaged across seed treatments (means ± SEM, n = 10).
Figure 3. Effects of seed treatment (PST and Control) on the total abundance, richness, and diversity (Shannon (H) and Simpson (D')) of the soil faunal community two weeks after planting. Soil faunal community is broken out into functional guilds: (a-d) Detritivores, (e-h) Herbivores, and (i-l) Mixed. Data are means ± SEM, n = 15. Asterisks denote significant differences between means (*p < 0.10, *p < 0.05, **p < 0.01, ***p < 0.001).
not differ between treatments (Fig. 3i, j, k, and l). The predator guild was also affected by the seed treatment early in the growing season; however, the nature of the response depended on the year (ANOVA, Abundance: Treatment*Year: $F_{2,23} = 3.32, P = 0.0541$; Richness: Treatment*Year: $F_{2,15} = 14.22, P = 0.0003$; Shannon: Treatment*Year: $F_{2,15} = 6.08, P = 0.0118$; Simpson’s: Treatment*Year: $F_{2,18} = 7.49, P = 0.0044$, Fig. 4). In 2013, predator abundance, richness, and diversity early in the growing season were similar in the PST and control treatments (Abundance: $t_7 = -0.47, P = 0.6560$, Richness: $t_8 = -1.29, P = 0.2381$, Shannon: $t_8 = -0.87, P = 0.4117$, Simpson: $t_8 = -0.52, P = 0.6185$, Fig. 4a, b, c, and d). In 2014, the total abundance, richness and diversity of the predator guild was higher in the PST compared to the control two weeks after planting (Abundance: $t_8 = 2.67, P = 0.0282$, Richness: $t_8 = 3.46, P = 0.0085$, Shannon: $t_8 = 2.35, P = 0.0468$, Simpson: $t_4 = 2.29, p = 0.0836$, Fig. 4e, f, g, and h).

Conversely, early in the growing season in 2015, predator richness and diversity were lower in the PST compared to the control treatment, and there was no effect of PST on predator abundance (Abundance: $t_8 = -0.66, P = 0.5266$, Richness: $t_8 = -3.79, P = 0.0053$, Shannon: $t_8 = -3.50, P = 0.0080$, Simpson: $t_8 = -3.42, P = 0.0091$, Fig. 4i, j, k, and l). In contrast to the detritivore and predator guilds, we did not detect any differences early in the season between the PST and control treatments in herbivore guild abundance, richness, or diversity (Fig. 3e, f, g, and h).

Effects of PST on guild-level abundance and diversity were less widespread at crop maturity. Only one guild, the predators, exhibited a response to PST; however, the response was inconsistent between years (ANOVA, Richness: Treatment*Year: $P = 0.0433$; Shannon: Treatment*Year: $P = 0.0222$; Simpson: Treatment*Year: $P = 0.0334$, Fig. 5). For example, in 2013, PST predator diversity was higher in the PST compared to the control (Shannon: $t_8 = 2.48$, Fig. 5b).
**Figure 4.** Effects of seed treatment (PST and Control) on the total abundance, richness, and diversity (Shannon (H) and Simpson (D’)) of the *Predator* guild two weeks after planting. Data are broken out by year: (a-d) 2013, (e-h) 2014, and (i-l) 2015. Data are means ± SEM, n = 5. Asterisks denote significant differences between means (•p < 0.10, *p < 0.05, **p < 0.01, ***p < 0.001).
Figure 5. Effects of seed treatment (PST and Control) on the richness and diversity (Shannon (H) and Simpson (D')) of the Predator guild at crop maturity. Responses are broken out by year: (a-c) 2013, (d-f) 2014, and (g-i) 2015. Data are means ± SEM, n = 5. Asterisks denote significant differences between means (*p < 0.10, *p < 0.05, **p < 0.01, ***p < 0.001).
\( P = 0.0382; \) Simpson: \( t_8 = 2.92, P = 0.0194 \), but predator richness was unaffected (Richness: \( t_8 = 1.77, P = 0.1151 \), Fig. 5a, b, and c). In 2014, predator richness was lower in the PST compared to the control treatment (Richness: \( t_8 = -1.90, P = 0.0943 \)), while diversity did not differ (H: \( t_8 = -1.37, P = 0.2068 \), D: \( t_8 = -1.07, P = 0.3167 \), Fig. 5d, e, and f). In 2015, predator richness and diversity both were similar in the PST and control treatments (Richness: \( t_8 = 0.63, P = 0.5447 \); H: \( t_8 = 1.00, P = 0.3454 \); D: \( t_8 = 0.87, P = 0.4089 \), (Fig. 5g, h, and i). Predator abundance did not vary between the PST and control treatments at crop maturity (ANOVA, Abundance: Treatment: \( P = 0.9149 \); Year: \( P = 0.2663 \); Treatment*Year: \( P = 0.4938 \)).

At crop harvest, effects of PST were observed only in the final year of the study (2015). Specifically, PST reduced the diversity of the mixed guild (PST: \( 0.0938 \pm 0.05 \), Control: \( 0.3784 \pm 0.08 \), \( t_8 = -2.93, P = 0.0189 \)) and marginally increased predator richness (Control: \( 1.0 \pm 0.45 \), PST: \( 2.2 \pm 0.37 \), \( t_8 = 2.06, P = 0.0736 \), Fig. 6g, h, and i).

**Response of individual taxon to seed treatment**

We used indicator species analysis to assess which individual taxon within the community were associated with either the PST or control treatment at the three sampling times each year. We observed that nine taxa were strongly associated with the treatments; however, these differed by sampling time (Table 2). Across all years, there were more indicator taxa associated with post-planting than any other sampling time. A total of seven taxa had significant associations with one of the two seed treatments at post-planting. Five of the seven taxa were most closely associated with PST and included one non-targeted herbivore, Symphyla, and four detritivores: one Diplopoda (Polydesmida), two Collembolan (Sminthuridae and Entomobryidae) and one Myriapoda (Pauropoda). One predatory taxon, Araneae, and one mixed taxon, Diplura Japygidae were associated with the control at the post-planting period. At crop maturity, only
Figure 6. Effects of seed treatment (PST and Control) on the richness and diversity (Shannon (H) and Simpson (D’)) of the mixed and predator guilds at crop harvest over the three-year field experiment. Responses are broken out by functional guild and community measure: (a-c) Shannon’s diversity (H) of Mixed guild, (d-f) Simpson’s diversity (D’) of Mixed guild, and (g-i) Richness (S) of Predator guild. Data are means ± SEM, n = 5. Asterisks denote significant differences between means (*p < 0.10, *p < 0.05, **p < 0.01, ***p < 0.001).
Table 2. Indicator values of taxa having significant ($P < 0.1$) associations with seed treatment (control or pesticide seed treatment (PST)) for each sampling event (Post-planting, Crop Maturity, and Harvest) and year (2013, 2014, and 2015).

<table>
<thead>
<tr>
<th>Sampling Event</th>
<th>Year</th>
<th>Taxa</th>
<th>Indicator Value</th>
<th>$P$</th>
<th>Taxa</th>
<th>Indicator Value</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Post-planting</em></td>
<td>2013</td>
<td>Symphyla</td>
<td>81.8</td>
<td>0.0464</td>
<td>Polydesmida</td>
<td>72.0</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Diplura</td>
<td>69.6</td>
<td>0.0616</td>
<td>Sminthuridae</td>
<td>80</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>Araneae</td>
<td>83.3</td>
<td>0.046</td>
<td>Entomobryidae</td>
<td>63.6</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pauropoda</td>
<td>73.3</td>
<td>0.0864</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Crop Maturity</em></td>
<td>2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Thysanoptera</td>
<td>76.7</td>
<td>0.0836</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Diplopora Sp. (immature)</td>
<td>83.3</td>
<td>0.0866</td>
</tr>
<tr>
<td><em>Harvest</em></td>
<td>2013</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
one detritivore taxon was associated with PST, immature Diplopoda (Polydesmida), and one mixed taxon was associated with the control (Thysanoptera) across the three-year experiment. No taxa were identified as indicator species at the harvest sampling period in any of the years.

**Effects of seed treatment on soil faunal community composition**

Despite observing effects of PST on individual taxon and at the guild-level, seed treatment effects at the whole-community level were not apparent in any of the sampling periods (perMANOVA, post-planting: Treatment: \( P = 0.338 \), year \( P = 0.001 \), Treatment*Year: \( P = 0.638 \); crop maturity: Treatment: \( P = 0.378 \), year \( P = 0.001 \), Treatment*Year: \( P = 0.445 \); harvest: Treatment: \( P = 0.615 \), year \( P = 0.001 \), Treatment*Year: \( P = 0.324 \)) (Fig. 7).

**Effects of seed treatment on aboveground decomposition**

We observed no effect of seed treatment on surface litter decomposition (Treatment: \( P = 0.9214 \), Treatment*Sampling event: \( P = 0.8896 \), Treatment*Year: \( P = 0.9548 \), Treatment*Year*Sampling event: \( P = 0.91436 \)). The interaction between year and time best explained the variation in decomposition (Year*Sampling event: \( F_{4,49.7} = 19.5, P < 0.0001 \), Fig. 8).

**Effects of seed treatment on plant-available soil nitrate**

Plant-available soil nitrate varied by year and sampling period, but was only marginally affected by seed treatment. Specifically, nitrate was lower in PST compared to control at crop maturity in 2013 (\( t_8 = -2.21, P = 0.0580 \), Fig. 9). Overall, plant-available soil nitrate was greater in maize (2013, Fig. 9a) than in soybean (2014, Fig. 9b) with the highest absorption rates occurring post-planting in maize. These substantially larger rates captured the diffusion of the recently applied fertilizers through the soil. Post-planting, there were no effects of PST on plant available soil nitrate (ANOVA, Treatment: \( P = 0.4778 \), Year: \( P < 0.0001 \), Treatment*Year: \( P = \))
Figure 7. Non-metric multidimensional scaling (NMS) ordinations of soil mesofaunal communities for each sampling event (Post-planting, Crop Maturity, and Harvest) using total abundance of each taxon. Filled symbols represent PST while open (white) symbols represent our control. Statistically similar groups identified with perMANOVA (p < 0.05) are circled.
Figure 8. Effects of seed treatment (PST and Control) on surface litter decomposition (percent ash free dry mass remaining) during the growing season each year: (a) 2013, (b) 2014, and (c) 2015. Data are means ± SEM, n = 5. Asterisks denote significant differences between means (•p < 0.10, *p < 0.05, **p < 0.01, ***p < 0.001).
Figure 9. Effects of seed treatment (PST and Control) on plant available soil nitrogen (NO$_3$-N absorption rates) during the growing season each year: (a) 2013 and (b) 2014. Data are means ± SEM, n = 5. Asterisks denote significant differences between means (•p < 0.10, *p < 0.05, **p < 0.01, ***p < 0.001).
0.5023). Similarly, seed treatment did not affect plant available nitrogen at harvest (ANOVA, Treatment: $P = 0.3211$, Year: $P = 0.0033$, Treatment*Year: $P = 0.3204$).

**Effects of seed treatment on yields**

PST did not affect harvested grain yield over the course of experiment (ANOVA, Treatment: $P = 0.1092$, Year: $P < 0.0001$, Treatment*Year: $P = 0.4899$). Mean yields for each year are reported in Table 3.

**DISCUSSION**

Pesticide seed treatments (PST) with neonicotinoids are intended to protect crops from soil-borne pests, especially herbivorous insects, early in the growing season when crops are most vulnerable. However, there is emerging evidence that PST can adversely affect beneficial non-targeted organisms including bees and predatory beetles, yet we still know relatively little about the effects of PST on all the other fauna inhabiting agricultural soils. In our study, we observed that PST does indeed affect the soil faunal community, resulting in significant changes in the abundance or composition of every feeding guild except the herbivore guild—the intended target of PST, and that these effects are greatest directly after planting (Figs. 3 and 4). Our analysis of the community at the feeding guild-level was critical to detecting these non-targeted effects of PST, as our analyses at the aggregated community-level revealed relatively few significant differences (Table 1 and Fig. 2). However, despite the fact that PST altered taxa abundance at multiple trophic levels, we observed little evidence that these effects on the soil community altered the soil functional processes that soil fauna help regulate, namely nitrogen mineralization, surface litter decomposition, or crop grain yield (Figs. 8 and 9).
Table 3. Table showing means and standard errors of the effects of pesticide seed treatment (PST) on yields during the 2013 (maize), 2014 (soybean), and 2015 (maize) growing seasons ($n = 5$).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (kg / ha)</th>
<th>2013 (maize)</th>
<th>2014 (soybean)</th>
<th>2015 (maize)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>11,850 ± 218</td>
<td>2,212 ± 63</td>
<td>12,801 ± 363</td>
</tr>
<tr>
<td>PST</td>
<td></td>
<td>12,367 ± 306</td>
<td>2,281 ± 104</td>
<td>13,022 ± 285</td>
</tr>
</tbody>
</table>
Given that concentrations of neonicotinoids and other PST toxins are highest immediately after planting (Goulson, 2013), it is not surprising that the effects on soil fauna we observed tended to be greatest early in the growing season (Figs. 3 and 4). Soil organisms can be exposed to neonicotinoid toxins directly not only through the intended pathway, the consumption of treated plant tissues, but also through unintended pathways, including contact with toxins adsorbed to soil particles, dissolved in water, transported as dust particles, and exuded as sap (UOH, 2013; Godfray et al., 2014; Hladik et al., 2014; Godfray et al., 2015). Moreover, soil organisms, including non-herbivorous organisms, tend to congregate near live plants (Curry and Ganley, 1977; Lussenhop and Fogel, 1991), and therefore likely come into direct contact with the seed treatment. This is probably further exacerbated in conventional row cropping systems, including at our field site, because there are typically few other living plants (e.g., weeds or cover crops) in the field at the time of planting and two weeks post-planting, as herbicides are commonly applied to kill weeds and cover crops in preparation for planting (Crossley et al., 1992). Therefore, the effect of seed treatments on non-targeted soil fauna may be greatest immediately after planting because treated crop plants are the only plants available for soil organisms to congregate around.

The differential responses of each soil faunal feeding guild to PST suggests (1) soil organisms vary in their susceptibility to PST and/or (2) PST changes the availability of resources utilized by each feeding guild. Previous research has shown that the susceptibility of soil organisms to neonicotinoids varies despite the fact that nicotinic acetylcholine receptors (nAChRs) are found in the central nervous system of many organisms (Meng et al., 2015). This is true among arthropods, which made up the majority of the soil organisms we assessed in this study. Specifically, non-insect arthropods tend to be less susceptible to neonicotinoids than
insects (Douglas and Tooker, 2016). These innate differences in susceptibility may explain why we did not observe a negative effect of PST on the abundance of detritivore taxa even though the diversity of the mixed guild was reduced (Fig. 3b, k, and l). However, differential susceptibility to neonicotinoids is not sufficient to explain the significant increase in detritivore richness we observed in the presence of PST. Alternatively, the increase in the number of detritivore taxa in the PST treatment may have been due to beneficial changes in resources available to the detritivore guild. For example, microbial driven degradation of pesticides can result in the production of novel metabolites (Masaphy et al., 1993) and these metabolites can modulate how arthropods interact with fungi (Rohlfs and Churchill, 2011), possibly making the fungi more palatable to some fungivores (which were included in the detritivore guild). It is therefore conceivable that more transient fungivores were encouraged to colonize the PST treatment because the fungi in this treatment were more palatable, thereby increasing the richness of the detritivore guild. Additional research would be necessary to test this hypothesis.

Somewhat surprisingly, we found that the intended target of the seed treatment insecticide, the herbivore guild (which includes crop pests), was unaffected by PST in all three years of our experiment (Fig 3e, f, g, and h). The lack of a PST effect on the herbivore guild could have been due to our field site having relatively low abundances of herbivores. Previous research examining both root and foliar feeding herbivores demonstrated that when pest densities were low there was no change in herbivore abundance with the use of seed applied neonicotinoids (Bredeson and Lundgren, 2015). With the abundance and composition of pest populations varying annually as a result of weather conditions (Lemic et al., 2016), management practices (e.g., crop rotation) (Gurr et al., 2003), and insect migration patterns (Chapman et al., 2015), preemptively managing for early season pests with seed treatments is not always
beneficial, especially when pest densities remain below their economic incidence levels (Bredeson and Lundgren, 2015; Krupke et al., 2017).

What was more surprising, however, was our observation that populations of at least one herbivore taxon actually increased in the presence of PST. Specifically, Symphyla (root-feeding myriapods) were higher in the presence of PST compared to the control in the first year of our study (Table 1). In high densities Symphyla, which are non-insects closely related to millipedes and centipedes (Diplopoda and Chilopoda), can severely damage crop roots and reduce stand densities (Umble et al., 2006) and have previously been shown to be less susceptible to neonicotinoids (Reynolds, 2008). It is possible, however, that Symphyla are not only tolerant to the PST toxins, but may also be experiencing some sort of ecological release, either bottom-up, through reduced competition for resources, or top-down, through the loss of predator control. Future research will be necessary to determine whether the apparent positive effects of PST on Symphyla are due to tolerance to neonicotinoids, ecological release, or a combination of these factors.

Because soil predators occupy the top trophic position in the soil food web, we might expect that variability in this guild would be correlated with the effects of PST on the taxa occupying the lower trophic positions within the soil food web. In our experiment, however, the predator guild did not respond uniformly to seed treatment across years (Fig. 3), and there was no apparent correspondence between the changes in the predator guild and patterns observed in their prey populations (i.e. detritivore and herbivore guilds). Seed-applied neonicotinoids have been shown to decrease natural enemy abundance compared to no-insecticide controls, even when their effects on potential-prey densities are taken into consideration (Douglas and Tooker, 2016). In some cases, PST may have no apparent direct effect on prey abundance because the
neonicotinoids are trophically transferred from neonicotinoid-tolerant prey that consume treated plant tissues to their predators resulting in increased predator mortality and decreased abundances (Bredeson et al., 2015; Douglas et al., 2015). The time lag between when neonicotinoid tolerant-prey consume treated plant tissues and when susceptible predatory taxa consume those preys may partially explain why in our study the effects of PST on predators persisted longer into the growing season (Fig. 4, 5, and 6) while the effects on most other guilds were observed primarily at the post-planting sampling period.

The effects of PST with neonicotinoids on the soil faunal community were apparent at the feeding guild level, but not when analyzed at the whole-community level (Table 1 and Fig. 2). For our study, we choose to organize the soil community into feeding guilds because previous literature suggested that each guild would respond differentially to PST due to the fact that the pathways in which members of specific guilds interact with the PST toxins differed (Girolami et al., 2009; Bonmatin et al., 2015; Douglas et al., 2015; Gibbons et al., 2015). Organizing the taxa by functional traits (McGill et al., 2006), or to the individual species level, as opposed to broader taxonomic classifications, may afford a more nuanced assessment of the effects of PST on the soil community. This type of approach could aid in determining the degree to which functional redundancies (Naeem, 1998) exist within the community (and at each trophic position) and whether such redundancies might help explain why PST appeared to have relatively little effect on the soil-driven processes we monitored (i.e., surface litter decomposition and plant available soil nitrogen) (Fig. 8 and 9). Even if functional redundancy is substantial, it is possible that thresholds exist (Groffman et al., 2006), such that prolonged PST use would eventually negatively affect agroecosystem processes such as decomposition or plant available nitrogen.
mineralization, particularly in systems where soil arthropod communities are already depauperate due to other disturbances (such as tillage or a history of PST use, etc.).

These data provide compelling evidence that pesticide seed treatments can restructure the soil community by affecting the abundance and diversity of non-targeted organisms at multiple trophic-levels, with the most dramatic effects occurring directly after planting. Changes in the soil community, however, did not alter the two soil-driven processes that we examined, nor did we detect a yield benefit from PST. Finer resolution approaches to measuring decomposition and nutrient cycling may be necessary to reveal how PST affect these agroecosystem processes. Future studies should also consider examining the effects of PST with neonicotinoids on other soil-driven processes, such as biological control of weeds (e.g., Smith et al. 2016) and carbon sequestration, and do so across a range of soil types and soil communities, so that ultimately farmers can better calculate the relative costs and benefits of using PST.

LITERATURE CITED


CHAPTER 4

DIMINISHED RETURNS? EFFECTS OF NEONICOTINOID SEED TREATMENTS ON SOIL ARTHROPOD COMMUNITIES VARY ALONG A PESTICIDE USE GRADIENT

ABSTRACT

The practice of using commodity crop seeds pretreated with insecticide-fungicide mixtures is common in row crop agriculture. Seeds treated with pesticide seed treatments (PST) that contain neonicotinoid insecticides are often being used successively in the same field. However, despite growing concern over the non-target impacts of PST on a variety of arthropod and non-arthropod populations, the impacts of repeated and long-term use of seed treatments on soil communities are poorly understood. We collected intact soil food web communities from four agricultural sites with varying histories of PST use and exposed them to maize seeds with and without PST with neonicotinoids. We assessed how PST affected the abundance and diversity of the soil arthropod community as well as maize growth over a 28-day period. Our findings demonstrate for the first time that soil arthropod communities dramatically change with the initial introduction of PST while communities with prolonged histories of seed treatment exposure are relatively unaffected by subsequent exposures. Our results suggest that the effects of PST on soil biota are strongest the first time they are used and that these effects diminish when PST is used successively. Farmers using PST may experience the greatest “observable returns” the first time they use PST, but the relative effects of PST may decline with repeated use, and thus may result in a case of diminishing returns with rising ecological and economic costs.
INTRODUCTION

Insecticide-fungicide mixtures are commonly applied to the seeds of commodity crops to protect them from early season insect and fungal pests (Tomizawa and Casida, 2005). These pesticide seed treatments (PST) typically include mixtures of systemic insecticides (often in the class neonicotinoid), systemic and contact fungicides, and proprietary adjuvants (e.g., surfactants, wetting agents, and activators) which collectively provide above- and belowground pest protection (Tomizawa and Casida, 2005; Bonmatin et al., 2015; Mullin et al., 2015). In 2011, more than 79% of maize and up to 44% of soybean hectares in the U.S. were planted with seeds pre-coated in a pesticide mixture containing neonicotinoids (Douglas and Tooker, 2015). Recently, the use of seed treatments has come under scrutiny because seed-applied neonicotinoids have been linked to decreased abundances of agronomically-beneficial organisms including bees and predatory ground beetles (Girolami et al., 2009; Krupke et al., 2012; Douglas et al., 2015; Rundlöf et al., 2015; Mogren and Lundgren, 2016) and impairment of important soil functions (Douglas et al., 2015; Smith et al., 2016). However, despite growing concern over the non-target impacts of PST on a variety of arthropod and non-arthropod populations, few studies have assessed the effects of PST on entire communities of organisms, and especially soil arthropod food web communities, which control soil functions and processes that are essential to the sustainability of agriculture. Limited too is our understanding of how repeated and long-term use of PST may influence the response of soil communities to subsequent PST use (Van der Sluijs et al., 2015).

Soil food webs are made up of a diverse assemblage of microbes, arthropods, and other soil organisms whose interactions regulate important agroecosystem services, including
decomposition and carbon sequestration, nutrient cycling, and biocontrol of agronomic pests (Coleman et al., 2004; Bardgett et al., 2005). Soil arthropods are an especially important component of most soil food webs, as they occupy a variety of trophic positions, including detritivore, herbivore, and predator trophic levels. Live and dead plant materials serve as basal resources for the soil food web, including seeds, leaf tissues, roots, and root exudates. Through consumption of these materials, soil organisms at lower trophic-levels immobilize plant-derived carbon and nutrients and then through predator-prey interactions these resources are transferred to higher trophic levels. Soil arthropods, such as Diplopoda (millipedes), facilitate decomposition and microbial colonization of litter by incorporating litter from the soil surface and increasing leaf litter surface area (Cárcamo et al., 2000; Coleman et al., 2004). While microbivorous soil fauna, including Collembola and Acari, alter nutrient cycling via their grazing activities which modifies microbial activity and growth (Wickings and Grandy, 2011; Crowther et al., 2012; Ngosong et al., 2014). Soil arthropods at higher trophic levels, including Chilopoda (centipedes), predatory Coleoptera (beetles), and Araneae (spiders), regulate populations of organisms at lower trophic levels contributing to the biological control of pests (Straub et al., 2008).

With crop plant-based resources taking a central role in the soil food web in agroecosystems, crops treated with PST have the capacity to impact the soil food web at all trophic positions through both direct and indirect pathways. This is especially true given that exposure to seed-applied neonicotinoids can occur not only through the direct consumption of treated plant tissues, but also through dust particles, plant exudates and sap, contaminated soils and water, and trophic transference (Godfray et al., 2014; Bonmatin et al., 2015; Douglas et al., 2015; Gibbons et al., 2015; Godfray et al., 2015; Van der Sluijs et al., 2015).
Seed-applied neonicotinoid insecticides, while intended to target herbivorous insects, have been shown to also affect non-targeted soil arthropods and the biocontrol services they provide (Seagraves and Lundgren, 2012; Bredeson et al., 2015; Douglas and Tooker, 2016; Smith et al., 2016). Some non-target taxa, such as Collembola, which are agriculturally important fungivores (Crossley et al., 1992) due to their beneficial function in the removal of pathogenic fungi and as a prey source for predaceous arthropods (Sabatini and Innocenti, 2000; Agustí et al., 2003; Eitzinger and Traugott, 2011), have been shown to increase in density (El-Naggar and Zidan, 2013) and surface activity (Zaller et al., 2016) with neonicotinoid PST use. In contrast, populations of natural enemies, including predatory ground beetles (Coleoptera Carabidae, *Chlaenius tricolor*), *Nabis americoferus* (Hemiptera Nabidae) and *Chrysoperla* (Neuroptera Chrysopidae), were shown to be negatively affected by neonicotinoid PST (Seagraves and Lundgren, 2012; Douglas et al., 2015; Douglas and Tooker, 2016). PST, due to their effects on the abundance of predatory soil fauna and the soil fungal community (Nettles et al., 2016), have been implicated in observed reductions in the biological control of both insect and mollusk pests (Douglas et al., 2015) and weed seeds in the soil (Smith et al., 2016). Collectively, this evidence suggests seeds pre-coated in neonicotinoids have the potential to affect not only the composition and diversity of soil food webs, but also the agroecosystem services they regulate.

What’s more, crop plant uptake of neonicotinoids in PST can be relatively low (i.e., 1.6-20% depending on the crop, (Sur and Stork, 2003)), suggesting that the majority of the active and inactive ingredients in PST remain in the soil ecosystem where neonicotinoids are capable of persisting for several years (Bonmatin et al., 2015). This suggests that previous research conducted in systems with a history of PST use may underestimate the effects of PST on already depauperate soil communities. As agriculture continues to both intensify and extensify in order
to meet the food, feed, fiber, and biofuel demands of a growing global population (Hunter et al., 2017), it is necessary that we better understand how soil food webs are affected by PST and whether cumulative use of PST and/or site history alters these effects.

Here we report the results of a greenhouse experiment in which we collected intact soil food web communities from four agricultural sites with varying histories of PST use and exposed them to maize seeds with and without PST containing neonicotinoids. We assessed how PST affected the abundance and diversity of the soil arthropod food web community as well as maize growth over a 28-day period. We hypothesized that soil communities with a history of successive exposure to PST would be less affected by PST compared to those communities that were naïve to PST. We also measured the incidence of pathogenic fungal root colonization to determine if the fungicidal components of the PST varied in effectiveness among soils with different PST histories.

METHODS

Experimental design

The experiment was conducted in a greenhouse using intact soil communities (collected as soil cores) from four agricultural sites with varying histories of PST use. Each soil core was placed in a pot and individual cores from each site were assigned to be planted with the same genotype of glyphosate resistant maize either with or without PST. The experiment was arranged in a completely randomized design with two destructive harvest times (14- and 28-days after planting). There were five replications for each site by PST treatment by harvest time combination (80 total experimental units). Each experimental unit (pot) was labelled with a unique identification number which contained no treatment information, thereby reducing the
chance that observer bias could inadvertently influence data collection. Additional details regarding site selection and soil community collection, treatment establishment, and sample processing and data analysis are described below.

Site histories

Intact soil communities were collected from four agricultural field sites near Durham, New Hampshire, USA (43.1340° N, 70.9264° W) that varied in PST use. The sites were all located within a 4-km area. In addition to history of PST use, each site also differed in the community that was present at the time of sampling, previous cropping history, frequency of tillage, as well as other management variables. Each site with a history of PST use was paired with a nearby site in which PST was not used in order to maintain similar soil types between the two sites. The soil community with the longest history of PST exposure (henceforth referred to as ‘Experienced’) was collected from a continuous maize field in Durham, NH where a variety of PST had been used for at least the previous ten years. Soils at this site were annually moldboard plowed and disked prior to planting. The PST most recently used at the Experienced site (i.e., the previous year) was a mixture of the systemic insecticide thiamethoxam (Class: Neonicotinoid), the contact fungicide fludioxonil (Group: Phenylpyrroles), and the systemic fungicides mefenoxam (Group: Phenylamides) and azoxystrobin (Group: Quinone outside inhibitor (QoI)), (Cruiser Extreme® Corn 250, Syngenta, Greensboro, NC, USA). Soil type at this site was a Buxton silt loam.

The soil community with the second longest history of PST exposure was collected from a site in Madbury, NH where PST had been used intermittently over the past five years (hereafter ‘Intermittent’). This site had been in a maize-soybean rotation for five years with PST used only in the maize rotation. Soils at this site were moldboard plowed annually then harrowed. PST had
been used at the *Intermittent* site one year prior to soil community sampling and consisted of a mixture of the systemic neonicotinoid insecticide thiamethoxam, the contact fungicide fludioxonil, and the systemic fungicides mefenoxam, azoxystrobin, and thiabendazole (Group: Methyl Benzimidazole Carbamates), (CruiserMaxx® Corn 250, Syngenta, Greensboro, NC, USA). Soil type at the *Intermediate* site was a Charlton fine sandy loam.

Two sites, and their associated soil communities, had no known history of PST use (hereafter ‘*Naïve* ’). One site was adjacent to and on the same soil type as the *Intermittent* site. Soils at this site had been under continuous untreated alfalfa for the previous five years (hereafter *Naïve alfalfa*). The other *Naïve* site was adjacent to and on the same soil type as the *Experienced* site. Soils at this site had been managed under hayfield production without pesticide applications for the previous 15 years (*Naïve hayfield*).

*Collection of soil communities*

Soil communities were collected as intact cores at each of the four field sites on 19 May 2016. This date was prior to planting maize at both the *Experienced* and *Intermittent* sites, and thus the soil communities at both these sites were representative of a pre-planting community. Each sample consisted of two soil cores (5 cm diameter x 10 cm depth) collected close to one another (i.e., side by side). A total of 20 two-core samples were collected from each of the four sites. Soil cores were carefully removed from the coring apparatus and transferred to an individual 10 mil thick plastic sheet which was wrapped around the soil sample preserving its physical characteristics and vertical orientation. Samples were placed into plastic bags, sealed, and then placed on ice while in the field. All samples were stored in a 4°C refrigerator overnight.
Pot setup and growth conditions in greenhouse

The experiment was conducted at the University of New Hampshire McFarlane Greenhouses facility in Durham, New Hampshire. Polypropylene tall Euro pots (17 cm diameter x 15 cm with 12 holes in the base, Euro Pot, EU170T5) were filled with uninoculated potting mix prior to adding field soil cores. The potting mix ingredients included Canadian sphagnum peat moss, perlite (horticultural grade), vermiculite, dolomitic and calcitic limestone, and wetting agent (ProMix BX, Premier Tech, USA). We saturated the potting mix in each pot with water and then removed two cores of potting mix from the center of each pot. Each core had the same dimensions as the field soil cores (5 cm diameter x 10 cm depth).

The next day (20 May 2016) we carefully placed the field soil cores into the pre-made holes in each pot such that the vertical orientation of the core was retained. A single maize seed (hybrid FC 397 3122, 1st Choice Seeds, Milton, IN, USA) with PST (PST) or without PST (Control) was planted 2.5 cm deep in between the two field soil cores such that seed to field soil contact was maximized (Fig. 1). Seeds with PST were pre-coated with a mixture of the systemic insecticide thiamethoxam (Class: Neonicotinoid), the contact fungicide fludioxonil, and the systemic fungicides mefenoxam (Group: Phenylamides), azoxystrobin (Group: Quinone outside inhibitor (QoI)), and thiabendazole (Group: Methyl Benzimidazole Carbamates), (CruiserMaxx® Corn 250, Syngenta, Greensboro, NC, USA). Five pots in each site x PST treatment combination were randomly assigned to each of the two harvest dates. Numeric identification labels devoid of treatment information were added to each pot during the planting process. Once all pots were labelled, their positions were randomized on the greenhouse bench and then hand-watered.

A single irrigation line was inserted into one of the field soil cores within each pot. For the first week of the experiment, each plant was irrigated with water. Beginning 27 May 2016,
Figure 1. Photo from greenhouse experiment immediately following the sowing of untreated (control) maize seed in between the two cores of *Experienced* field soils surrounded by a sterile potting mix.
plants were fertigated with NPK 15-4-15 fertilizer at 150 ppm in two pulses (80 mL/pulse) every other day. The frequency of fertilizer pulsing increased as the plants grew such that for the third week of the experiment two pulses of fertilizer were provided every day and in the final week two pulses of fertilizer were provided twice daily.

_Destructive harvest_

The soil communities were sampled destructively after 14 and 28 days (3 June 2016 and 17 June 2016, respectively). For each sampling event, aboveground maize biomass was clipped at the soil surface, bagged, labelled, and then placed in a 60°C oven until constant mass was maintained prior to weighing. Remaining material in each pot was then inverted over a labelled plastic bag and the entire root ball was carefully removed by hand. Loose soil was carefully removed from the roots. Root balls were then placed into clean, labelled plastic bags and stored in a 4°C fridge for subsequent analysis of fungal root pathogens (see below). All remaining soils and the associated soil arthropod community within the pot were sealed in their respectively labelled bags and placed directly into a 4°C fridge for arthropod extraction (see below).

_Maize response to PST_

In addition to aboveground biomass, we also measured maize leaf chlorophyll 13 and 27 days after planting. Leaf chlorophyll is a proxy for leaf nitrogen content and therefore offers an indirect assessment of plant nitrogen status (Alcántar et al., 2002). Similar to Piekkielek and Fox (1992), we used a handheld chlorophyll meter (SPAD 502 Plus; Konica Minolta, Spectrum Technologies, Inc., Aurora, IL) and collected ten readings on the newest fully mature leaf between the leaf margin and central vein for each plant. All plant nitrogen status data are reported in SPAD units.
**Soil faunal community**

To assess the soil arthropod communities, we used Berlese-Tullgren funnels to extract all live and active fauna from the soil in each pot at each harvest period. Extractions were conducted for 72 hours, during which time temperatures slowly increased from room temperature (22°C) to a maximum of 50°C. This method extracts live and active fauna by slowly desiccating the soil from the top down, encouraging organisms to burrow downward into a collection vial. Extracted organisms were stored in 90% ethyl alcohol at room temperature for later identification. All soil arthropods were identified using distinguishable morphological characteristics based on Triplehorn and Johnson (2005) and assistance from the curator of the University of New Hampshire’s Insect Collection (Dr. Donald Chandler, Professor of Zoology). Soil arthropods are reported as the number of individuals per experimental unit (an individual pot).

Over the course of the greenhouse study, several common greenhouse pests likely colonized our soils. These include shoreflies (Ephydridae), fungus gnats (Mycetiophilidae), thrips (Thysanoptera), and parasitoid wasps of shoreflies (Hymenoptera Figitidae *Hexacola neoscatellae*). While these taxa were likely not initially present in the field-collected soil cores, the fact that each pot was managed under identical conditions (in terms of watering and fertilization) and randomly placed on the greenhouse bench provides the opportunity to examine the effects of the treatments on natural processes of colonization.

**Root fungal pathogens**

After the root balls were carefully removed from the intact soil cores, loose soils were manually removed from the roots. A clean moist paper towel was then wrapped around each root ball prior to it being placed into a clean plastic bag. Within 72 hours of collection, samples were shipped overnight to the root fungal analysis laboratory.
Pathogenic fungal colonization was evaluated by the North Dakota State University Plant Diagnostic Laboratory on 8 June 2016 and 22 June 2016 using their standard diagnostic procedures. Briefly, this involved isolating any diseased root tissue, cleaning the tissue with 10% bleach solution, rinsing the tissue with autoclaved distilled water, air drying the tissue in a sterile biosecurity hood, and then plating portions of tissue into two culturing environments: (1) molecular grade water and (2) one-half strength acidified potato dextrose agar (1/2aDPA) and water agar (WA). For roots that appeared completely healthy, random tissue sections were taken from the whole plant and processed in the same manner as the symptomatic root tissue. Pathogens were identified over the course of the next couple of weeks, and if necessary plates were sub-cultured to better identify the pathogens. Fungi were identified under a compound microscope based on colony and spore characteristics.

Statistical analyses

Soil arthropod total abundance, richness, evenness, and diversity data were analyzed with two-factor ANOVA in SAS (Version 9.4, SAS Institute, Cary, NC) using the MIXED procedure. In all analyses, data for each site (Experienced, Intermittent, Naïve Alfalfa, and Naïve Hayfield) were analyzed separately with seed treatment (PST or Control) and harvest time (14- and 28-days) as fixed factors. To achieve homogeneity of variances, abundance data were log_{10} (x+1) transformed prior to statistical analyses. Subsequent t-tests were conducted using the PROC TTEST procedure in SAS if the interaction between seed treatment and harvest time was significant (significance level at p < 0.05). Treatment effects on select taxa groups were also analyzed using two-factor ANOVA and t-tests when applicable (significance level at p < 0.05). Soil arthropod abundances are reported as the number of individuals per pot ± standard error of the mean (SEM).
In addition to univariate analyses, we conducted several multivariate analyses to test for significant differences in soil arthropod community composition between treatments and harvest times. We conducted a permutation-based multivariate analysis of variance (PerMANOVA, Anderson, 2001) on a distance matrix of Bray-Curtis dissimilarity coefficients calculated with species-level raw abundances using the adonis2 command in the ‘Vegan’ package in R (Oksanen et al., 2007; R Core Team, 2014). All pairwise perMANOVA comparisons were made using the package “RVAideMemoire” pairwise.perm.manova command in R (Hervé, 2015). Non-metric multidimensional scaling ordination based on Bray-Curtis dissimilarity coefficients was used to visualize treatment and harvest time effects on soil community composition and abundance in PCord (McCune and Mefford, 1999).

We calculated the frequency of plants colonized by each pathogen based on site history, seed treatment, and harvest date. Statistical analyses were not conducted on these data due to the fact that these were presence-absence data with small sample sizes (n = 5).

RESULTS

Soil arthropod taxa at each site

We recorded a total of 53 taxa across all sites, seed treatments, and harvest dates. On average, 102.55 ± 12.50 individuals were collected per pot. The soil arthropod communities consisted of Ephydridae, Collembola, Acari, Thysanoptera and Aphididae, Figitidae, Mycetiophilidae, and Diptera larvae which contributed 39.15 ± 3.49%, 20.49 ± 2.59%, 15.81 ± 1.85%, 7.34 ± 0.92%, 6.37 ± 1.28%, 6.29 ± 1.13%, and 2.18 ± 0.44% to total arthropod community abundance, respectively.
In the *Experienced* soil, a total of 35 taxa were recorded across all seed treatments and harvest dates. On average, 131.15 ± 32.32 individuals were collected per pot. The most abundant arthropods consisted of Ephydridae (53.54 ± 7.88%), Collembola (15.87 ± 4.57%), Figitidae (9.71 ± 3.78%), Mycetiophilidae (6.01 ± 2.53%), Acari (5.64 ± 1.43%), Thysanoptera and Aphididae (4.99 ± 1.21%), and Diptera larvae (2.41 ± 0.86%), (Fig. 2).

A total of 35 arthropod taxa were recorded for the *Intermittent* soils across all seed treatments and harvest dates. An average of 62.70 ± 6.12 individuals was collected per pot. Arthropods consisted of Ephydridae (40.82 ± 6.37%), Collembola (14.51 ± 3.34%), Acari (14.07 ± 2.74%), Thysanoptera and Aphididae (9.44 ± 1.93%), Mycetiophilidae (8.62 ± 2.41%), Figitidae (6.54 ± 2.02%), Diptera (2.77 ± 1.19%), and Myriapoda (Symphyla and Chilopoda, 1.65 ± 0.58%), (Fig. 2).

In *Naïve Alfalfa*, a total of 37 taxa were recorded with an average of 170 ± 30.84 individuals per pot. The most abundant arthropod groups included Ephydridae (38.35 ± 7.61%), Collembola (33.56 ± 7.63%), Acari (10.79 ± 3.38%), Figitidae (5.89 ± 2.59%), Mycetiophilidae (5.88 ± 2.58%), Thysanoptera and Aphididae (3.66 ± 0.85%), and Diptera larvae (1.00 ± 0.36%), (Fig. 2).

A total of 40 taxa were recorded for the *Naïve Hayfield* soils across all seed treatments and harvest dates. An average of 45.8 ± 4.92 individuals was collected per pot. The most abundant arthropods consisted of Acari (32.77 ± 3.72%), Ephydridae (23.87 ± 4.42%), Collembola (18.03 ± 3.06%), Thysanoptera and Aphididae (11.27 ± 2.46%), Mycetiophilidae (4.66 ± 1.45%), Figitidae (3.33 ± 1.15%), Diptera larvae (2.54 ± 0.95%), and Coleoptera adults and larvae (1.49 ± 0.70%), (Fig. 2).
Figure 2. Abundances of major groups of soil arthropods in pots treatments (Control and PST) containing soils collected from agricultural sites representing a PST use gradient in SE New Hampshire. Sites were characterized by frequent PST use, Experienced; intermittent PST use, Intermittent; and no previous history of PST use, Naive Alfalfa and Naive Hayfields. Data are means of harvest periods (14- and 28-days), n = 10; SEM is based on mean of total abundance, n = 10.
Effects of PST on soil arthropod communities

The effect of PST on soil arthropod communities depended on the site from which the soil communities were collected (perMANOVA, Site*Treatment*Harvest: P < 0.001). Communities originating from the two sites with a history of prior PST use (i.e., the Experienced and Intermittent) were not affected by the PST treatment applied in the greenhouse (perMANOVA, Experienced - Treatment: P = 0.130, Harvest: P < 0.001, Treatment*Harvest: P = 0.186; Intermittent - Treatment: P = 0.661, Harvest: P < 0.001, Treatment*Harvest: P = 0.222). Not surprisingly, harvest date did affect soil arthropod community composition and species abundance; however, there was no interaction between harvest date and PST treatment on soil communities collected from these two sites. In contrast, soil arthropod communities that were collected from the two sites with no prior exposure to PST use (i.e., Naïve Alfalfa and Naïve Hayfield) were strongly affected by both PST treatment and harvest time, and these two factors interacted, indicating that the composition and species abundance of these communities was highly responsive to the pesticides contained within the PST (perMANOVA, Naïve Alfalfa - Treatment: P = 0.002, Harvest: P = 0.001, Treatment*Harvest: P = 0.004; Naïve Hayfield - Treatment: P = 0.014, Harvest: P = 0.001, Treatment*Harvest: P = 0.012, Fig. 3). Soil communities at both sites were affected by PST at the 14-day harvest. Differences were also significant at the 28-day harvest in the Naïve Hayfield community, but not the Naïve Alfalfa community (Pairwise perMANOVA, Naïve Hayfield: 14-day harvest: P = 0.025, 28-day harvest: P = 0.010; Naïve Alfalfa: 14-day harvest: P = 0.009, 28-day harvest: P = 0.059).
Figure 3. Nonmetric multidimensional scaling ordinations showing the effects of PST on soil arthropod community composition in (a) *Naïve Alfalfa* (NMDS Stress = 11.985, p = 0.0120) and (b) *Naïve Hayfield* (Stress = 17.488, p = 0.0199). Ellipses denote that groups are significantly different p < 0.05, based on perMANOVA.
Effects of PST on total soil arthropod abundance

The total abundance of soil arthropods was not affected by PST in soils collected from either the Experienced (ANOVA, Treatment: $F_{1,16} = 1.04, P = 0.3231$; Harvest: $F_{1,16} = 4.07, P = 0.0606$; Treatment*Harvest: $F_{1,16} = 0.3566, P = 0.5666$) or Intermittent site (ANOVA, Treatment: $F_{1,16} = 1.23, P = 0.2835$; Harvest: $F_{1,16} = 2.34, P = 0.1455$; Treatment*Harvest: $F_{1,16} = 0.55, P = 0.4701$; Fig. 4 a and b). In contrast, in soils collected from both of the sites with no previous history of PST use, total arthropod abundance was significantly higher in the Control compared to the PST treatment (ANOVA, Naïve Alfalfa: Treatment: $F_{1,16} = 6.99, P = 0.0177$; Harvest: $F_{1,16} = 5.87, P = 0.0276$; Treatment*Harvest: $F_{1,16} = 16.54, P = 0.0009$; Naïve Hayfield: Treatment: $F_{1,16} = 4.30, P = 0.0547$; Harvest: $F_{1,16} = 17.98, P = 0.0006$; Treatment*Harvest: $F_{1,16} = 7.13, P = 0.0168$, Fig. 4 c and d), and these differences were most pronounced at the 28-day harvest (T-test, Naïve Alfalfa: 14-day harvest: $t_8 = -1.69, P = 0.1295$; 28-day harvest: $t_8 = 3.70, P = 0.0060$; Naïve Hayfield: 14-day harvest: $t_8 = -0.63, P = 0.5453$; 28-day harvest: $t_8 = 2.69, P = 0.0275$).

Effects of PST on abundance of taxa groups

We examined the effect of PST treatment on taxa groups for which abundances were large enough to permit statistical analysis. These included the Ephydridae, Collembola, Acari, Thysanoptera and Aphididae, Figitidae (at 28-day harvest only), Mycetiophilidae, and Diptera larvae. Among these groups, only the Ephydridae, Collembola, and Figitidae exhibited significant differences in abundance due to PST treatment.

Ephydridae abundance in soils collected from the Experienced and Naïve Alfalfa sites was nearly two-times higher in the PST compared to the Control treatment, and this effect was consistent across the two harvest times (ANOVA, Experienced: Treatment: $F_{1,16} = 4.95, P =$
Figure 4. Effects of PST on the total abundance of soil arthropods collected from sites representing a PST use intensity gradient in SE New Hampshire. Soil communities were collected from sites characterized by (a) frequent PST use, *Experienced*; (b) intermittent PST use, *Intermittent*; and no previous history of PST use, (c) *Naïve Alfalfa* and (d) *Naïve Hayfields*. Data were broken out by harvest time when ANOVA indicated a significant PST treatment*harvest time interaction (*p < 0.05*). Box plots are based on an *n* = 10 for *Experienced* and *Intermittent*, and *n* = 5 for both *Naïve* sites. The line within the box represents the median; the box represents 50% of these data; whiskers represent the 10th and 90th percentiles; dots indicate outliers. Asterisks denote significant differences between means (*p < 0.05*, **p < 0.01*, ***p < 0.001*).
0.0409; Harvest: $F_{1,16} = 77.68$, $P < 0.0001$; Treatment*Harvest: $F_{1,16} = 4.16$, $P = 0.0584$; Naïve Alfalfa: Treatment: $F_{1,16} = 11.85$, $P = 0.0033$; Harvest: $F_{1,16} = 56.67$, $P < 0.0001$; Treatment*Harvest: $F_{1,16} = 0.03$, $P = 0.8697$). Ephydridae abundance was also higher in the PST compared to Control treatment in soils collected from the Naïve Hayfield, but only at the 14-day harvest period (ANOVA, Treatment: $F_{1,16} = 1.34$, $P = 0.2634$; Harvest: $F_{1,16} = 2.95$, $P = 0.1051$, Treatment*Harvest: $F_{1,16} = 6.97$, $P = 0.0178$). There was no difference in Ephydridae abundance between PST and Control treatments in soils collected from the Naïve Hayfield site at the 28-day harvest (T-test: 14-day harvest: $t_8 = -4.11$, $P = 0.0034$, 28-day harvest: $t_8 = 0.83$, $P = 0.4280$).

Ephydridae abundance did not differ between treatments at either harvest time in soils collected from the Intermittent site (ANOVA: Treatment: $F_{1,16} = 0.39$, $P = 0.5390$; Harvest: $F_{1,16} = 3.91$, $P = 0.0654$; Treatment*Harvest: $F_{1,16} = 2.41$, $P = 0.1403$).

In soils collected from both of the naïve sites the PST treatment reduced the abundance of Collembola, particularly those in the Sminthuridae family; however, this effect was significant only at the 28-day harvest (ANOVA, Naïve Alfalfa: Treatment: $F_{1,16} = 44.41$, $P < 0.0001$; Harvest: $F_{1,16} = 5.44$, $P = 0.0330$, Treatment*Harvest: $F_{1,16} = 15.73$, $P = 0.0011$; Naïve Hayfield: Treatment: $F_{1,16} = 23.04$, $P = 0.0002$; Harvest: $F_{1,16} = 0.15$, $P = 0.7045$, Treatment*Harvest: $F_{1,16} = 8.21$, $P = 0.0112$; Fig. 5). The most dramatic reduction in Collembola abundance with PST treatment was observed in soils collected from the Naïve Alfalfa site, where there were on average 64 times more Collembola in the Control compared to the PST treatment at the 28-day harvest (T-test: 14-day harvest: $t_8 = 2.11$, $P = 0.0680$, 28-day harvest: $t_8 = 6.91$, $P = 0.0001$).

Similarly, there were on average 16 more Collembola individuals per pot in the Control compared to the PST treatment at the 28-day harvest in soils collected from the Naïve Hayfield (T-test: 14-day harvest: $t_8 = 1.51$, $P = 0.1697$, 28-day harvest: $t_8 = 4.99$, $P = 0.0011$). Differences
Figure 5. Effects of PST on the abundance of major groups of Collembola in soils collected from sites representing a PST use intensity gradient in SE New Hampshire. Soil communities were collected from sites characterized by (a) frequent use of PST, Experienced; (b) intermittent use of PST, Intermittent; and no previous use of PST (c) Naïve Alfalfa and (d) Naïve Hayfield. Data were broken out by harvest time when ANOVA indicated a significant PST treatment*harvest time interaction (p<0.05). Bars depict the means of n = 10 for Experienced and Intermittent, and n = 5 for both Naïve sites. SEM is based on mean of total abundance, n = 10. Asterisks denote significant differences between means (*p < 0.05, **p < 0.01, ***p < 0.001).
in Collembola abundance between PST and Control treatments were not significant in soils collected from either the Experienced or Intermittent sites (ANOVA, Experienced: Treatment: $F_{1,16} = 2.72, P = 0.1188$; Harvest: $F_{1,16} = 0.00, P = 0.9481$, Treatment*Harvest: $F_{1,16} = 0.68, P = 0.4230$; Intermittent: Treatment: $F_{1,16} = 1.54, P = 0.2321$; Harvest: $F_{1,16} = 0.41, P = 0.5297$, Treatment*Harvest: $F_{1,16} = 0.82, P = 0.3796$).

The PST treatment reduced the abundance of the parasitoid wasp group Figitidae at the 28-day harvest in soils collected from the Naïve Hayfield (ANOVA, Naïve Hayfield: Treatment: $F_{1,8} = 5.83, P = 0.0422$). Specifically, there were over four times more Figitidae in the Control compared to the PST treatment. Similar trends were observed in soils collected from the Experienced and Naïve Alfalfa sites (ANOVA, Experienced: Treatment: $F_{1,8} = 1.14, P = 0.3165$, Naïve Alfalfa: Treatment: $F_{1,8} = 1.85, P = 0.2106$).

**Effects of PST on soil arthropod diversity**

Soil arthropod richness was lower in the PST compared to the Control treatment in soils collected from the two naïve sites (Fig. 6). In soils collected from the Naïve Alfalfa site there were on average two fewer taxa present in the PST compared to the Control treatment (ANOVA: Treatment: $F_{1,16} = 4.72, P = 0.0452$; Harvest: $F_{1,16} = 0.64, P = 0.4352$, Treatment*Harvest: $F_{1,16} = 1.06, P = 0.3188$) and in the Naïve Hayfield there were nearly four fewer taxa present in the PST treatment compared to the control (ANOVA: Treatment: $F_{1,16} = 9.53, P = 0.0071$; Harvest: $F_{1,16} = 8.47, P = 0.0102$, Treatment*Harvest: $F_{1,16} = 0.19, P = 0.6651$). Soil arthropod richness did not differ between treatments in soils collected from either of the sites with a previous history of PST use (ANOVA, Experienced: Treatment: $F_{1,16} = 3.22, P = 0.0916$, Harvest: $F_{1,16} = 0.42, P = 0.5273$, Treatment*Harvest: $F_{1,16} = 1.49, P = 0.2399$; Intermittent: Treatment: $F_{1,16} = 0.42, P = 0.5263$, Harvest: $F_{1,16} = 4.43, P = 0.0514$, Treatment*Harvest: $F_{1,16} = 0.10, P = 0.7502$).
Figure 6. Effects of PST on community richness (number of taxa per pot) in soils collected from sites representing a PST use intensity gradient in SE New Hampshire. Soil communities were collected from sites characterized by (a) frequent use of PST, Experienced; (b) intermittent use of PST, Intermittent; and no previous use of PST (c) Naïve Alfalfa and (d) Naïve Hayfield. Bars depict the means and SEM of n = 10. Asterisks denote significant differences between means (*p < 0.05, **p < 0.01, ***p < 0.001).
Soil arthropod evenness also differed between the PST and Control treatments in the naïve soils; however, the nature of the responses varied with harvest time (ANOVA, Naïve Alfalfa: Treatment: $F_{1,16} = 0.33$, $P = 0.5760$; Harvest: $F_{1,16} = 4.64$, $P = 0.0469$; Treatment*Harvest: $F_{1,16} = 29.30$, $P < 0.0001$; Naïve Hayfield: Treatment: $F_{1,16} = 7.53$, $P = 0.0144$; Harvest: $F_{1,16} = 0.52$, $P = 0.4829$; Treatment*Harvest: $F_{1,16} = 3.25$, $P = 0.0905$; Fig. 7). In soils collected from the Naïve Alfalfa site, evenness was greater in the Control compared to the PST treatment at the 14-day harvest, while the opposite response was observed at the 28-day harvest (T-test: 14-day harvest: $t_8 = 4.84$, $P = 0.0013$, 28-day harvest: $t_8 = -3.08$, $P = 0.0151$). Conversely, in soils collected from the Naïve Hayfield site, evenness was higher in the Control compared to the PST treatment at both harvest times. In contrast to the soils collected from the naïve sites, soils collected from the two sites with histories of PST use exhibited no differences in arthropod evenness in response to the PST treatment (ANOVA, Experienced: Treatment: $F_{1,16} = 0.01$, $P = 0.9127$; Harvest: $F_{1,16} = 23.17$, $P = 0.0002$; Treatment*Harvest: $F_{1,16} = 0.07$, $P = 0.7956$; Intermittent: Treatment: $F_{1,16} = 1.06$, $P = 0.3180$; Harvest: $F_{1,16} = 7.90$, $P = 0.0126$; Treatment*Harvest: $F_{1,16} = 0.98$, $P = 0.3377$).

Effects of PST treatment on soil arthropod diversity (Shannon’s $H'$) were similar in nature to those observed for taxa evenness. Significant PST treatment effects were observed only in the soils collected from the two naïve sites (ANOVA, Naïve Alfalfa: Treatment: $F_{1,16} = 2.41$, $P = 0.1402$; Harvest: $F_{1,16} = 5.58$, $P = 0.0311$; Treatment*Harvest: $F_{1,16} = 27.37$, $P < 0.0001$; Naïve Hayfield: Treatment: $F_{1,16} = 11.14$, $P = 0.0042$; Harvest: $F_{1,16} = 3.66$, $P = 0.0740$; Treatment*Harvest: $F_{1,16} = 0.67$, $P = 0.4248$; Fig. 8). In soils collected from the Naïve Alfalfa
Figure 7. Effects of PST on community evenness (equitability of abundance) in soils collected from sites representing a PST use intensity gradient in SE New Hampshire. Soil communities were collected from sites characterized by (a) frequent use of PST, Experienced; (b) intermittent use of PST, Intermittent; and no previous use of PST (c) Naïve Alfalfa and (d) Naïve Hayfield. Data were broken out by harvest time when ANOVA indicated a significant PST treatment*harvest time interaction (p<0.05). Bars depict the means of n = 10 for Experienced, Intermittent, and Naïve Hayfield, and n = 5 for Naïve Alfalfa. SEM is based on mean of total abundance, n = 10. Asterisks denote significant differences between means (*p < 0.05, **p < 0.01, ***p < 0.001).
Figure 8. Effects of PST on community diversity (Shannon-Weiner diversity (H)) in soils collected from sites representing a PST use intensity gradient in SE New Hampshire. Soil communities were collected from sites characterized by (a) frequent use of PST, Experienced; (b) intermittent use of PST, Intermittent; and no previous use of PST (c) Naïve Alfalfa and (d) Naïve Hayfield. Data were broken out by harvest time when ANOVA indicated a significant PST treatment*harvest time interaction (p<0.05). Bars depict the means of n = 10 for Experienced, Intermittent, and Naïve Hayfield, and n = 5 for Naïve Alfalfa. SEM is based on mean of total abundance, n = 10. Asterisks denote significant differences between means (*p < 0.05, **p < 0.01, ***p < 0.001).
site, the diversity response depended on the harvest time, and was lower in the PST treatment at the 14-day harvest but higher in the PST treatment at the 28-day harvest (T-test: 14-day harvest: \( t_8 = 5.39, P = 0.0007 \), 28-day harvest: \( t_8 = -2.37, P = 0.0454 \)). There were no significant effects of PST treatment on arthropod diversity in soils collected from either of the two sites with histories of PST use (ANOVA, Experienced: Treatment: \( F_{1,16} = 0.88, P = 0.3624 \); Harvest: \( F_{1,16} = 15.32, P = 0.0012 \); Treatment*Harvest: \( F_{1,16} = 0.21, P = 0.6525 \); Intermittent: Treatment: \( F_{1,16} = 0.21, P = 0.6506 \); Harvest: \( F_{1,16} = 8.67, P = 0.0095 \); Treatment*Harvest: \( F_{1,16} = 0.66, P = 0.4273 \)).

**Effects of PST on maize root colonization by pathogenic fungi**

We assessed infection of maize roots by three genera of pathogenic fungi at each of the two harvest times. At the 14-day harvest, none of the maize plants in the Control treatment exhibited infection by *Pythium* spp. In contrast, *Pythium* infection was observed in at least some of the plants with the PST treatment in soils from all sites except the Naïve Hayfield (Table 1). Similarly, *Rhizoctonia* infection was observed in some of the plants in the Control treatment in soil from the Experienced site, but not in soils from the other three sites. In contrast, *Rhizoctonia* was observed in plants grown with the PST treatment in soils from all of the sites except the Naïve Alfalfa. *Fusarium* was observed to occur in plants grown both with and without PST, regardless of where the soil was collected.

By the 28-day harvest, all three pathogenic fungi were observed in at least some of the plants grown in the Control treatment in soils from all four sites (Table 1). None of the three pathogen groups were present in plants grown with the PST treatment in soils collected from the Naïve Hayfield site; however, plants grown with the PST treatment in soils collected from the other three sites had at least two of the pathogens present.
Table 1. Percent of individual maize plants colonized by three genera of pathogenic fungi, *Pythium spp.*, *Rhizoctonia spp.* and *Fusarium spp.* by seed treatment (PST, Control), harvest date, and soil collection site. Data are means ± SEM, n = 5.

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Site</th>
<th>Pythium spp.</th>
<th>Rhizoctonia spp.</th>
<th>Fusarium spp.</th>
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<td></td>
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<td>Control</td>
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<td>0 ± 0</td>
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<td><em>Intermittent</em></td>
<td>20 ± 20</td>
<td>0 ± 0</td>
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<tr>
<td></td>
<td><em>Naïve Alfalfa</em></td>
<td>20 ± 20</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td><em>Naïve Hayfield</em></td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>20 ± 20</td>
</tr>
<tr>
<td>28-days</td>
<td><em>Experienced</em></td>
<td>40 ± 25</td>
<td>40 ± 25</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td><em>Intermittent</em></td>
<td>40 ± 25</td>
<td>60 ± 25</td>
<td>40 ± 25</td>
</tr>
<tr>
<td></td>
<td><em>Naïve Alfalfa</em></td>
<td>80 ± 20</td>
<td>40 ± 25</td>
<td>40 ± 25</td>
</tr>
<tr>
<td></td>
<td><em>Naïve Hayfield</em></td>
<td>0 ± 0</td>
<td>40 ± 25</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>
Maize growth responses to PST

There were no effects of the PST treatment on maize aboveground biomass or plant nitrogen status in any of the soils collected from the four sites (Table 2).

DISCUSSION

The objectives of this study were to (a) quantify the effects of PST on communities of soil arthropods and maize growth, (b) determine if the effects of PST are site-specific, and (c) determine whether effects of PST on soil communities are stronger at sites with no previous history of PST use compared to those with a history of repeated exposure to PST. Our data indicate that PST can have dramatic impacts on soil arthropod community abundance, species richness, diversity, and species composition, and that these effects primarily involve taxa that are not the intended targets of PST. Our data also indicate that these effects vary among sites. In accordance with our hypothesis, our data show that soil communities in sites with histories of PST use were affected less strongly by PST than communities in sites with no previous history of PST. Communities that were naïve to seed treatments exhibited significant changes in composition and reductions in total arthropod abundance, richness, and diversity within 28-days of their first exposure to PST. These results suggest that first-time use of PST can result in quantifiable impacts on soil communities, which raises concern about the larger-scale implications of increased adoption of this management practice globally (Douglas and Tooker, 2015).

In naïve soils, the introduction of PST changed soil community composition primarily through the elimination of apparently susceptible community members (Fig. 6). In our soils, Collembola, especially species in the family Sminthuridae, exhibited the greatest loss in
Table 2. ANOVA table showing p-values for tests of the effect of seed treatment on aboveground biomass and plant nitrogen status of maize grown in soil collected from four sites representing a gradient in PST use.

<table>
<thead>
<tr>
<th>Site</th>
<th>Experienced</th>
<th>Intermittent</th>
<th>Naïve Alfalfa</th>
<th>Naïve Hayfield</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aboveground Biomass</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Treatment</td>
<td>0.4709</td>
<td>0.2512</td>
<td>0.7298</td>
<td>0.7171</td>
</tr>
<tr>
<td>Harvest</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Seed Treatment*Harvest</td>
<td>0.7358</td>
<td>0.3901</td>
<td>0.9102</td>
<td>0.5668</td>
</tr>
<tr>
<td><strong>Leaf nitrogen (SPAD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed Treatment</td>
<td>0.8768</td>
<td>0.6814</td>
<td>0.9172</td>
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</tr>
<tr>
<td>Harvest</td>
<td>0.0186</td>
<td>0.1291</td>
<td>0.2432</td>
<td>0.5692</td>
</tr>
<tr>
<td>Seed Treatment*Harvest</td>
<td>0.0868</td>
<td>0.3859</td>
<td>0.7096</td>
<td>0.6124</td>
</tr>
</tbody>
</table>
abundance when exposed to PST. These small, oval-bodied hexapods (0.75-3 mm) occupy various feeding guilds within the soil food web, depending on species; however, most are considered fungivorous or detritivorous and contribute to decomposition and nutrient cycling services in agroecosystems (Triplehorn and Johnson, 2005). In previous studies, another Collembolan family, Entomobryidae, has been shown to increase in abundance and activity with neonicotinoid PST use (El-Naggar and Zidan, 2013; Zaller et al., 2016). Together these data suggest that the families within Collembola vary in their susceptibilities to PST.

Ecologically, the loss of species diversity within a community can lower the community’s ability to respond to and recover from disturbances (Brussaard et al., 2007). This is concerning given that most agricultural soils are already frequently disturbed due to agricultural management practices such as tillage and crop harvest, and are increasingly subject to disturbance in the form of weather extremes, which are expected to increase in both intensity and frequency with climate change (Hayhoe et al., 2008).

Our observation that soil arthropod communities in sites with histories of PST use exhibited relatively little change with subsequent PST exposure suggests that soil arthropod taxa that are susceptible to PST had already been eliminated by prior PST use, leaving behind only those taxa which tend not to be susceptible. With respect to seed-applied neonicotinoids, non-insect arthropods like spiders (Araneae) and mites (Acari) are generally thought to be more tolerant compared to insects (Douglas and Tooker, 2016). In contrast, we observed the smallest proportion (and total abundance) of Acari observed in our study at the site with the most frequent PST use (Experienced, Fig. 2). We suspect, however, that this primarily reflects the minimal diversity and amounts of surface residues (e.g. crop leaf tissues) present at this site compared to
the other sites during sampling considering litter complexity and composition are known to affect mite diversity and abundance (Hansen and Coleman, 1998).

An alternative explanation for the lack of a PST effect is that the susceptible populations evolved tolerance or resistance to the pesticides in the PST used in this study. Disturbances associated with agricultural management practices often tend to select for organisms that are tolerant of disturbance and can shift community composition towards taxa that are disturbance specialists (McIntyre and Lavorel, 1994). Similarly, widespread and frequent use of neonicotinoids has been shown to lead to insect populations that are resistant to the pesticide. To date, species-level resistance to neonicotinoids has been documented in several pest species including aphids, whiteflies, and Colorado potato beetles (*Leptinotarsa decemlineata*) (Nauen and Denholm, 2005; Bass et al., 2015). The extensive use of neonicotinoids as seed treatments therefore represents an additional source of selection for resistance, which could compromise the efficacy of these insecticides (Bass et al., 2015). This situation is similar to the dramatic increase in glyphosate resistant weeds that occurred following the rapid and widespread adoption of glyphosate-resistant crops (NRC, 2010; Powles, 2010).

Similarly, the fungicides used in PST often include major site-specific groups such as Quinone outside inhibitors (QoIs; e.g. azoxystrobin) and phenylamides (e.g. mefenoxam), (see Monsanto, 2017; Pioneer, 2017; Syngenta Crop Protection, 2017). Both of these fungicide groups are at high risk for selecting for resistance (Fungicide Resistance Action Committee, 2017) because they have site-specific modes of action, which means only a single mutation must occur at the target site for a resistant pathogen population to develop (Hahn, 2014). Although mixtures of fungicides and insecticides are commonly used in agriculture, our current understanding of their combined effect on the soil fungal community is quite limited, particularly
when they are mixed with neonicotinoids. The PST used in our study included three fungicides, azoxystrobin, mefenoxam, and thiabendazole. We found that this mixture provided only limited apparent protection from the three fungal pathogen genera assessed in our study, and protection appeared to vary by site or soil community. For example, compared to the control, the seed treatment completely protected the maize seedlings from *Pythium*, *Rhizoctonia*, and *Fusarium* through 28-days of growth in one of the soils with no history of PST-use; however, the seed treatment provided no added benefit in the other naïve soil (Table 1). Similar site-dependent effects of fungicides have been found by others (Weems et al., 2015).

*Limitations of the experiment*

There are several important aspects of our experiment that limit our ability to conclusively ascribe the relative lack of PST treatment effects we observed to the previous history of PST use at our sites. First, both of the sites with a previous history of PST use (‘Experienced’ and ‘Intermittent’) were also annually tilled and planted to annual crops, while the plant communities at the two naïve sites were both dominated by perennial species and had not been tilled for at least the previous five (*Naïve Alfalfa*) or fifteen (*Naïve Hayfield*) years. Hence, it is not possible to determine whether soil arthropod communities in the sites with previous PST exposure were less responsive to PST because susceptible organisms had indeed already been eliminated by (or evolved resistance due to) prior PST exposure or because frequent tillage and/or other aspects of annual crop production systems effectively constrained community membership to taxa that tend to be less responsive to PST. Second, the soils for this experiment were collected from only four sites, each with a unique PST management history and plant community, and across two soil types, limiting our ability to generalize beyond these four sites. Additional site replication, including sampling across chrono-sequenced seed treatment
applications in otherwise similarly managed annual cropping systems, would aid in further unravelling the relationships between use of PST and changes in the soil arthropod community. Third, the soil arthropod communities in our study had relatively low trophic-level diversity because communities were only collected by soil coring in the early spring. Only using soil cores for community collection likely limited the abundance of vagile epigeal species included in our study. An early spring sampling date was chosen to avoid exposing soil arthropod communities to the seed treatment twice in the same year and because this sampling time captured the soil community present at the time that crops with PST are typically planted. Fourth, our community analyses were all species-based. A trait-based approach (e.g., McGill et al., 2006) would likely provide additional insights into how seed treatments affect soil arthropod communities and the agroecological processes and functions they regulate. Finally, while our data do not indicate that the effects of PST on soil arthropod communities result in substantive impacts on maize growth, this was the only metric of agroecological function that we measured, and this was limited to 14 and 28-days after planting. This time period may not have been sufficient to detect longer-term effects on maize yield. Seed treatments have been shown to affect other agroecosystem services, such as the biocontrol of insect pests and weed seeds (Douglas et al., 2015; Smith et al., 2016). Future studies should consider investigating the effects of repeated applications of seed treatments on other ecosystem services and be conducted over longer timespans.

Agricultural and ecological implications

Our results suggest that the effects of PST on soil biota are strongest the first time they are used and that these effects diminish when PST is used successively. If this is true, farmers using PST may experience the greatest “observable returns” the first time they use PST. In the context of agricultural decision-making, farmers typically follow a process of learning and
experience when considering whether or not to adopt a new practice, with on-farm trial
evaluations ultimately contributing to the farmer’s final decision (Pannell et al., 2006). Thus, if
significant protective benefits are observed the first-time PST are used it is likely the farmer will
scale up use of the practice in anticipation of sustained benefits. Our results, however, suggest
that the relative effects of PST may decline with repeated use, and thus may result in a case of
diminishing returns with rising ecological and economic costs. Such costs may include
development of more neonicotinoid- or fungicide-tolerant or resistant pest communities, which
would result in reduced efficacy of the seed treatment ingredients.

Our study also raises questions with regard to the on-going discussions of agricultural
extensification and sustainable intensification (Pretty and Bharucha, 2014; Hunter et al., 2017),
since our study suggests that soil arthropod communities in sites recently converted to more
intensive forms of agriculture (i.e., extensification) will be particularly susceptible to the effects
of PST. Such conversions have recently occurred in former CRP (Conservation Reserve
Program) land in the Midwest due to increasing demand for grain-based biofuels (Morefield et
al., 2016) and in South America and other regions where extensive tracts of rainforest and
grassland have given way to soybean and other row crop production (Gibbs et al., 2010). Will
the change in soil arthropod communities due to PST use in these “naïve” sites undermine
essential soil functions and processes and exacerbate the overall negative environmental impacts
of agricultural extensification in these regions compared to if no PST is used? With regard to
intensification, if the protective benefits of PST diminish over time or are minimal to begin with,
continued use of PST in intensively managed agroecosystems may be at best ineffective and at
worst counterproductive and environmentally damaging. Would reducing or eliminating the use
of PST in intensively managed systems result in “recovery” of the soil arthropod community?
And if so, how long would this take and would this substantively improve agroecosystem services in otherwise intensively-managed production systems? Answers to these questions could help farmers and other agriculture professionals make better land use decisions.

LITERATURE CITED


Monsanto, 2017. Seed treatments. Monsanto, St. Louis, MO, USA.


CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

The results of this dissertation provide novel insights into how soil functional zone management (SFZM) and pesticide seed treatments affect the soil faunal community in both intended and untended ways. In Chapter 2, I demonstrated that by using SFZM, a conservation tillage strategy, we can create unique zonation of the row and inter-row soil arthropod communities compared to uniform tillage. However, there were tradeoffs associated with this strategy, as the higher abundance of soil arthropods and more non-pest taxa associated with the crop rows under ridge tillage were offset by a more depauperate community inhabiting the crop inter-row compared to the uniformly tilled system. In Chapter 3, I demonstrated with a field experiment that pesticide seed treatments (PST) with neonicotinoids, which are widely used in commodity cropping systems and are intended to target early season crop pests, can alter the abundance and diversity of non-targeted soil fauna spanning multiple trophic-levels with no detectable effect on herbivores — the guild that is the intended target of PST. Lastly, in Chapter 4, I demonstrate for the first time that the initial introduction of PST into a soil community results in dramatic changes in soil community abundance and diversity, while communities with prolonged histories of seed treatment exposure appear to be relatively unaffected by subsequent exposure.

Recognition of the importance of diverse soil faunal communities in our agroecosystems is growing among scientists and growers (Brussaard et al., 2007; Coleman and Wall, 2007; Briones, 2014; Grandy et al., 2016). My results add to our growing understanding of the effects of several common agricultural management practices on soil faunal communities. My research
on PST, in particular, addresses two questions growers who use PST might find informative: What are the effects of PST on soil biodiversity? and (2) Do PST maintain the same level of effectiveness when used successively in the same field? In my field experiments, we managed each system according to best management practices (BMP), so that our experiment closely matched the way growers in the region manage their own fields. In doing so, I hope growers find the results of these studies practical and informative.

**Future directions**

The first experiment of this dissertation addresses how SFZM affects the abundance and diversity of the soil faunal community within the distinct zones created under ridge tillage. Additional studies will be needed to address how these changes in the soil community affect soil-driven processes and agronomic yields. There is emerging evidence that SFZM facilitates the creation of distinct functional zones, where nutrient cycling and water retention are more readily synchronized with the needs of a cash crop compared to uniformly tilled soils (Kane et al., 2015; Williams et al., 2016b, a). The agroecosystem benefits of SFZM, however, may also include enhanced biocontrol of pests and accelerated surface litter decomposition as the soil food web inhabiting the row under SFZM was a more diverse and abundant soil faunal community compared to the uniformly managed soils. Of course, consideration of how well the soil community inhabiting the inter-row position under SFZM performs soil-derived services should also be accounted for as my data provide compelling evidence that this zone quickly becomes less hospitable to soil organisms. Additionally, my research was conducted over the first several years of establishing the SFZM system. Future research should assess whether soil communities and functions become more differentiated as SFZM systems “mature”.
Future research is also needed to determine the mechanisms underpinning the disconnect between the PST-induced changes in the soil community and lack of effect on the soil-driven soil processes we monitored. A functional traits approach (McGill et al., 2006) is a potential strategy for isolating these mechanisms and could aid in determining the degree to which functional redundancies (Naeem, 1998) exist within the community (and at each trophic position). If redundancies exist, this might help explain why PST appeared to have relatively little effect on surface litter decomposition and plant available soil nitrogen. However, even if there is functional redundancy within the community, it is possible that thresholds exist (Groffman et al., 2006), such that prolonged PST use would eventually negatively affect soil-derived agroecosystem processes.

Elucidation of the mechanisms driving the restructuring of the soil food web following the addition of PST would explain how PST affects the soil food web, more specifically which food web pathways the components of PST are disturbing or bolstering. A future field experiment could utilize the isotopic signatures of key organisms present in both PST and untreated systems to determine if these organisms shift in trophic position following PST addition (Schmidt et al., 2004; Kadoya et al., 2012; Klarner et al., 2014). These data could be used to determine the degree to which PSTs alter soil faunal interactions, or “rewire” consumer-resource linkages within the soil food web, as alterations to these interactions could have significant consequences for the important agroecosystem services soil fauna provide.

More work will also be needed to determine why soil arthropod communities in sites with previous PST exposure were less responsive to PST. This may be because susceptible organisms had already been eliminated by prior PST exposure or because other aspects of annual crop production systems (e.g. tillage, crop diversity) effectively constrained community membership
to taxa that tend to be less responsive to PST. Moreover, future studies should increase the amount of site replication, sample across chrono-sequenced seed treatment applications in otherwise similarly managed annual cropping systems, and conduct the experiment over longer timespans to aide in further unravelling the relationships between use of PST and changes in the soil arthropod community and agroecological function.

LITERATURE CITED


**APPENDICES**

**Appendix A.** Functional group and taxonomic classifications of all organisms collected from both crop rows and interrows in a maize-soybean site in Rock Springs, PA at 12 August 2013 and 1 August 2014. These data include organisms found in both the ridge-tillage and chisel plow systems (Chapter 2).

<table>
<thead>
<tr>
<th>ID</th>
<th>Functional group</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Subfamily</th>
<th>Genus</th>
<th>Species</th>
<th>Finest grouping</th>
</tr>
</thead>
<tbody>
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<td>Arthropoda</td>
<td>Arachnida</td>
<td>Sarcoptiformes</td>
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<td>na</td>
<td>na</td>
<td>na</td>
<td>Oribatei</td>
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<td>na</td>
<td>Diplopo (imm.)</td>
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<td>na</td>
<td>Diplopo (a)</td>
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</tr>
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<td>Diplopo (b)</td>
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**Appendix B.** Functional group and taxonomic classifications of all organisms collected from a maize-soybean site in Rock Springs, PA during the 2013, 2014, and 2015 growing seasons (May-October). These data are from the pesticide seed treatment field experiment (Chapter 3).

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