Fall 2020

PESTICIDE TREATED CROP SEEDS AND TILLAGE ALTER SEED COAT FUNGAL COMMUNITIES ON AMARANTHUS RETROFLEXUS IN A MAIZE-SOYBEAN CROPPING SYSTEM

Samuel Arrington Palmer
University of New Hampshire, Durham

PESTICIDE TREATED CROP SEEDS AND TILLAGE ALTER SEED COAT FUNGAL COMMUNITIES ON *AMARANTHUS RETROFLEXUS* IN A MAIZE-SOYBEAN CROPPING SYSTEM

By

Samuel Palmer IV

Baccalaureate of Arts Degree in History

The University of Massachusetts Dartmouth, 2009

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Natural Resources

September 2020

This thesis was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Natural Resources by:

Thesis Director, Richard G. Smith, Associate Professor of Cropping Systems Ecology and Management

Serita Frey, Professor of Microbial Ecology, Ecosystem Ecology, and Global Change Biology

Anissa Poleatewich, Assistant Professor of Plant Pathology

On August 10th, 2020

Approval signatures are on file with the University of New Hampshire Graduate School.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am thankful for the support of my wife Kendra and children Sam and Wren. I am also grateful for the help and camaraderie from my lab mates Nick Warren, Natalie Lounsbury, and Issac Avitor. Smith Lab technicians Julia Hobbie and Benjamin Fehr provided indispensable help. Drs Kirsten Peterson and Elisabeth Rowan of the Pennsylvania State University's Tooker Lab conducted all field work at our PA site. The project would not have been possible without Dr. Mark Anthony's coaching through our first round of amplicon sequencing. I am particularly grateful to Dr. Richard Smith, my primary advisor, for his willingness to take a risk on a student with no scientific background. This work was funded, in part, by USDA NIFA AFRI Grant No. 2017-67013-2659. New Hampshire Agricultural Experiment Station Hatch Project 1016232, the UNH Graduate School, and the National Science Foundation Graduate Research Fellowship Program.

LIST OF TABLES

LIST OF FIGURES

Abstract

Soil fungi, by damaging or decaying weed seeds in the soil seed bank, are important agents of biological weed control. Pesticide seed treatments (PST) that include fungicides may alter the communities of soil fungi that colonize weed seeds in the soil and therefore the nature and efficacy of this important source of biological weed control. Tillage, by disrupting fungal networks and spatially redistributing the fungicides associated with PST throughout the soil profile, may mediate the effects of PST on seed coat fungi. We conducted a two-year experiment at two sites with two levels of PST (treated and untreated crop seeds) and three levels of tillage (Full, Strip, and No-Till) and analyzed the fungal community on *Amaranthus retroflexsus* seed coats. We found that at our no-till site, fungal communities were less diverse in the presence of PST. We also found simplification of seed coat fungal communities between tillage treatments. These results suggest that both PST and tillage may modify the weed control effects of soil fungal communities and these effects should be further studied and considered when employing these common management practices.

Introduction

Soil seedbanks consist of seeds that have been shed from maternal plants but that have not yet germinated and are the source of most weeds in agricultural systems (Davis, 2006). Soil seedbanks are in continuous cycles of depletion and replenishment, with seeds leaving the seedbank through the processes of germination, granivory, and decay, and new seeds entering the seedbank through seed rain and migration (Davis, 2006). As such, seeds play an important role in the establishment and persistence of plant communities.

Since the first commercial herbicide became available in 1945, herbicides have become the primary tool for managing weeds that emerge from the soil seed bank (Cobb and Reid, 2010). Herbicides are often effective at preventing new inputs of seeds into the soil because they kill weeds before they produce new seeds, thereby depleting seedbanks over time. However, because herbicides exert such strong selective pressure on weed populations, an increasing number of agronomically important weeds have evolved resistance to herbicides, making herbicides less effective as the sole tool for weed management (Heap, I., 2020.; Mortensen et al., 2012).

Due to growing concerns over the evolution of herbicide resistance, as well as other non-target effects of herbicides, farmers are becoming increasingly interested in integrated weed management (IWM) (Norsworthy et al., 2012). Under an IWM approach, farmers attempt to manage weeds with a diverse set of cultural, physical, and chemical practices that target not just emerged weeds, but the entire weed lifecycle, including the soil seedbank (Swanton and Murphy, 1996). While cultural weed management practices common to IWM, such as the use of cover crops and crop rotation, can help reduce weed populations, they tend to have lower efficacy than herbicides (Liebman and Gallandt, 1997; Weisberger et al., 2019).

The lower efficacy of IWM practices increases the importance of biotic sources of weed mortality; the mammals, insects, and fungi which destroy weeds and weed seeds in the soil (Smith and Mortensen, 2017). From a community assembly perspective, herbicides can be thought of as strong abiotic filters on the assembly of weed communities, while biotic filters, such as cover crops, crop rotation, and the natural enemies of weeds (e.g., herbivores, granivores, and plant pathogens), tend to be weaker filters on the assembly process (Booth and Swanton, 2002; Ryan et al., 2010; Smith and Mortensen, 2017). Hence, for IWM to enable farmers to reduce their reliance on herbicides, strategies for increasing the effectiveness of biotic filters— especially the natural enemies of weeds— are needed. Modeling and understanding the efficiency of biotic filters requires improving our mechanistic understanding of when and how specific natural enemies influence weed populations and quantifying the rates at which they deplete the weed seedbank, and how these factors are affected by common agricultural practices (Kremer and Li, 2003; Liebman and Davis, 2000).

Saprotrophic fungi are important natural enemies of weed seeds (Chee-Sanford et al., 2006; Wagner and Mitschunas, 2008). Largely responsible for the decomposition process in soils, fungal saprotrophs possess the traits to decompose seeds despite their physical and chemical defenses (Schneider et al., 2012). While numerous studies have characterized the microbial communities present in bulk soils (Katulanda et al., 2018; Zhang et al., 2018) and in plant rhizospheres (Blagodatskaya et al., 2014, Sauvadet et al., 2018), few have characterized those present on agricultural weed seeds. While substantial research has been conducted on non-weed seed microbiomes (Nelson, 2018), much of this work has focused on the seed microbiome's role in seed germination and subsequent plant growth, rather than decay of dormant seeds. Gómez et al. (2014) identified a link between *Pythium ultimum* (an Oomycete

plant pathogen) and *Setaris faberi* (a monocot weed) viability in a greenhouse experiment, but we know little about how plant pathogen (Harding and Raizada, 2015) and agricultural management practices impact seedbanks (Davis et al., 2006; Sommermann et al., 2018).

Tillage, the preparation of soil by mechanical agitation, is an important management practice that can alter soil microbial communities, and potentially their role as biotic filters of weeds. Tillage has long been recognized to alter soil physical properties, soil microbial communities (Frey et al., 1999; Lee and Schmidt, 2014), and weed communities (Gómez et al., 2014). Farmers employ tillage for a variety of purposes, to terminate weeds, incorporate soil amendments, and to prepare seedbeds for planting. The use of tillage has declined in some regions owing to concerns over soil conservation and health. More recently, adoption of large-scale no-till systems have been enabled by the commercialization of transgenic, herbicide-resistant crops capable of tolerating post emergence applications of certain herbicides, particularly glyphosate. This system has largely eliminated the need for tillage as a weed management tool, while accelerating development of herbicide resistance (Bredeson and Lundgren, 2019; Douglas and Tooker, 2015; Jeschke et al., 2011). In regions with high incidences of herbicide resistant weeds, or systems which prohibit the use of certain herbicides, such as organic systems, tillage remains important. Strip tillage been proposed, and implemented, as a hybrid system where only the crop row is tilled to capitalize on the benefits of tillage with a reduced impact on soil health and structure (Williams et al., 2017).

Crop seeds coated in insecticides and fungicides also known as, pesticide seed treatments (hereafter "PST") are nearly ubiquitous in major cropping systems in the US (Douglas and Tooker, 2015; Jeschke et al., 2011). The prophylactic use of PST is intended to give crops an early advantage against possible yield loss due to insect pests and fungal disease (Labrie et al.,

2020; Papiernik et al., 2018). Despite their widespread use, research has documented non-target effects of PST components on insect populations, rhizosphere microbial communities (Nettles et al., 2016), and weed communities (Smith et al., 2016). In addition, PST components have been detected in non-target crop plants, including cover crops (Bredeson and Lundgren, 2019), as well as sensitive habitats well away from the point of application, suggesting that some components of PST are highly mobile (Anderson et al., 2015; Radolinski et al., 2018). As a consequence of these non-target effects, the justification for prophylactic use of PST is increasingly being questioned (Lamichhane et al., 2020).

Given the important role that soil fungi play in mediating weed population dynamics and the decomposition of weed seeds in the soil, surprisingly little attention has been payed to determining if PST with fungicides may alter the community of fungi that colonize weed seeds in the soil. Several lines of evidence suggest that PST use could impact the soil fungi that colonize weed seeds in the soil. Nettles et al. (2016) characterized changes in rhizosphere microbial communities associated with PST and found that crop rhizospheres were enriched in several fungal OTUs in the presence of PST. While the fungicide components of PST typically target *Pythium spp.*, *Fusarium spp.*, and other fungi which commonly lead to damping off in plant seedlings, these fungicides are broad spectrum and therefore other fungal taxa could be impacted by PST as well (Lamichhane et al., 2020; Syngenta, 2012). What's more, many of the same pathogenic fungi that attack crop seeds in the soil have also been found to colonize weed and attack seeds (Mohler et al., 2012).

Previous work investigating the roles that soil fungal communities play in mediating weed seed persistence in the soil have been inconclusive. Frost et al. (2019) found no difference in viablility of weed seeds treated with fungicide compared to untreated weed seeds. In contrast,

Nikolić et al. (2020) observed higher rates of weed seed decay in seeds buried in a no-till agricultural field compared to seeds buried in an adjacent planted riparian buffer zone. Other studies examining viability in seeds with minimal exposure to soil fungi, either by placing seeds in sterilized soils or by excluding fungi using fungicide coated weed seeds, and comparing to seeds in field soils with robust fungal communities, have yielded mixed results (Davis and Renner, 2007; Kumar et al., 2008; Leishman et al., 2000). Ullrich et al. (2011) reported inconsistent changes in weed seed viability in soils with high vs lower microbial biomass, and recommended targeting microbial function in order to better characterize the relationship. In the majority of these studies, characterization of the fungi present on weed seeds was done with phospholipid fatty-acid (PLFA) markers and media-based culture, methods which provide little insight into the functional capacity of fungal communities. These studies also focused on pathogens, most of which do not act on weed seeds until they initiate germination, rather than saprotrophs that break down dead or dormant seeds.

In this study we characterized the fungal community present on the coats of seeds of *Amaranthus retroflexus*, a warm season annual weed, overwintered in field soils with and without histories of PST and under differing intensities of tillage. We also assessed overwintered *A. retroflexus* seed viability and examined variation in this parameter in relation to fungal community composition. We hypothesized that both PST use and tillage intensity would alter the diversity of the fungal community that colonize weed seed surfaces, as well as the relative abundance of pathogenic taxa (the target organisms of PST). We predicted that PST and tillage would interact, with higher tillage intensity resulting in a dilution of the PST effect due to soil mixing. Lastly, we hypothesized that in PST treated plots, weed seed viability would be greater because of a suppression of fungal natural enemies.

Methods

Overview

In order to assess the impacts of PST and tillage on weed seed coat fungal communities and seed viability we conducted a two-year field experiment at two sites. At both sites we buried seeds of *Amaranthus retroflexsus* L. (Redroot Pigweed) in mesh bags and allowed them to overwinter following the 2018 and 2019 growing seasons. *A. retroflexsus* has a generally long lived seedbank (Korres et al., 2018; Ullrich et al., 2011) and is a common component of the weed flora at both sites. Several members of the genus are considered serious agricultural weeds throughout the US, with a number of genotypes having developed resistance to multiple herbicide modes of action, making it an ideal model weed (Korres et al., 2018; Heap 2020).

Site Descriptions

The study was conducted at two locations, the University of New Hampshire's Kingman Research Farm in Madbury, NH, USA (43°10'25.6"N 70°55'24.0"W) and The Pennsylvania State University's Russell E. Larson Agricultural Research Center at Rock Springs, PA, USA (40°42'37.5"N 77°57'04.5"W). Soils at the NH site are a Hollis-Charlton fine sandy loam (Charlton series: coarse-loamy, mixed, superactive, mesic Typic Dystrudepts; Hollis series: loamy, mixed, superactive, mesic Lithic Dystrudepts) (National Cooperative Soil Survey, Freyre and Loy, 2000). Soils at the PA site are shallow, well-drained lithic Hapludalfs formed from limestone residuum, and the dominant soil type is a Hagerstown silt loam (fine, mixed, semiactive, 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.

Experimental Treatments

Experimental treatments at the NH site were PST (two levels: treated and untreated crop seeds sown each year) and tillage (three levels: full-till, strip-till, and no-till) applied as a full factorial. The crop rotation was not an experimental factor, with a single crop, either maize or soybean, grown in all treatments each year. Additionally, we included a treatment planted to alfalfa to serve as a perennial crop "control". The tillage and alfalfa treatments were initiated in the spring of 2013 and were originally intended to investigate the effectiveness of interseeding legume cover crops into corn and soybean. Consequently, each tillage treatment had two levels (interseeded with legume cover crops or no interseeding); however, the interseeding treatments were never successful and therefore the two levels of each tillage treatment were essentially identical for the three years that the interseeding treatment was applied. All treatments except the alfalfa control were sown with pesticide-coated maize (2013 and 2015) or soybean (2014) seeds. PST treatments were initiated in spring 2016 by assigning the previous "interseeded" treatment associated with each tillage level to PST (maize and soybean planted with pesticide-coated seeds) while the "no interseeding" treatment was assigned the control treatment (maize and soybean planted without PST) (Table 1). Hence, the tillage treatments were in place for three years prior to the initiation of the PST treatments, and all PST treatments were in place for two years prior to the installation of seed bags. Treatments were randomly assigned to 12.2 m x 26 m plots (eight crop rows 76 cm apart) within each of four blocks, with no buffers between plots within a block. Blocks were separated by a 12.2 m buffer of mowed grass. Within each block two additional plots that were vestiges of the previous tillage/interseeding study were maintained as cropped "buffers" so as to reduce the potential for edge effects in the other plots. These buffer

plots were planted to either untreated maize or untreated soybean, depending on the year, and were managed identically to the other treatments, but were not considered part of the experiment.

Tillage treatments were initiated prior to planting each year. Full-till plots received a burn-down application of glyphosate and were moldboard plowed to 30 cm, disked, and then rolled with a Perfecta II (Unverferth Manufacturing Co., Inc., Kalida, OH). Strip-till plots received a burn-down application of glyphosate followed by strip-tillage with a Model 330 Ripper-Stripper (Unverferth Manufacturing Co., Inc., Kalida, OH). No-till plots received a burn-down application of glyphosate but were left otherwise undisturbed. Both maize (2013, 2015, 2017, and 2019; 32,000 seeds per acre) and soybean (2014, 2016, and 2018; 206,000 seeds per acre) were planted with a four-row planter (John Deere MaxEmerge, Moline, IL). Each year, crop genotype, planting date, row spacing, and crop density were identical between the PST treated and untreated plots. All treatments were seeded on the same day each year; however, untreated seeds were planted before treated seeds in order to prevent contamination in the planter. All plots received identical herbicide programs, pre and post plant applications of Round-up (1 Quart per acre @ 48.8% AI Roundup weatherMAX split between the pre and a post planting application that typically occurred around mid-July).

Table 1. Pesticide seed treatments by year at the NH site.

Year	Manufacturer	Seed	Treatment Trade Name	Insecticide	Fungicides
2016	BASF	Soybean, BG 7171 RR2 Soybeans	Alert soybeans 2020	Imidacloprid	Integral Liquid bio fungicide (*Bacillus subtillis*), Thiabendazole
2017	Syngenta	Maize, TA290-00	Cruiser-Maxx 250	Thiamethoxam	Mefenoxam, Fludioxonil, Mefenoxam, Azoxystrobin, Thiabendazole
2018	TA Seeds	Soybean, TS1759R2	Trius Elite	Imidacloprid	Metalaxyl, Fuldioxoinil, Thiabendazole, Azoxystrobin

Experimental treatments at the PA site were PST (three levels: treated and untreated crop seeds sown each year, and Integrated Pest Management (IPM)) and cover crop (two levels: cover crop, no cover crop) applied as a 6x6 Latin square. While the IPM treatment was intended to represent a third approach to managing pest insects, no IPM actions were initiated; therefore, the IPM plots were identical to the untreated plots. The cover crop treatment was grown from 11 April to 4 June 2017 (*Avena sativa*) and then 22 November 2017 to 23 May 2018 (*Secale cereale*). Cover crop performance was poor, with weeds exceeding cover crop biomass in all plots both years. Therefore, while cover crop is included in our models, we did not attempt to interpret the effects of cover crop on our response variables. The experimental rotation was maize-soybean initiated in the 2017 growing season, with a single crop, either maize or soybean, grown in all treatments each year as in NH. In 2016, 5 rows were planted to soybean, while the

6th row was planted to a Maize, soybean, sorghum, sunflower mix as part of another experiment. Treatments were randomly assigned to 12.2m x 33.5m plots (eight crop rows 76 cm apart) within each of 6 rows and 6 columns to minimize variation in the field, with no buffers between plots.

No tillage was conducted at the PA site, plots received a burn-down application of herbicide but were left otherwise undisturbed. Each year (2017 Maize; 79074 seeds per hectare; 2016 and 2018 Soybean; 439,874 seeds per acre) crop genotype, planting date, row spacing, and crop density were identical between the PST treated and untreated plots. All treatments were seeded on the same day each year; however, untreated seeds were planted before treated seeds in order to prevent contamination in the planter. All plots received identical herbicide programs (2017; post-plant, Impact, Accent, Banvil, and Degree Extra; 2018; pre-plant, 22 fl oz/acre, Roundup powerMAX).

Table 2. Pesticide seed treatments by year at the PA site.

Year	Manufacturer	Seed	Treatment Trade Name	Insecticide	Fungicides
2017	Syngenta	Maize, MC5250	Cruiser-Maxx 250	Thiamethoxam	Mefenoxam, Fludioxonil, Mefenoxam, Azoxystrobin, Thiabendazole
2018	TA Seeds	Soybean, TS2849R2S	Trius Elite	Imidacloprid	Metalaxyl, Fuldioxoinil, Thiabendazole, Azoxystrobin

Seed Bags

Fifty *A. retroflexsus* seeds from the same seed lot were placed in 10 cm x 10 cm nylon mesh bags 10 cm^2 and then surface sterilized in a 1% NaCL, 1% Tween (surfactant) solution for five minutes prior to installation in order to reduce the number of fungal sequences detected which did not originate from our treatments.

Three seed bags were buried 5 cm below the soil surface in each experimental plot at the NH and PA sites in November of 2017. Each experimental unit was eight crop rows wide and seed bags were installed in the three middle alleyways between crop rows, staggered ¼, ½, and ¾ of the length of the plots. This installation spread the bags across the central portion of the plots to avoid edge effects. Bags were removed in April of 2018 and moved to -80^o C cold storage within 2 hours. Exhumed seed bags from the PA site were bagged, placed in coolers with dry ice, and shipped overnight to NH where they were put into -80^o C cold storage until processing could be carried out. We repeated this process in year two (November 2018 to April 2019), but increased the number of seeds per bag to 100 and the number of bags per plot to five to allow for viability testing. Seeds used for viability testing were placed in 4^{o}C cold storage and assayed within 30 days of removal from soil (see details below).

In year two, control seed bags were also buried in the buffer plots at the NH site. Each control mesh bag was placed in plastic Ziploc bags so that seeds experienced the same soil temperatures as the other bags but without contact with soilborne fungi. Seeds from these bags were used to assess viability and the relative abundance of fungi on the sterilized seeds.

Seed Viability

In both 2018 and 2019, subsets of recovered seeds from the NH site were assayed for viability using methods similar to Ullrich et al. (2011). Seeds were examined with a hand lens after removal from mesh bags, and 200 in-tact seeds from each NH plot were placed in petri dishes with #2 filter paper. Petri dishes were placed in the greenhouse to reach optimal germination temperatures for *Amaranthus* spp. (Ghorbani et al., 1999), watered with 5 ml of RO water, and re-watered as necessary. Germinated seeds were counted weekly for three weeks. At the end of three weeks we removed seeds from dishes and performed the imbibed seed crush test (Borza et al., 2007) on any seeds which had not germinated. Seeds which crushed under light forceps pressure were counted as non-viable. Remaining seeds were cut in half, placed in a tetrazolium solution for 24 hours, and those with stained embryos were counted as viable. Seed viability (V) was calculated as:

$$V = (T_S - S_{FC} - S_{FT})/(T_S)$$

Where T_S is total seeds in the dish, S_{FC} is seeds that fail the crush test, and S_{FT} are seeds which did not have stained embryos in the tetrazolium test.

Seed Coat Fungal Community Characterization

ITS metabarcoding was used to assess seed coat fungal diversity and community composition. Once removed from mesh bags, seeds were rinsed in autoclaved water to remove loosely adhering soil. Then DNA was extracted from 0.5 g of seeds (approximately 50 seeds) from each plot using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA). It is important to note that our protocol of rinsing seeds in PCR grade water prior to DNA extraction, likely left all but the most loosely adhering soil fungi on seed surfaces. Therefore, the

seed coat fungal data we present here represent all soil fungi that were on the seeds in addition to those that had specifically colonized the seed coat.

The ITS2 region was amplified using polymerase chain reaction (PCR) and the primer pair fITS7 (Ihrmark et al., 2012) and ITS4 (Schoch et al., 2012). Custom PCR primers contained the Illumina adaptor sequence, an 8 base pair (bp) pad sequence, a 2 bp linker sequence, and one of 16 unique 8 bp index sequences. PCR reactions were performed in triplicate as in Anthony et al. (2017). Products were cleaned with an AxyPrep MAG PCR Clean-up kit (Corning, Tewksbury, Massachusetts, USA), inspected on an agarose gel, and DNA concentration was measured by fluorometry on a Qubit 3.0 Fluorometer (Life Technologies, Grand Island, New York, USA). Equimolar amplicon libraries (2018 and 2019) were sequenced on an Illumina MiSeq using v2 chemistry and a paired end read (2x 250 bp) at the Center for Genomics and Bioinformatics at Indiana University, Bloomington, Indiana, USA.

From the raw sequence data, Trimmomatic (Bolger et al., 2014) was used to remove sequences with phred scores < 2 and shorter than 100 bp and demultiplex the remaining sequences. USEARCH v11 (Edgar, 2010) was used to merge reads prior to extracting the ITS2 region and eliminating chimeric sequences using ITSx v1.1.1 (Bengtsson-Palme et al., 2013). The pipeline following the ITSx extraction was run on data sets with and without ITSx and produced identical results. USEARCH v11 was used to cluster Organizational Taxonomic Units (OTUs) at 97% similarity with the UPARSE algorithm and assign taxonomy using SINTAX and the Untie UTAX v8 database (Edgar, 2018, UNITE, 2018). Taxonomic assignments were processed through the FUNGuild database to assign functional guild information (Nguyen et al., 2016). This bioinformatics pipeline produced reads from a total of 2010 fungal OTUs.

Data Analysis

Seed viability was analyzed using ANOVA with orthogonal contrasts in R 3.6.3 core functions (R core development team, 2020) with an alpha of 0.05 for all tests. The assumptions of normality of residuals, homogeneity of variance, and block-treatment additivity were checked using Shapiro-Wilk, Levine's, and Tukey 1 degree of freedom tests for all univariate analyses.

Communities from the two sites were analyzed separately due to their different experimental designs and underlying treatment structures. Fungal community data from both sites were relativized (general relativization) by plots to produce relative abundance values, since total sequence abundance is an artifact of the process rather than of treatment (Hugerth and Andersson, 2017; McMurdie and Holmes, 2014). Prior to conducting multivariate analyses (described below), OTUs which occurred in less than 5 percent of plots were removed in order to reduce the influence of the rare OTUs on the analyses (McCune et al., 2002) leaving 1116 OTUs for analysis in NH of which 495 were assigned to a guild, and 1355 OTUs in PA, of which 625 were assigned to a guild.

PerMANOVA in R was run with the package 'vegan' to test for group differences in the seed coat fungal communities by treatment with a Bray-Curtis dissimilarity matrix and the function 'adonis2' (Oksanen et al., 2019, Anderson, 2001). The initial model for the NH site showed an interaction between year and tillage, so the two years were analyzed separately. No interactions were apparent for the PA site and years were analyzed together.

We used Nonmetric Multidimensional Scaling (NMS) of plots in OTU space in order to visualize differences in seed coat fungal community composition and structure between treatments. NMS was run in R with 'vegan' using Bray-Curtis dissimilarity. Ordinations were run with up to 10 axes; best fit was selected based on a reduction in stress on .05 from the next

lowest number of axes. Once the appropriate number of axes was selected, a Monte-Carlo simulation was run in order to determine if the ordination was significantly better than random. The resulting axes were plotted against each other using the 'ggplot2' package for visualization (Wickham, 2016)

In order to identify differences in the fungal communities at the OTU level, we conducted an Indicator Species Analysis (ISA) (Dufrêne and Legendre, 1997) with the R package 'indicspecies' and the function 'indval.g' with 4999 permutations (De Caceres & Legendre, 2009). ISA was performed using PST as the grouping factor in PA and PST and tillage in NH.

Seed coat fungal community diversity indexes (richness, evenness, Shannon's, and Simpson's Diversity, all calculated prior to deletion of rare OTUs) were calculated by plot in r using the vegan package and transformed to the -0.4625 power in order to meet ANOVA assumptions. Type II sums of squares were analyzed using the 'Anova' function of the 'car' package (Fox and Weisberg, 2019) in order to account for imbalance from missing plots. For the NH site, block, PST, and tillage were treated as fixed factors with year treated as a non-independent repeated measure.

To analyze fungal diversity metrics from the PA site, a Type II ANOVA was performed, again treating year as a repeated measure. Because the design of the PA experiment was a Latin Square with 6 treatment levels rather than the explicit factorial, we used the emmeans package (Lenth, 2020) to generate least squares adjusted means and orthogonal contrasts to examine the effects of cover crop, PST, and their interaction.

Funguild output of guilds (functional groups) were relativized (general relativization) by plot and relative abundance was analyzed using multivariate methods identical to those we used

on the communities by OTU. I then used JMP14 to generate stacked bar charts by functional group.

Results

Seed Coat Fungal Diversity and Community Composition at the PA site

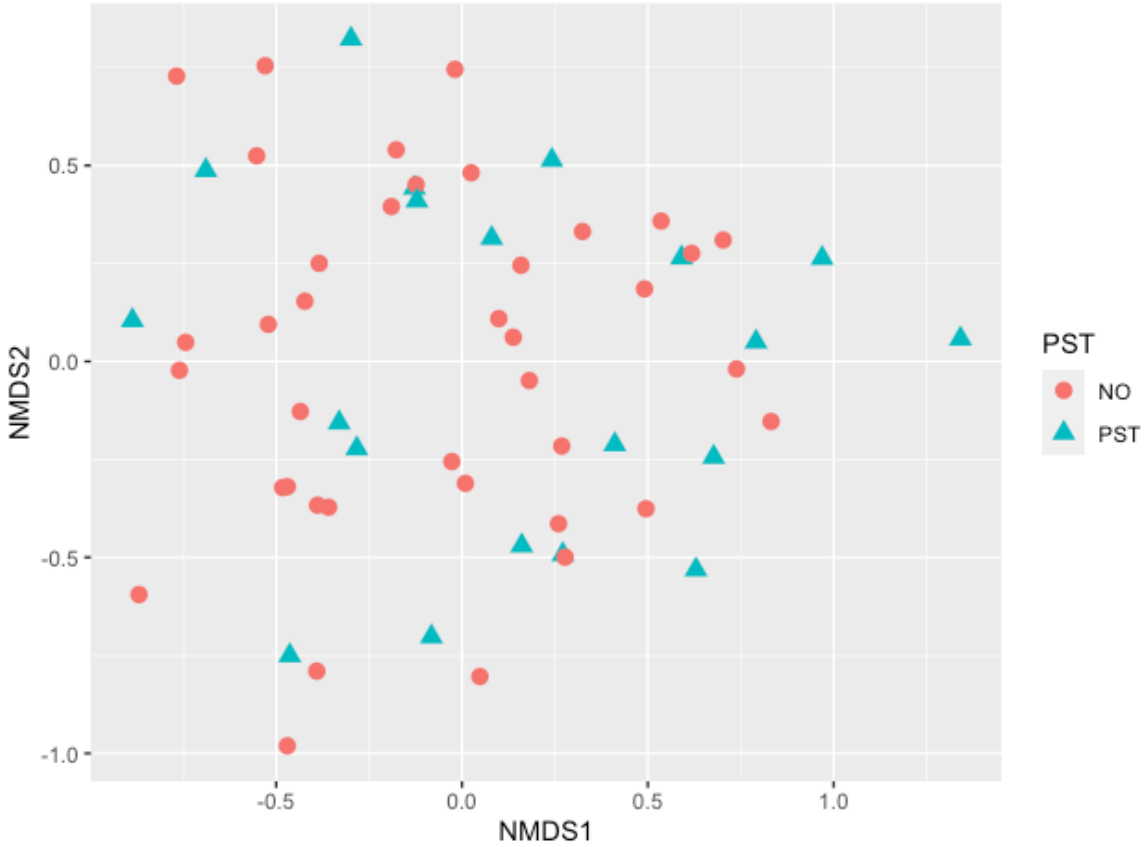

Figure 1: NMDS ordination of seed coat fungal communities at the PA site. Stress = .16; p=0.0001.

PerMANOVA of the seed coat fungal community data at the PA site indicated that both PST (p = 0.05) and year (p = 0.001) affected the composition of the soil fungal community that colonized the coats of overwintered seeds (Table 3). PST-treated plots had lower seed coat fungal community richness (p = 0.049), evenness (p = 0.041), and Shannon's diversity (p = 0.027) compared to the treatments without PST (Figure 2). Cover crops did not affect the composition of the community. Richness and Shannon's diversity also varied by year (p =

0.00001 (richness), p = 0.011 (Shannon Diversity)). Neither the PerMANOVA of OTUs or the ANOVA of diversity indexes indicated any interactions between PST, cover crops, and years.

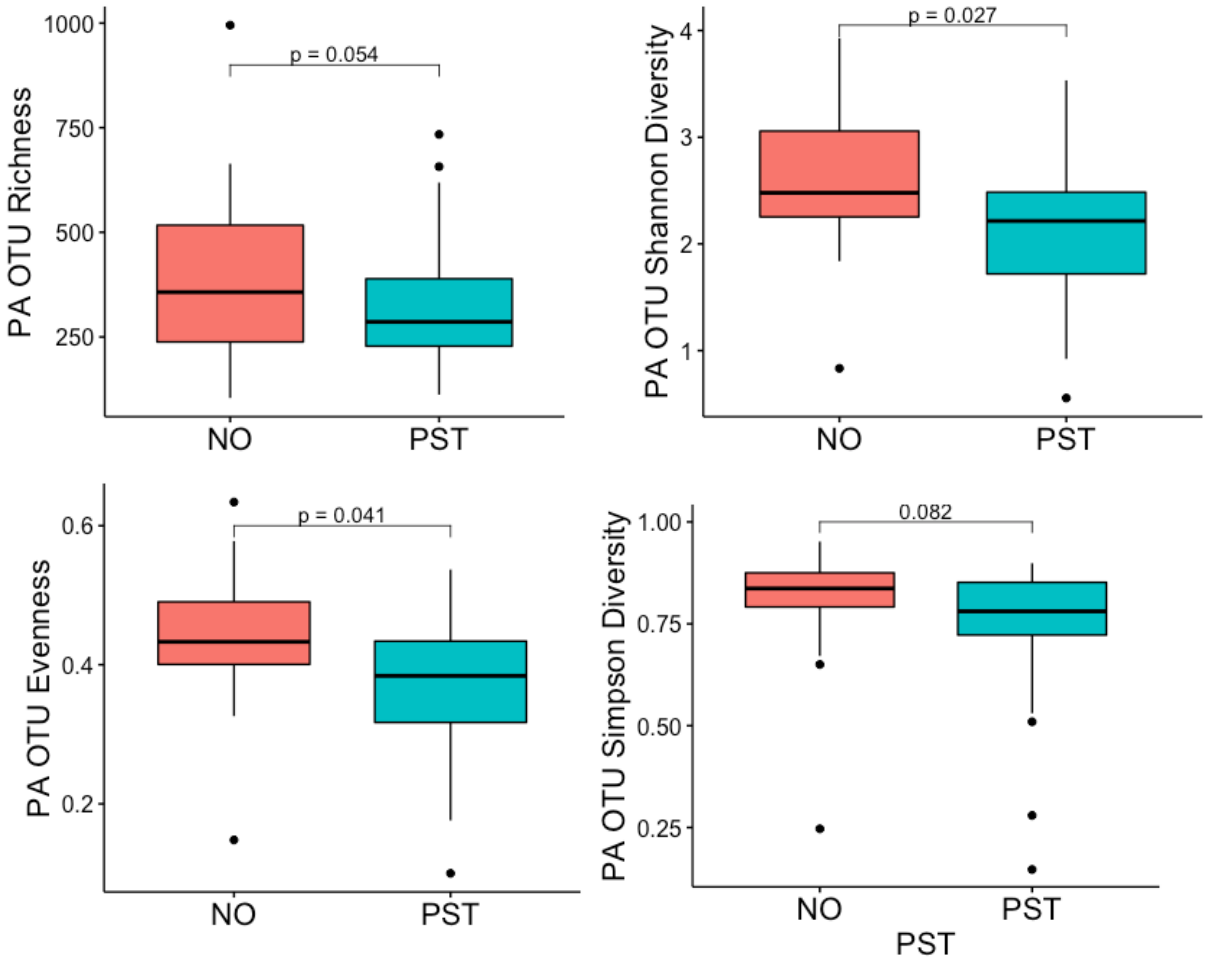

Figure 2. Diversity of fungal communities on *A. retroflexus* seeds overwintered in field soils over two separate years at the PA site. Soils were previously planted with crop seeds coated with insecticides and fungicides (PST) or without the pesticide coating (NO).

Seed Coat Fungal Diversity and Community Composition at the NH Site

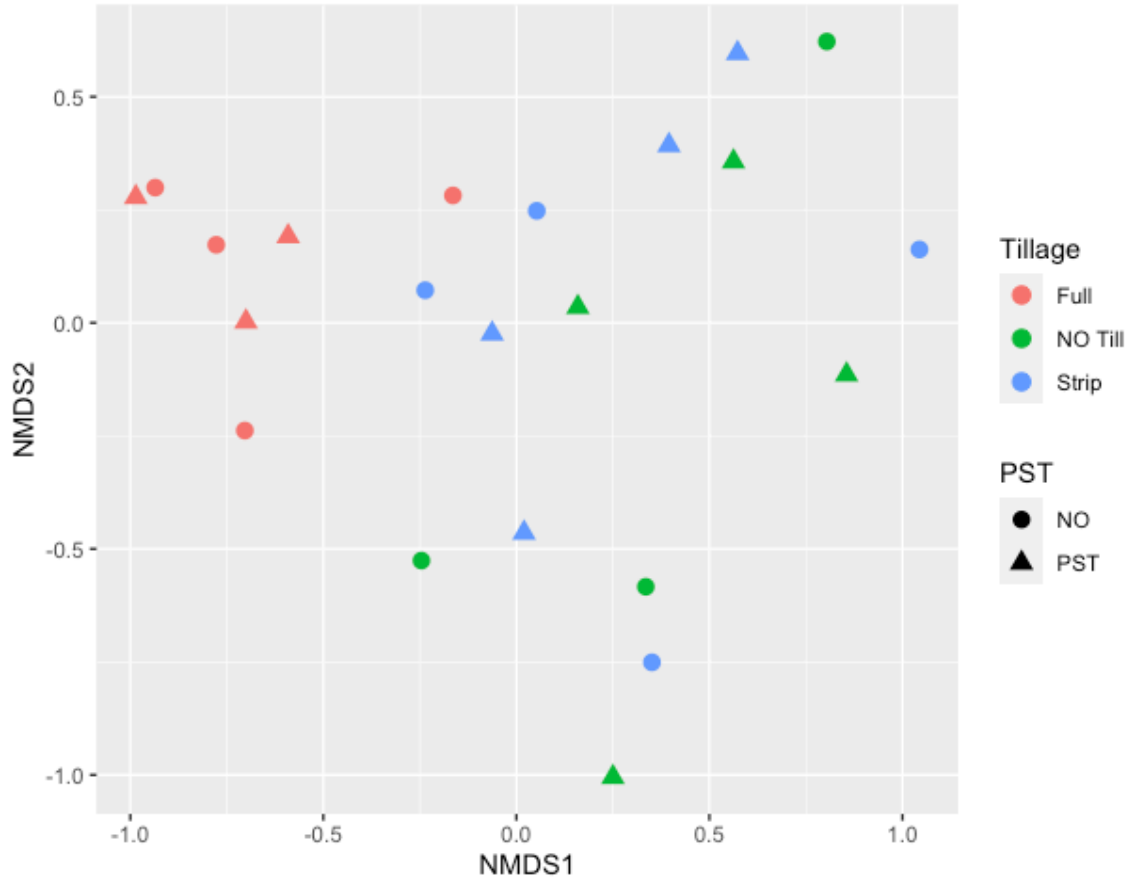

Figure 3: NMDS ordination of seed coat fungal communities at the NH site in 2018. Stress = .09; p=0.01.

At the NH site, where tillage and PST were experimental factors, we detected a significant effect of tillage in 2018 (PerMANOVA, p = 0.001), but not in 2019, and no effect of PST in either year (Table 3). NMDS ordination of the 2018 NH site data indicated distinct seed coat fungal communities in the full till plots compared to the strip and no-till plots, which were intermingled (stress = 0.09, p = 0.01) (Figure 3). While there was no main effect of PST in our model, all four diversity indexes were lower when looking at the effect of PST in only the no-till plots (Figure 6). Tillage also affected the Simpson's dominance index (p = 0.039), with seed coat fungal communities having lower Simpson's diversity in the full-till treatments compared to the

strip- and no-till treatments (Figure 5). Seed coat fungal community richness varied by year (p = 0.004) but was not affected by the treatments.

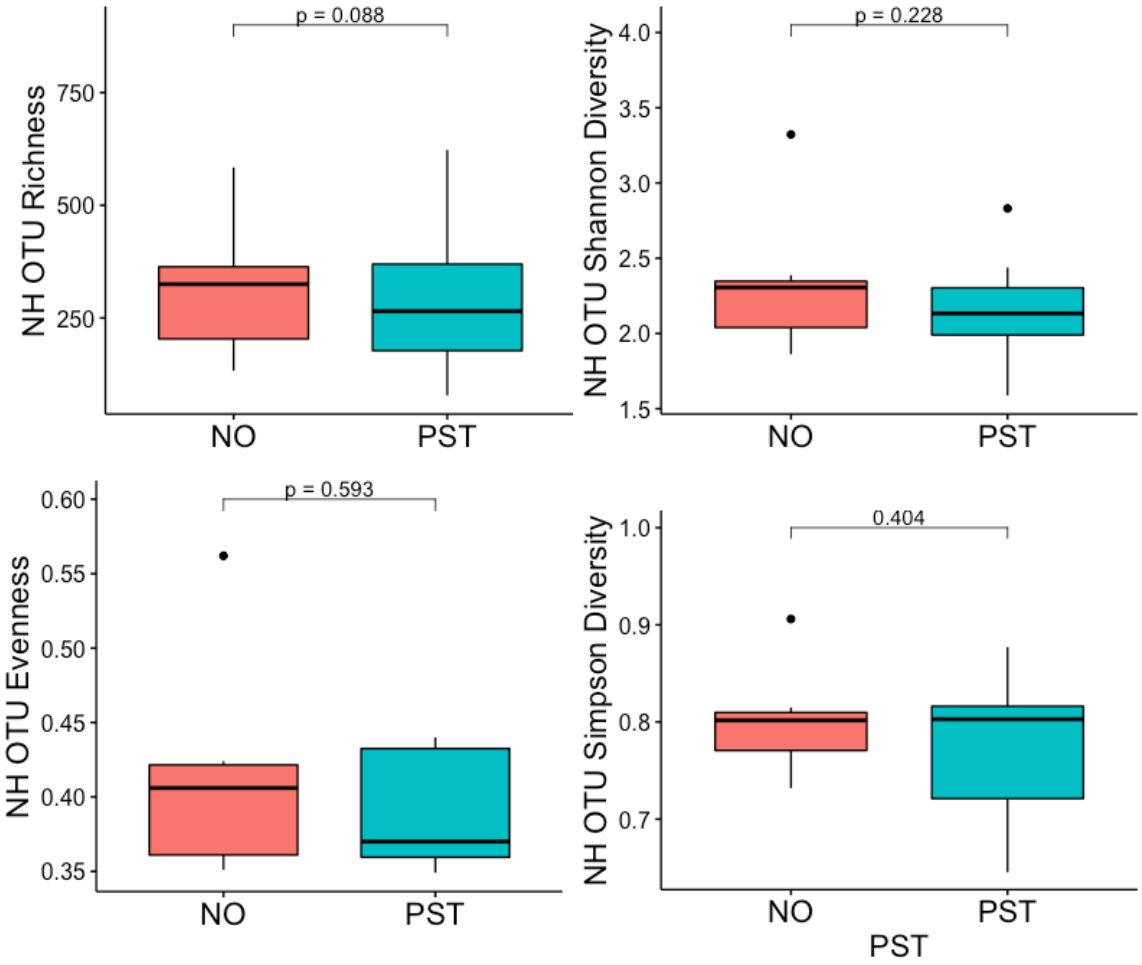

Figure 4: Diversity of fungal communities on *A. retroflexus* seeds overwintered in no-till field soils over two separate years at the NH site. Soils were previously planted with crop seeds coated with insecticides and fungicides (PST) or without the pesticide coating (NO).

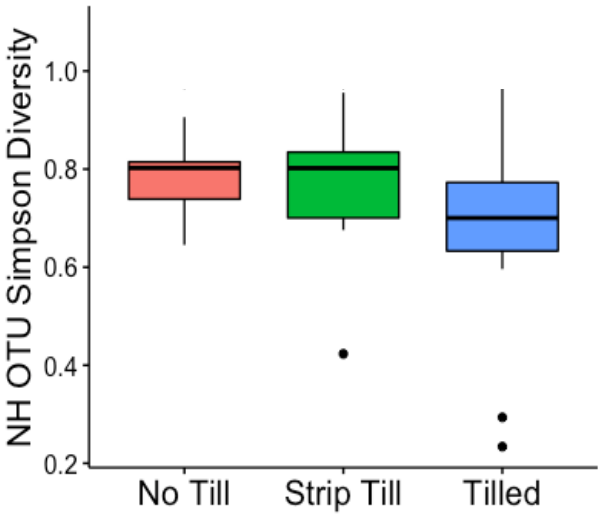

Figure 5: Effects of tillage on the Simpson diversity index of seed coat fungal communities at the NH site.

Table 3: Results of PerMANOVA of the seed coat fungal communities (OTUs) at the PA and NH sites.

Factor/site	R2	Pr(>F)
PA site 2018 & 2019		
PST	**0.02855**	**0.05**
Cover	0.01229	0.703
Year	**0.07107**	**0.001**
PST:Cover	0.01514	0.509
PST:Year	0.01717	0.353
Cover:Year	0.01287	0.649
PST:Cover:Year	0.02201	0.162
Residual	0.8209	
NH site 2018		
PST	0.01165	0.983
Tillage	**0.29047**	**0.001**
PST:Tillage	0.03103	0.989
Residual	0.66685	
NH site 2019		
PST	0.05605	0.475
Tillage	0.10143	0.606
PST:Tillage	0.0981	0.631
Residual	0.74443	

Indicator Species and Functional Groups at the PA site

We used indicator species analysis (ISA) to identify seed coat fungal OTUs that were significantly associated with each treatment. At the PA site, ISA identified 27 OTUs with significant single group associations to untreated plots, and 14 that were significantly associated with PST-treated plots (Table 4).

Table 4: Indicator species analysis results by site. Total OTUs associated with each treatment are shown in bold, associated OTUs which make up at least 1% of the community of any plot are displayed with the lowest level of taxonomic assignment made.

Site/factor/OTU	**Known taxonomy**	**stat**	**p.value**
PA			
Seed Treatment			
Untreated, 27 OTUs			
OTU 7	Fungi	0.954	0.0006
OTU 3	*Helicodendron spp.*	0.83	0.0168
OTU 82	*Sporisorium spp.*	0.807	0.039
OTU 41	Phylum:Ascomycota	0.764	0.0182
Treated, 14 OTUs			
OTU 57	Fungi	0.796	0.0108
NH 2018			
Seed Treatment			
Treated, 7 OTUs			
OTU 8	*Tetracladium sp.*	0.972	0.0494
OTU 31	*Monographella nivalis*	0.739	0.0106
Untreated, 7 OTUs			
Tillage			
Full Till, 6 OTUs			
OTU 1	Fungi	0.866	0.0002
Strip, 26 OTUs			
OTU 7	Fungi	0.882	0.0008
OTU 2	*Tetracladium maxilliforme*	0.708	0.023
OTU 82	*Sporisorium sp.*	0.707	0.0178
OTU 415	*Cryptococcus magnus*	0.699	0.0292
No Till, 38 OTUs			
OTU 6	*Tetracladium sp.*	0.78	0.0432
NH 2019			
Seed Treatment			
Treated, 5 OTUs			
Untreated, 0 OTUs			
Tillage			
Full Till, 9 OTUs			
OTU 11	*Fusarium tricinctum*	0.971	0.013
OTU 24	Phylum: Ascomycota	0.92	0.0026
OTU44	*Guehomyces pullulans*	0.906	0.0106

OTU125	Class:Tremellomycetes	0.806	0.0454
Strip Till, 2 OTUs			
No Till, 3 OTUs			
OTU 2	*Tetracladium maxilliforme*	0.794	0.022

Analysis of the seedcoat fungal community at the PA site by functional guild showed differences between PST treated and untreated plots ($p = 0.043$) and between years ($p = 0.002$). Specifically, seeds overwintered in the PST-treated plots had a lower relative abundance of unknown taxa (62.1% treated plots, 71.2% in untreated) and higher relative abundance of general (16.3% in treated plots, 12.7% in untreated) and soil saprotrophs (13.4% in untreated, 7% in untreated plots). There was a change of less than .5% relative pathogen abundance (4.9% in treated plots, 4.5% of untreated plots) and other identified fungi made 2.0% treated plots and 3.1% of untreated plots (Figure 6).

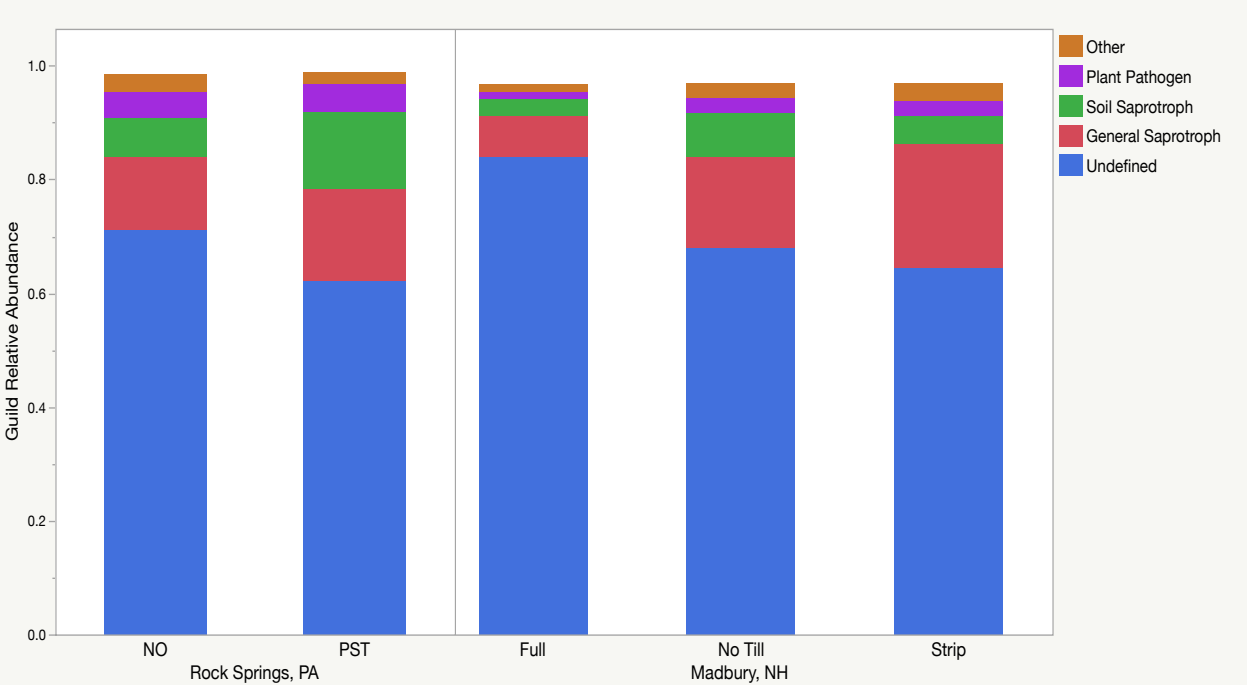

Figure 6: Relative abundance of seed coat fungal functional groups as affected by PST (PA site) and tillage (NH site in 2018).

Table 5. Results of PerMANOVA conducted on seed coat fungal communities based on functional groups. Significant factors are bolded ($p<0.05$).

Factor/site	R2	Pr(>F)
PA 2018 & 2019		
PST	**0.04114**	**0.043**
Cover	0.00463	0.869
Year	**0.10648**	**0.002**
PST:Cover	0.03321	0.081
PST:Year	0.01105	0.549
Cover:Year	0.02285	0.185
PST:Cover:Year	0.02666	0.147
Residual	0.75397	
NH 2018		
PST	0.00074	0.95
Tillage	**0.33426**	**0.025**
PST:Tillage	0.00784	0.972
Residual	0.65716	
NH 2019		
PST	0.00555	0.928
Tillage	0.05058	0.801
PST:Tillage	0.21872	0.129
Residual	0.72515	

Indicator Species and Functional Groups at the NH site

At the NH site, we conducted ISA and functional group PerMANOVA separately by year, due to the significant year by tillage interaction when communities were analyzed at the OTU level (PerMANOVA, $p = 0.008$). In 2018, ISA based on PST treatment identified seven OTUs that were associated with untreated plots and seven that were associated with PST-treated plots (Table 4). In 2019, we found no OTUs associated with the untreated plots and five

associated with PST-treated plots. Conducting ISA based on tillage treatment in 2018 identified six OTUs associated with full till, 26 with strip till, and 38 with no-till (Table 4). Interestingly, OTU1, for which no taxonomic information was assigned, was associated with full till and made up 57% of the sequences in those plots. This OTU also had the highest relative abundance of any OTU in our dataset. In 2019, we found nine OTUs associated with full tillage, two associated with strip tillage, and three associated with no-till (Table 3).

PST did not alter the relative abundance of functional guilds (PerMANOVA, $p = 0.95$); however, tillage did in 2018 (PerMANOVA, $p = 0.025$) but not in 2019 (PerMANOVA, $p = 0.801$). In full till plots in 2018 the relative abundance of taxa in the "unknown" functional group was higher (86.7% in full till, 66.5% in strip tilled, and 70.1% in no-till plots). The relative abundance of taxa in all other guilds were lower in full till plots compared to the strip and no-till plots (general saprotrophs, 7.6% of full till, 22.5% of strip till plots, and 16.7% on no-till plots; soil saprotrophs, 3.1% in full till plots, 5.3% in strip tilled, and 7.9% in no-till; plant pathogens, 1.3% of full till plots, 2.6% in strip tilled plots, and 2.7% in no-till plots; other fungi made up 1.4%, 3.1%, and 2.5%. in full strip, and no-till plots respectively.)

Seed Colonization by Soil Fungi and Impacts on Viability

Seed colonization and viability were assessed at the NH site in 2019 only. Fungal OTU richness after overwintering was 600% greater in buried seeds exposed to soil (mean = 187 OTUs) compared to control seeds which were buried but not exposed to soil (mean = 31 OTUs) (ANOVA, $p<0.0001$; Figure 7). This change in OTUs indicates that weed seeds were rapidly colonized by soil fungi, and that most of the sequences we found on our buried seeds represent organisms which originated from the soil in our experimental field plots. Seed viability was

reduced 35.6% by overwinter exposure to soil and was just 16.4% in seeds with soil contact compared to seeds overwintered in plastic bags, which had a mean viability of 25.5% (ANOVA, p=0.0064; Figure 7). This reduction in seed viability can be attributed primarily to microbial decay and physical destruction, as seeds lost due to predation or prior fatal germination were not factored into the viability calculation. Neither PST or tillage affected overwintered seed viability (ANOVA, $p > 0.05$).

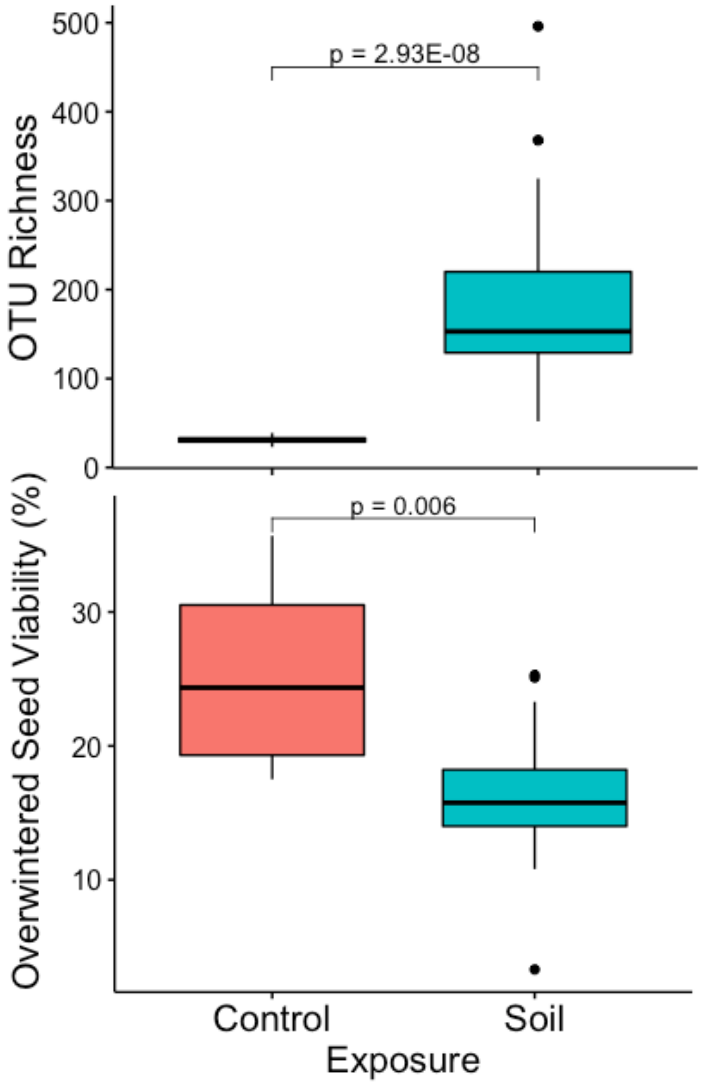

Figure 7: Effects of soil burial in mesh bags (Soil) and in plastic bags (Control) on seed coat fungal OTU richness and seed viability.

Discussion

Our data on *A. retroflexsus* seed coat fungal colonization and viability (Figure 6) indicate that, as we expected, *A. retroflexsus* seeds were colonized by a diverse suite of soil fungi during the overwintering period, and that exposure to the soil over this time period resulted in substantial declines in seed viability (>35%). Given that our control seeds experienced the same soil temperatures, we can rule out temperature fluctuations as contributing to this decline in viability. While we attribute the decline in viability largely to microbial processes, as other mortality factors such as seed predation and fatal germination were excluded by our methods, we also cannot rule out other soil processes, such as gas or nutrient exchange or moisture conditions which likely also differed between the mesh and control (plastic) bags and which could have influenced viability (Dalling et al., 2011; Davis, 2007; Davis et al., 2006). We saw no treatment related differences in the overwintered viability rates of our weed seeds and saw no differences in the seed coat fungal communities in that year.

At our PA site where the entire experiment was conducted under no-till, we detected differences in the communities of soil fungi that colonized weed seeds overwintered in soils where PST treated crop seeds were planted compared to where those same crops were planted without PST. We observed differences in fungal community composition and diversity, as well as the relative abundance of different functional guilds. Reductions in fungal community richness, evenness, and Shannon's diversity associated with PST use at the PA site indicate that PST can act as a meaningful "filter" on the community of soil fungi that colonize weed seeds in the soil, supporting our second hypothesis. This is especially significant given the fact that PST treated crop seeds contain relatively small amounts of fungicide and that our seed assays

occurred well after PST treated crops were planted and harvested each year. We found no evidence at the PA site that effects of PST on seed coat fungal communities were altered by cover crops despite previous research indicating they can take up PST components (Bredeson and Lundgren, 2019). The lack of a cover crop effect may be a result of the timing of cover crop planting and growth in this study, as the fall sown cover crop would have had the fastest growth, and therefore highest potential for pesticide uptake, in the subsequent spring when PST concentrations in the soil would be expected to be at their lowest(Anderson et al., 2015; Radolinski et al., 2018).

We did detect evidence that the fungicide component of PST reduced the occurrence of at least one soil borne pathogen, *Fusarium tricinctum*, a pathogen which PST are specifically labeled for use against. While this species was identified as an indicator species of untreated plots at the PA site, our analysis of functional guilds indicated that the difference in the relative abundance of plant pathogens between the treated and untreated plots was less than 0.5% and not statistically significant. Previous research has found that use of PST is often not associated with a reduction in the pests and pathogen's they target (Douglas and Tooker, 2015; Labrie et al., 2020; Lamichhane et al., 2020). Perhaps more significantly, two other taxa that are not intended to be targeted by PST were also found to respond to PST use, the litter saprotrophic genus *Helicodendron* (six species in dataset) and *Mrakia frigida,* a cold-tolerant yeast (Hua et al., 2010; Mudur et al., 2006). The abundance of *Helicodendron spp.* was lower in the presence of PST, indicating it may be susceptible to at least one of the PST components. In contrast, the relative abundance of *M. frigida* was higher in the presence of PST, suggesting that this cold adapted yeast may have benefited from reduced competition during the winter months.

Seed coat fungal communities in PST-treated plots at the PA site had more fungi assigned to saprotrophic guilds than seed coats in untreated plots, which may indicate an increased capacity for seed decay in the presence of PST in contrast to our hypothesis. Unfortunately, we did not measure seed viability rates in overwintered seeds from the PA site. Where we did measure viability (seeds from the NH site in 2019), we did not see a similar effect of PST on the weed seed coat fungal community; therefore, the role that PST-mediated shifts in saprotrophic seed coat fungi might play in weed seed overwinter survival remains unknown.

We did not detect an effect of PST on weed seed coat fungal communities at the NH site, nor did we detect an interaction between PST and tillage as we had hypothesized. It is possible that our lower experimental power at the NH site (four replicates) compared to the PA site (6 replicates) resulted in an inability to detect the PST effect. In accordance with this explanation, we had expected that the effects of PST would be strongest in the no-till treatment and it was indeed the case that while not significantly different, all four seed coat fungal community diversity measures were numerically lower in the PST-treated plots in the no-till treatment compared to the untreated plots, while this was no discernable trends in these indices in the other tillage treatments. It is also possible that other factors such as climate, soil type, or a host of other site-specific biotic or abiotic factors which likely differed between the two sites contributed to the lack of a detectible PST effect at the NH site.

In contrast to PST, tillage did have a strong effect on seed coat fungal communities at the NH site, both at the OTU level and functionally, as we had hypothesized. The change in Simpson's dominance index and greater relative abundance of OTU1 in the full till plots in 2018, may be indicative of the simplified soil environment that high soil disturbance practices like moldboard tillage create. Disturbed environments favor simpler communities both above and

below ground (Anthony et al., 2017; Smith, 2015) but this is, to the best of our knowledge, the first report of tillage system influencing the community of soil fungi that colonize weed seeds. As such, these data could have important implications for our understanding of weed community dynamics and how these are altered by changes in tillage.

We were unable to fully address our first hypothesis, as we only reliably tested viability of seeds from the NH site in the year (2019) where we did not detect a difference in the seed coat fungal communities between treatments. While we did attempt to collect viability data in 2018, the year we did detect a strong effect of tillage in the fungal community, methodological problems made those data unreliable and so they were excluded from analysis. Because of this; we are unable to link our findings of functional changes in the seed coat fungal community to changes in weed seed overwinter survival in soil.

Taxonomic or guild classifications were unavailable for a significant portion of the soil fungi that colonized the weed seed coats in our study, a problem common to most amplicon sequencing projects (Hugerth and Andersson, 2017). Inability to classify all members of the fungal community could lead to biases in our analyses based on which taxa are described and which are not. For example, the difference in fungi assigned to the "unknown" guild between the PST-treated and untreated treatments was ~10% at the PA site and ~20% between the full and strip/no-till plots at the NH site, differences of magnitude close to the sum of all other differences in those communities at each site. However, given that the taxa we are most interested in, i.e., the plant pathogens and saprotrophs, are taxa of broad interest, they are likely better described than fungi at large; therefore, this particular bias may be less of a concern.

Our study involved only a single weed species, *A retroflexus*, and so it remains unknown the degree to which our results are weed species-specific. It is not unrealistic to expect that other

weed species may have varying responses to soil fungal communities, PST, and/or tillage based on seed defense syndrome (Dalling et al., 2011) or other factors (Buyer et al., 1999; Sarmiento et al., 2017).

Taken together, these data provide strong evidence that PST alters the community of soil fungi that successfully colonize weed seeds during the overwintering period in row crop agroecosystems. Data from the PA site, and congruent trends from the NH site, suggest that the effects of PST on weed seed coat fungal communities is strongest in no-till cropping systems. Data from the NH site provide evidence that tillage, independent of PST, can also strongly influence weed seed coat fungal communities. Given that PST use is common in no-till cropping systems, systems that also frequently feature high numbers of herbicide-resistant weeds (Heap 2020), our data provide compelling evidence for the need to better understand the potential relationships between soil fungi, PST, and weed population dynamics.

Conclusions

We conducted two-year experiments at two sites, one in PA and another in NH, to investigate the effect of PST use on the community of soil fungi that colonized weed seeds over the winter fallow period in a typical maize/soybean rotation. At the PA site we found strong and consistent evidence that PST alter the composition and diversity of the soil fungal community that colonized the coats of weed seeds. At the NH site we had lower power to detect PST effects but found that tillage resulted in increased seed coat fungal community dominance by a single species compared to strip- and no-till. Unfortunately, we were not able to assess the impact of these changes on weed seed viability and decay rates and therefore the need to understand the impact of PST and tillage-mediated changes in fungal communities on weed seedbanks remains important and unanswered.

Despite an experimental design that meant weed seeds were exposed to the soil when pesticide levels in the soils would theoretically be expected to be at their lowest concentrations– we were able to detect functional changes in the fungal communities on the coats of weed seeds when pesticide treated crop seeds were used. We found that in cases where the effect of PST was present, fungal saprotrophs and yeasts—species which may be expected to mediate seed decay and the effects of soil pathogens (Yurkov, 2018) —were the most responsive members of the seed coat fungal community, while the relative abundance of plant pathogens was unchanged. Taken together, these results suggest that while we have yet to understand the full impact of these PST-mediated changes in weed seed coat fungal communities and weed population dynamics, given the fact that PST use has been shown to have little to no impact on crop yields (Labrie et al., 2020), it may be wise to reserve the use of these products to a more strictly IPM

context, rather than prophylactically as they are used now. Further, our results show that chemical and non-chemical agricultural management practices alter the fungal community that have access to weed seeds, and that more research is required to understand the implication of these effects on weed community dynamics.

References

Anderson, J.C., Dubetz, C., Palace, V.P., 2015. Neonicotinoids in the Canadian aquatic environment: A literature review on current use products with a focus on fate, exposure, and biological effects. Science of The Total Environment 505, 409–422. doi:10.1016/j.scitotenv.2014.09.090

Anthony, M.A., Frey, S.D., Stinson, K.A., 2017. Fungal community homogenization, shift in dominant trophic guild, and appearance of novel taxa with biotic invasion. Ecosphere 8, e01951. doi:10.1002/ecs2.1951

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A.S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution n/a-n/a. doi:10.1111/2041-210X.12073

Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., Kuzyakov, Y., 2014. Microbial Growth and Carbon Use Efficiency in the Rhizosphere and Root-Free Soil. PLoS ONE 9, e93282. doi:10.1371/journal.pone.0093282

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. doi:10.1093/bioinformatics/btu170

Borza, J.K., Westerman, P.R., Liebman, M., 2007. Comparing Estimates of Seed Viability in Three Foxtail (Setaria) Species Using the Imbibed Seed Crush Test with and Without Additional Tetrazolium Testing. Weed Technology 21, 518–522. doi:10.1614/WT-06-110

Bredeson, M.M., Lundgren, J.G., 2019. Neonicotinoid insecticidal seed-treatment on corn contaminates interseeded cover crops intended as habitat for beneficial insects. Ecotoxicology 28, 222–228. doi:10.1007/s10646-018-02015-9

Buyer, J.S., Roberts, D.P., Russek-Cohen, E., 1999. Microbial community structure and function in the spermosphere as affected by soil and seed type 45, 7.

Chee-Sanford, J.C., Williams, M.M., Davis, A.S., 2006. Do microorganisms influence seed-bank dynamics? Weed Science 54, 575–587. doi:10.1614/WS-05-055R.1

Dalling, J.W., Davis, A.S., Schutte, B.J., Elizabeth Arnold, A., 2011. Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community: Seed survival in soil. Journal of Ecology 99, 89–95. doi:10.1111/j.1365-2745.2010.01739.x

Davis, A.S., 2007. Nitrogen Fertilizer and Crop Residue Effects on Seed Mortality and Germination of Eight Annual Weed Species. Weed Science 55, 123–128. doi:10.1614/WS-06-133.1

Davis, A.S., 2006. When does it make sense to target the weed seed bank? Weed Science 54, 558–565. doi:10.1614/WS-05-058R.1

Davis, A.S., Anderson, K.I., Hallett, S.G., Renner, K.A., 2006. Weed seed mortality in soils with contrasting agricultural management histories. Weed Science 54, 291–297. doi:10.1614/WS-05-54.2.291

Davis, A.S., Renner, K.A., 2007. Influence of Seed Depth and Pathogens on Fatal Germination of Velvetleaf (*Abutilon theophrasti*) and Giant Foxtail (*Setaria faberi*). Weed Science 55, 30–35. doi:10.1614/W-06-099.1

Douglas, M.R., Tooker, J.F., 2015. Large-Scale Deployment of Seed Treatments Has Driven Rapid Increase in Use of Neonicotinoid Insecticides and Preemptive Pest Management in U.S. Field Crops. Environmental Science & Technology 49, 5088–5097. doi:10.1021/es506141g

Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species:the need for a flexible asymmetrical approach. Ecological Monographs 67, 345–366. doi:10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2

Edgar, R.C., 2018. Accuracy of taxonomy prediction for 16S rRNA and fungal ITS sequences. PeerJ 6, e4652. doi:10.7717/peerj.4652

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. doi:10.1093/bioinformatics/btq461

Frey, S.D., Elliott, E.T., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biology and Biochemistry 31, 573–585. doi:10.1016/S0038-0717(98)00161-8

Freyre, R., Loy, J.B., 2000. Evaluation and Yield Trials of Tomatillo in New Hampshire. HortTechnology 10, 374–377. doi:10.21273/HORTTECH.10.2.374

Frost, M.D., Haramoto, E.R., Renner, K.A., Brainard, D.C., 2019. Tillage and Cover Crop Effects on Weed Seed Persistence: Do Light Exposure and Fungal Pathogens Play a Role? Weed Science 67, 103–113. doi:10.1017/wsc.2018.80

Ghorbani, R., Seel, W., Leiferr, C., 1999. Effects of Environmental Factors on Germination and Emergence of Amaranthus retroflexus. Weed Science 47, 505–510.

Gómez, R., Liebman, M., Munkvold, G., 2014. Weed seed decay in conventional and diversified cropping systems. Weed Research 54, 13–25. doi:10.1111/wre.12052

Harding, D.P., Raizada, M.N., 2015. Controlling weeds with fungi, bacteria and viruses: a review. Frontiers in Plant Science 6. doi:10.3389/fpls.2015.00659

Heap, I., n.d. The International Herbicide-Resistant Weed Database [WWW Document]. URL http://www.weedscience.org/Home.aspx (accessed 7.26.20).

Hua, M.-X., Chi, Z., Liu, G.-L., Buzdar, M.A., Chi, Z.-M., 2010. Production of a novel and cold-active killer toxin by Mrakia frigida 2E00797 isolated from sea sediment in Antarctica. Extremophiles 14, 515–521. doi:10.1007/s00792-010-0331-6

Hugerth, L.W., Andersson, A.F., 2017. Analysing Microbial Community Composition through Amplicon Sequencing: From Sampling to Hypothesis Testing. Frontiers in Microbiology 8, 1561. doi:10.3389/fmicb.2017.01561

Ihrmark, K., Bödeker, I.T.M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K.E., Lindahl, B.D., 2012. New primers to amplify the fungal ITS2 region – evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiology Ecology 82, 666–677. doi:10.1111/j.1574-6941.2012.01437.x

Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the Status and Global Strategy for Neonicotinoids. Journal of Agricultural and Food Chemistry 59, 2897–2908. doi:10.1021/jf101303g

Katulanda, P.M., Walley, F.L., Janzen, H.H., Helgason, B.L., 2018. Land use legacy regulates microbial community composition in transplanted Chernozems. Applied Soil Ecology 129, 13–23. doi:10.1016/j.apsoil.2018.03.001

Korres, N.E., Norsworthy, J.K., Young, B.G., Reynolds, D.B., Johnson, W.G., Conley, S.P., Smeda, R.J., Mueller, T.C., Spaunhorst, D.J., Gage, K.L., Loux, M., Kruger, G.R., Bagavathiannan, M.V., 2018. Seedbank Persistence of Palmer Amaranth (*Amaranthus palmeri*) and Waterhemp (*Amaranthus tuberculatus*) across Diverse Geographical Regions in the United States. Weed Science 66, 446–456. doi:10.1017/wsc.2018.27

Kremer, R.J., Li, J., 2003. Developing weed-suppressive soils through improved soil quality management. Soil and Tillage Research 72, 193–202. doi:10.1016/S0167-1987(03)00088-6

Kumar, V., Brainard, D.C., Bellinder, R.R., 2008. Suppression of Powell Amaranth (Amaranthus powellii), Shepherd's-Purse (Capsella bursa- pastoris), and Corn Chamomile (Anthemis arvensis) by Buckwheat Residues: Role of Nitrogen and Fungal Pathogens. Weed Science 56, 271–280.

Labrie, G., Gagnon, A.-È., Vanasse, A., Latraverse, A., Tremblay, G., 2020. Impacts of neonicotinoid seed treatments on soil-dwelling pest populations and agronomic parameters in corn and soybean in Quebec (Canada). PLOS ONE 15, e0229136. doi:10.1371/journal.pone.0229136

Lamichhane, J.R., You, M.P., Laudinot, V., Barbetti, M.J., Aubertot, J.-N., 2020. Revisiting Sustainability of Fungicide Seed Treatments for Field Crops. Plant Disease 104, 610–623. doi:10.1094/PDIS-06-19-1157-FE

Lee, Z.M., Schmidt, T.M., 2014. Bacterial growth efficiency varies in soils under different land management practices. Soil Biology and Biochemistry 69, 282–290. doi:10.1016/j.soilbio.2013.11.012

Leishman, M.R., Masters, G.J., Clarke, I.P., Brown, V.K., 2000. Seed Bank Dynamics: The Role of Fungal Pathogens and Climate Change. Functional Ecology 14, 293–299.

Liebman, Davis, 2000. Integration of soil, crop and weed management in low-external-input farming systems. Weed Research 40, 27–47. doi:10.1046/j.1365-3180.2000.00164.x

Liebman, M., Gallandt, E.R., 1997. Many Little Hammers: Ecological Management of Crop-Weed Interactions, in: Ecology in Agriculture. Elsevier, pp. 291–343. doi:10.1016/B978-012378260-1/50010-5

McCune, B., Grace, J.B., Urban, D.L., 2002. Analysis of ecological communities, 2nd printing. ed. MjM Software Design, Gleneden Beach, Or.

McMurdie, P.J., Holmes, S., 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Computational Biology 10, e1003531. doi:10.1371/journal.pcbi.1003531

Mohler, C.L., Dykeman, C., Nelson, E.B., Ditommaso, A., 2012. Reduction in weed seedling emergence by pathogens following the incorporation of green crop residue: Seedling emergence and pathogens. Weed Research 52, 467–477. doi:10.1111/j.1365-3180.2012.00940.x

Mortensen, D.A., Egan, J.F., Maxwell, B.D., Ryan, M.R., Smith, R.G., 2012. Navigating a Critical Juncture for Sustainable Weed Management. BioScience 62, 75–84. doi:10.1525/bio.2012.62.1.12

Mudur, S.V., Swenson, D.C., Gloer, J.B., Campbell, J., Shearer, C.A., 2006. Heliconols A−C: Antimicrobial Hemiketals from the Freshwater Aquatic Fungus *Helicodendron g iganteum*. Organic Letters 8, 3191–3194. doi:10.1021/ol060916w

Nelson, E.B., 2018. The seed microbiome: Origins, interactions, and impacts. Plant and Soil 422, 7–34. doi:10.1007/s11104-017-3289-7

Nettles, R., Watkins, J., Ricks, K., Boyer, M., Licht, M., Atwood, L.W., Peoples, M., Smith, R.G., Mortensen, D.A., Koide, R.T., 2016. Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. Applied Soil Ecology 102, 61–69. doi:10.1016/j.apsoil.2016.02.008

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20, 241–248. doi:10.1016/j.funeco.2015.06.006

Nikolić, N., Squartini, A., Concheri, G., Stevanato, P., Zanin, G., Masin, R., 2020. Weed Seed Decay in No-Till Field and Planted Riparian Buffer Zone. Plants 9, 293. doi:10.3390/plants9030293

Norsworthy, J.K., Ward, S.M., Shaw, D.R., Llewellyn, R.S., Nichols, R.L., Webster, T.M., Bradley, K.W., Frisvold, G., Powles, S.B., Burgos, N.R., Witt, W.W., Barrett, M., 2012. Reducing the Risks of Herbicide Resistance: Best Management Practices and Recommendations. Weed Science 60, 31–62. doi:10.1614/WS-D-11-00155.1

Papiernik, S.K., Sappington, T.W., Luttrell, R.G., Hesler, L.S., Allen, K.C., 2018. Overview: Risk Factors and Historic Levels of Pressure From Insect Pests of Seedling Corn, Cotton, Soybean, and Wheat in the United States. Journal of Integrated Pest Management 9, 18. doi:10.1093/jipm/pmx026

Radolinski, J., Wu, J., Xia, K., Stewart, R., 2018. Transport of a neonicotinoid pesticide, thiamethoxam, from artificial seed coatings. Science of The Total Environment 618, 561–568. doi:10.1016/j.scitotenv.2017.11.031

Sarmiento, C., Zalamea, P.-C., Dalling, J.W., Davis, A.S., Stump, S.M., U'Ren, J.M., Arnold, A.E., 2017. Soilborne fungi have host affinity and host-specific effects on seed germination and survival in a lowland tropical forest. Proceedings of the National Academy of Sciences 114, 11458–11463. doi:10.1073/pnas.1706324114

Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. The ISME Journal 6, 1749–1762. doi:10.1038/ismej.2012.11

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Fungal Barcoding Consortium, Fungal Barcoding Consortium Author List, Bolchacova, E., Voigt, K., Crous, P.W., Miller, A.N., Wingfield, M.J., Aime, M.C., An, K.-D., Bai, F.-Y., Barreto, R.W., Begerow, D., Bergeron, M.-J., Blackwell, M., Boekhout, T., Bogale, M., Boonyuen, N., Burgaz, A.R., Buyck, B., Cai, L., Cai, Q., Cardinali, G., Chaverri, P., Coppins, B.J., Crespo, A., Cubas, P., Cummings, C., Damm, U., de Beer, Z.W., de Hoog, G.S., Del-Prado, R., Dentinger, B., Dieguez-Uribeondo, J., Divakar, P.K., Douglas, B., Duenas, M., Duong, T.A., Eberhardt, U., Edwards, J.E., Elshahed, M.S., Fliegerova, K., Furtado, M., Garcia, M.A., Ge, Z.-W., Griffith, G.W., Griffiths, K., Groenewald, J.Z., Groenewald, M., Grube, M., Gryzenhout, M., Guo, L.-D., Hagen, F., Hambleton, S., Hamelin, R.C., Hansen,

K., Harrold, P., Heller, G., Herrera, C., Hirayama, K., Hirooka, Y., Ho, H.-M., Hoffmann, K., Hofstetter, V., Hognabba, F., Hollingsworth, P.M., Hong, S.-B., Hosaka, K., Houbraken, J., Hughes, K., Huhtinen, S., Hyde, K.D., James, T., Johnson, E.M., Johnson, J.E., Johnston, P.R., Jones, E.B.G., Kelly, L.J., Kirk, P.M., Knapp, D.G., Koljalg, U., Kovacs, G.M., Kurtzman, C.P., Landvik, S., Leavitt, S.D., Liggenstoffer, A.S., Liimatainen, K., Lombard, L., Luangsa-ard, J.J., Lumbsch, H.T., Maganti, H., Maharachchikumbura, S.S.N., Martin, M.P., May, T.W., McTaggart, A.R., Methven, A.S., Meyer, W., Moncalvo, J.-M., Mongkolsamrit, S., Nagy, L.G., Nilsson, R.H., Niskanen, T., Nyilasi, I., Okada, G., Okane, I., Olariaga, I., Otte, J., Papp, T., Park, D., Petkovits, T., Pino-Bodas, R., Quaedvlieg, W., Raja, H.A., Redecker, D., Rintoul, T.L., Ruibal, C., Sarmiento-Ramirez, J.M., Schmitt, I., Schussler, A., Shearer, C., Sotome, K., Stefani, F.O.P., Stenroos, S., Stielow, B., Stockinger, H., Suetrong, S., Suh, S.-O., Sung, G.-H., Suzuki, M., Tanaka, K., Tedersoo, L., Telleria, M.T., Tretter, E., Untereiner, W.A., Urbina, H., Vagvolgyi, C., Vialle, A., Vu, T.D., Walther, G., Wang, Q.-M., Wang, Y., Weir, B.S., Weiss, M., White, M.M., Xu, J., Yahr, R., Yang, Z.L., Yurkov, A., Zamora, J.-C., Zhang, N., Zhuang, W.-Y., Schindel, D., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109, 6241–6246. doi:10.1073/pnas.1117018109

Smith, R.G., 2015. A succession-energy framework for reducing non-target impacts of annual crop production. Agricultural Systems 133, 14–21. doi:10.1016/j.agsy.2014.10.006

Smith, R.G., Atwood, L.W., Morris, M.B., Mortensen, D.A., Koide, R.T., 2016. Evidence for indirect effects of pesticide seed treatments on weed seed banks in maize and soybean. Agriculture, Ecosystems & Environment 216, 269–273. doi:10.1016/j.agee.2015.10.008

Smith, R.G., Mortensen, D.A., 2017. A Disturbance-based Framework for Understanding Weed Community Assembly in Agroecosystems: Challenges and Opportunities for Agroecological Weed Management, in: Agroecological Practices for Sustainable Agriculture. WORLD SCIENTIFIC (EUROPE), pp. 127–154. doi:10.1142/9781786343062_0005

Sommermann, L., Geistlinger, J., Wibberg, D., Deubel, A., Zwanzig, J., Babin, D., Schlüter, A., Schellenberg, I., 2018. Fungal community profiles in agricultural soils of a long-term field trial under different tillage, fertilization and crop rotation conditions analyzed by high-throughput ITS-amplicon sequencing. PLOS ONE 13, e0195345. doi:10.1371/journal.pone.0195345

Swanton, C.J., Murphy, S.D., 1996. Weed Science Beyond the Weeds: The Role of Integrated Weed Management (IWM) in Agroecosystem Health. Weed Science 44, 437–445. doi:10.1017/S0043174500094145

Syngenta, U., 2012. CruizerMaxx 250 Seed treatment [WWW Document]. URL https://www.syngenta-us.com/current-label/cruiser-5fs; https://www.syngenta-us.com/current-label/maxim-quattro

Ullrich, S.D., Buyer, J.S., Cavigelli, M.A., Seidel, R., Teasdale, J.R., 2011. Weed Seed Persistence and Microbial Abundance in Long-Term Organic and Conventional Cropping Systems. Weed Science 59, 202–209. doi:10.1614/WS-D-10-00142.1

Wagner, M., Mitschunas, N., 2008. Fungal effects on seed bank persistence and potential applications in weed biocontrol: A review. Basic and Applied Ecology 9, 191–203. doi:10.1016/j.baae.2007.02.003

Weisberger, D., Nichols, V., Liebman, M., 2019. Does diversifying crop rotations suppress weeds? A meta-analysis. PLOS ONE 14, e0219847. doi:10.1371/journal.pone.0219847

Williams, A., Davis, A.S., Jilling, A., Grandy, A.S., Koide, R.T., Mortensen, D.A., Smith, R.G., Snapp, S.S., Spokas, K.A., Yannarell, A.C., Jordan, N.R., 2017. Reconciling opposing soil processes in row-crop agroecosystems via soil functional zone management. Agriculture, Ecosystems & Environment 236, 99–107. doi:10.1016/j.agee.2016.11.012

Yurkov, A.M., 2018. Yeasts of the soil - obscure but precious. Yeast 35, 369–378. doi:10.1002/yea.3310

Zhang, Y., Hao, X., Alexander, T.W., Thomas, B.W., Shi, X., Lupwayi, N.Z., 2018. Long-term and legacy effects of manure application on soil microbial community composition. Biology and Fertility of Soils 54, 269–283. doi:10.1007/s00374-017-1257-2