Responses of soil faunal food webs to pesticide seed treatments

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Project Information

Summary:

Insecticide-fungicide seed treatments are widely used in commodity cropping systems in the United States, but little is known about how these pesticide seed treatments (PST) affect non-targeted soil faunal composition and structure and their ability to function in agroecosystems. We conducted a two-year field experiment to examine the effects of PST on soil faunal composition, structure, and function. Corn (2013) and soybean (2014) with and without pesticide seed treatments were planted as a two-year rotation in a completely randomized design with five replications. We measured soil faunal community composition, the carbon and nitrogen isotopic signatures of seven soil fauna taxa, aboveground litter decomposition, nutrient cycling, and crop response. Our results suggest this management practice does not cause community-level shifts in soil faunal composition, but can reduce detritivore biomass. Our results also suggest pesticide seed treatments can benefit Collembola Isotomidae (fungivores) and a non-targeted pest population, Symphylan, while adversely affecting two predatory taxa, Carabidae *Bembidion spp*. and Diplura Japygida. We also found marginal decreases in $\delta^{15}N$ signatures with the use of PST for a group of generalist predators (Lithobiomorpha, Campodeidae, and Japygidae) suggesting these organisms predate more on prey that occupy lower trophic positions when PST are used. These effects on soil faunal composition and community structure, however, did not result in measurable differences in the decomposition of surface litter, plant available nitrogen, or grain yields between our treatments. This research contributes to the ongoing debate regarding the environmental consequences of using PSTs in our agroecosystems. Our results reveal new unforeseen consequences of using insecticide-fungicide seed treatments and raise further questions about the

effectiveness of this management practice in commodity cropping systems. It is our hope that these results can help us identify less deleterious ways of managing pests and ultimately improve the overall sustainability of our agroecosystems.

Introduction:

In non-organic agriculture, crop seeds are commonly pre-coated with broad spectrum insecticides and fungicides to control crop seed predators and pathogens. Neonicotinoids, a class of insecticides, are often included in pesticide seed treatment (PST) mixtures because of their effectiveness at killing agronomic pests, low toxicity to vertebrates, and systemic properties (i.e. plants can absorb and translocate the toxins throughout the plant tissue). In 2011, seventeen years after the release of neonicotinoids, it is estimated that 34-44% of soybean and 79-100% of maize hectares grown in the United States had seed-applied neonicotinoids (Douglas and Tooker 2015). Although this pest management practice is widely integrated into our commodity cropping systems, we know surprisingly little about how PSTs with neonicotinoids affect non-targeted organisms and their ability to function in agroecosystems.

Emergent evidence suggests commonly used PSTs can exert unforeseen effects on soil-inhabiting non-targeted populations at multiple trophic positions (Seagraves and Lundgren 2012, El-Naggar and Zidan 2013, Douglas et al. 2015, Nettles et al. 2016, Smith et al. 2016). In the field, PSTs have been shown to shift bacterial community composition in the crop rhizosphere (Nettles et al. 2016), increase densities of fungivore populations (El-Naggar and Zidan 2013), reduce densities of generalist predators (Seagraves and Lundgren 2012), and increase densities of weed seeds (Smith et al. 2016). Furthermore, PSTs with neonicotinoids are not always effective at killing important agronomic pests, such as slugs and soybean aphids (Seagraves and Lundgren 2012, Douglas et al. 2015). Yet, when ground beetles predate on slugs that have accumulated insecticidal toxins from treated plant tissue, the predatory beetles can rapidly succumb to the harbored toxins, exemplifying one way PSTs with neonicotinoids can affect non-targeted soil fauna through indirect pathways (Douglas et al. 2015). Collectively, these data suggest PSTs alter the abundance of non-targeted soil-inhabiting organisms at multiple trophic-positions, which may be inadvertently re-structuring, via “rewiring” consumer-resource linkages, the soil food web.

Alterations in the structure of the soil food web consequently may result in diminished performance of the soil community, specifically their ability to provide agroecosystem services. Soil food webs are comprised of highly interconnected assemblages of plants, microbes and fauna (Brussaard 1997) that through their daily activities regulate organic matter decomposition, nutrient cycling, and pest suppression (Coleman et al. 2004). When disruptions between consumers and resources occur the functional capacity of the food web can be affected. For example, in a simplistic mesocosm experiment where only two mesofaunal species were manipulated (Collembola: *Sinella curviseta* Brook, Earthworm: *Lumbricus terrestris* L.), the addition of wheat seeds coated in PSTs with neonicotinoids resulted in increased collembolan surface activity while earthworm activity was unaffected (Zaller et al. 2016). Interestingly, plant decomposition rates in mesocosms with PST-wheat seeds were reduced compared to systems without PSTs (Zaller et al. 2016). The results from this controlled experiment demonstrate that PSTs with neonicotinoids can disrupt soil food web performance even in simplified soil communities. Agricultural soils, however, house diverse soil faunal communities and, thus, warrant community-level investigations which can elucidate the full impact of PSTs with neonicotinoids on agroecosystem services.

With its widespread use, it is important for farmers and regulatory agencies to better understand the unintended impacts of crop seeds pre-coated with insecticide-fungicide mixtures on the soil food web and its ability to function. We hypothesized PSTs with neonicotinoids would change the composition of the soil food web such that the abundance of fauna with higher trophic positions (e.g. predators) would decrease. We also expected soil faunal communities to restructure themselves with measurable shifts in the trophic positions of the fauna in the wake of these changes in community-level composition. Finally, we hypothesized community-level performance would decrease because of PST induced changes in community composition and structure. To test these hypotheses, we conducted a two-year field experiment where we grew crops with and without PSTs at Penn State University's Russell E. Larson Agricultural Research Center in Rock Springs, PA.

References

Brussaard, L. 1997. Biodiversity and Ecosystem Functioning in Soil. Ambio 26:563-570.

Coleman, D. C., D. A. Crossley Jr, and P. F. Hendrix. 2004. Fundamentals of soil ecology. Academic press.

Douglas, M. R., J. R. Rohr, and J. F. Tooker. 2015. EDITOR'S CHOICE: Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. Journal of Applied Ecology 52:250-260.

Douglas, M. R., and J. F. Tooker. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. Environmental science & technology 49:5088-5097.

El-Naggar, J. B., and N. E.-H. A. Zidan. 2013. Field evaluation of imidaclorpid and thiamethoxam against sucking insects and their side effects on soil fauna. Journal of Plant Protection Research 53.

Nettles, R., J. Watkins, K. Ricks, M. Boyer, M. Licht, L. W. Atwood, M. Peoples, R. G. Smith, D. A. Mortensen, and R. T. Koide. 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.

Seagraves, M. P., and J. G. Lundgren. 2012. Effects of neonicitinoid seed treatments on soybean aphid and its natural enemies. Journal of Pest Science 85:125-132.

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

Zaller, J. G., N. König, A. Tiefenbacher, Y. Muraoka, P. Querner, A. Ratzenböck, M. Bonkowski, and R. Koller. 2016. Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material. BMC Ecology 16:1.

Project Objectives:

With a field experiment, we addressed the following objectives to better understand the effects of pesticide seed treatments on *in situ* soil food web composition, structure, and function. Broadly, we hypothesized that pesticide seed treatments would change the composition of the soil faunal community which would result in re-structuring of the soil food web and decreases in its functional capacity.

Objective 1. Determine to what extent pesticide seed treatments alter the composition of the soil faunal community.

We completed a two-year field experiment (corn-soybean rotation) in 2013 and

2014 where we collected *in situ* soil mesofauna three times each growing season. As of December 2015, we reached this objected as all soil faunal samples (2013 and 2014) were processed and subsequent data was analyzed. Minor revisions to this objective were made in 2014 so the objective was more succinct with emerging literature in this field. This included a change of emphasis from soil faunal diversity measures to community compositional changes.

Objective 2. Determine whether pesticide treated seeds alter the trophic position and/or resource preferences of commonly occurring soil faunal species.

Due to the small quantities (<10 individuals / plot) and small individual biomass (< 0.01 mg) of each soil faunal taxon collected from our field experiment, we are not able to analyze all consumer-resource links in our system as initially proposed. To accurately measure $\delta^{15}N$ and $\delta^{13}C$, a sample must contain a minimum amount of carbon and nitrogen; specifically, 20-150 µg N and 200-2000 µg C. Because we are interested in how pesticide seed treatments affect consumer-resource links, we narrowed our investigation to include only the most commonly occurring taxa found at our field site. We identified seven taxa that frequently occurred among plots, time points, and years which met the minimum sample mass required for isotopic analyses. These taxa include:

1. Acari Oribatida
2. Acari Mesostigmata
3. Collembola Onychiuridae
4. Collembola Entomobryidae
5. Diplura Japygidae
6. Diplura Campodeidae
7. Chilopoda Lithobiomorpha.

After isolating adults of each taxon, drying the specimen, and transferring individuals to sample tins, we submitted our samples to the UNH Stable Isotope Laboratory for analysis (March 2016). NE-SARE granted us an extension to complete this objective because we underestimated the amount of time needed to prepare these samples and were unaware of the scheduled renovations at the UNH Stable Isotope Laboratory. We received all data from the UNH Isotope Laboratory by July 2016, and completed all subsequent analyses therein after.

Objective 3. Determine the effects of pesticide seed treatments on agriculturally important ecosystem services including decomposition and nitrogen cycling.

Decomposition bags and anion resin strips were used to measure decomposition and nitrate cycling, respectively, in the field experiment. Sets of decomposition bags and anion resin strips were deployed at the beginning of each field season and were processed and analyzed before December 2015. Each growing season, we also measured differences in crop growth, including plant heights and leaf chlorophyll content, and grain yields.

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Research

Materials and methods:

Experimental Design. The main objective of this research was to assess the effects of PSTs with neonicotinoids on *in situ* soil food webs and its regulation of important agroecosystem services including decomposition and nitrogen cycling. Our two-year single factor experiment was established 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) in May 2013. Each year the same genotype of a glyphosate resistant cash crop (maize in 2013 and soybean in 2014) 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, and plot treatments were maintained throughout the duration of the experiment. Planting and crop management followed standard agronomic practices for the region.

Site Description. Soils at the field 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. 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).

Maize (2013). Prior to planting, 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 2013). The field was then S-tined, disked, and cultimulched (14-15 May 2013). On 16 May 2013, maize (hybrid TA510-18, TA Seeds, Jersey Shore, PA, USA) was planted in 76 cm-spaced rows at a seed density of 78,300 seeds ha^{-1}. The seed treatment applied to maize was CruiserMaxx Corn 250 (Syngenta, Greensboro, NC, USA), which is a mixture of the systemic insecticide thiamethoxam (class neonicotinoid), the contact fungicide fludioxonil, and the systemic fungicides mefenoxam, azoxystrobin, and thiabendazole. Urea was applied 358 kg ha^{-1} rate on 31 May 2013, and a post-emergence application of glyphosate (1,390 g ha^{-1}) was applied on 20 June 2013 to control emerged weeds.

Soybean (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 2014). On 30 May 2014, soybean (TS2849R2S, TA Seeds) was no-till planted into the maize residue in 76 cm-spaced rows at a seed density of 432,250 seeds ha^{-1}. The seed

treatment applied to the treated soybean was CruiserMaxx Beans with Vibrance (Syngenta). This pesticide mixture includes the systemic insecticide thiamethoxam (class neonicotinoid), and the contact fungicide fludioxonil, and the systemic fungicides mefenoxam and sedaxane. On 16 June 2014, a post-emergence application of glyphosate (1,390 g ha^{-1}) was applied to control emerged weeds.

Litterbag experiment. A litter decomposition experiment (approximately 130-days long) was initiated each year at the time the crop was planted and ending before harvest. Decomposition bags measuring 18 cm x 18 cm were constructed from nylon mesh with 1.5 mm square openings to allow mesofaunal entry. Approximately 9 g dry wt. of cereal rye (*Secale cereal*) were added to each litter bag. All litter was harvested as living shoots and stems from a nearby non-organically managed field in the springs of 2013 and 2014. The litter was oven dried at 60°C until constant mass was maintained, and then cut into 3-5 cm pieces and homogenized. Litter bags were placed on the soil surface, ensuring uniform contact with the soil by removing both living and dead plant biomass where necessary. Stainless steel nails were used at each corner of the bag to secure it to the soil surface. Six litter bags were randomly tacked to the soil in each plot such that there was at least one meter between litter bags and the bags were in-line with the crop rows. In addition to the bags placed in the field, we also constructed 10 “handling bags” to 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 of decomposition. During management activities, litterbags remained in the field because bags were aligned with the crop rows and thus undisturbed by field operations.

In each plot, we collected two litter bags at each sampling time, which were immediately placed in sealed plastic bags, and stored in a cooler with ice. Once in the laboratory, litter bags were weighed to determine the percent moisture. Litter bags were initially dried through the arthropod extraction process (described below) and then placed in a 60°C oven until constant mass was maintained. 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 litter bag we followed a soil correction equation first proposed by Blair (1998) which accounts for differences in organic matter content and contaminating soil (Harmon et al. 1999):

$$FLi=(SaAFDM-SlAFDM)¸(LiAFDM-SlAFDM)$$

where FLi is the proportion of litterbag sample mass that is actually litter; SaAFDM is the % AFDM of the entire litterbag sample; SlAFDM is the % AFDM of the surface soil at the site; and LiAFDM is the % AFDM of the initial litter.

Soil fauna collection, extraction, and identification. To assess the soil mesofaunal community, we collected *in situ* soil fauna inhabiting the bulk soil and organic matter layer with soil cores and litter bags (previously described), respectively. Soil fauna samples were collected three times during the growing season; these dates correspond with litter bag collection dates. Soil cores measuring 5 cm width x 17 cm depth were collected from directly below each litter bag following litter bag removal. In each plot, we collected two litter bags and soil cores which were immediately placed in separate sealed plastic bags, and stored in a cooler with ice. In the laboratory, all samples were stored at 4°C prior to fauna extraction using collapsible Berlese funnels (Bioquip Rancho Dominquez, CA). A 40 g sub-sample of soil was

weighed and dried at 60°C for 48 hours to determine percent soil moisture (Michigan State 2013). Fauna extractions were conducted for 48 hours during which extraction temperatures slowly increased from room temperature (22°C) to a maximum of 50°C. This method extracts all active fauna by slowly desiccating the soil/litter encouraging live organisms to crawl into a collection vial. Organisms were stored in 90% ethyl alcohol at room temperature for later identification and isotopic analyses. All soil organisms were identified using distinguishable morphological characteristics and then organized into trophic species. Total arthropod abundances and richness per bag will be documented. Arthropod densities will be reported as biomass per gram of litter or volume of soil.

Ion exchange resins. Buried anion exchange resins were used to capture turnover rates of plant available N-NO_3^- in the soil. Ion exchange resins are strips of an organic polymer that can adsorb ions from soil solutions. We used 2.5 cm x 10 cm strips cut from sheets of anion-absorbing resins. These strips were vertically buried 15 cm deep (spanning 5-15 cm below the soil surface). To account for the variation in inorganic nitrogen across small spatial scales (Nyiraneza et al. 2011), three ion exchange resin strips were buried in each plot. Strips were randomly located within the plot and were deployed approximately two weeks prior to each litter bag collection time on the following dates: 2013 – June 5, July 12, September 26; 2014 – May 30, June 13, July 23. There were a total of three sampling periods with sampling periods ranging from 9 to 27 days.

At each removal date, all three strips from each plot were rinsed with distilled water to remove any adhering soil. The clean strips were then transferred to a 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 the application of fertilizers in 2013.

Crop productivity. We measured plant height, crop leaf chlorophyll content, and grain yields. Plant height was deemed the distance from the soil surface to the highest point on the plant in its natural resting state. Crop leaf chlorophyll content was measured on 10 randomly selected plants in each plot using a handheld chlorophyll meter (SPAD 502 Plus; Konica Minolta, Spectrum Technologies, Inc., Aurora, IL). These measurements can be used as an indirect assessment of plant N status (Alcántar et al. 2002). Similar to the protocol described by Piekkielek and Fox (1992), for each plant we took five readings on the newest fully mature leaf between the leaf margin and central vein. At the end of the growing season, all harvestable grains from the four experimental rows in each plot were harvested with a 2-row combine. Grain yields are reported as kg / ha at the standardized moisture for each crop (e.g. 15.5% for corn, 13.0% for soybean).

$\delta^{15}N$ and $\delta^{13}C$ analyses. To determine the effect of PSTs on soil arthropod trophic position and/or resource preferences, we isotopically analyzed the most common arthropods collected from the litter bags and soil samples. All samples were stored in 90% ethyl alcohol at room temperature for about 2 years prior to analysis. This storage method was found to have little effect on the ^{15}N signatures of soil arthropods (Fábián 1998). Soil arthropods were separated under a dissecting microscope and then entire specimen were added to tins. To meet a minimum of 10

µg of N per sample, tins with larger taxa (i.e. D. Japygidae, D. Campodeidae, C. Lithobiomorpha) included less than ten individuals and tins with smaller taxa (i.e. A. Oribatida, A. Mesostigmata, C. Onchiuridae, and C. Entomobryidae) included ten to 70 individuals. In most cases samples included individuals from different soil cores and litter bags. All samples were then dried for 48 hours at 60°C and stored in a desiccator until submitted to the UNH Isotope Lab. This lab uses a coupled system consisting of an elemental analyzer (Vario PYRO cube, Elementar, Hanau, Germany) and a gas isotope mass spectrometer (VisION, Isoprime, Stockport, United Kingdom). Isotope natural abundance is expressed using the delta (δ) notation with, for example, $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$. R_{sample} and $R_{standard}$ refer to the $^{15}N/^{14}N$ ratio in samples and standard, respectively. Delta values are in units per mil ($^{o}/_{oo}$). Only samples with >10 µg of N were included in our results. All samples C. Onchiuridae contained < 10 µg of N and were therefore excluded from all analyses.

Statistical analysis. For most of our analyses, we used linear mixed-effects models using the statistical software R (version 3.31) with seed treatment (PST or Control) and sampling time as factors. To assess the relationships between PST, faunal community composition, and sampling time, we conducted PerMANOVA's using the Vegan package in the statistical software R and created non-metric multidimensional scaling (NMS) ordinations using PCORD (version 6, MjM Software Design, Gleneden Beach, OR). NMS analyses were conducted using the Sorensen (Bray-Curtis) distance measure and an instability criterion of 0.0005 (McCune et al. 2002).

References

Alcántar, G., M. Sandoval, J. Z. Castellanos, F. Méndez, P. Sánchez, and M. Nieves Rodríguez. 2002. Diagnostic methods to evaluate nutrient status of garlic, onion, and broccoli. Communications in soil science and plant analysis 33:2585-2598.

Braker, W. L. 1981. Soil survey of Centre County, Pennsylvania. USDA-SCS, Washington, D.C.

Doane, T. A., and W. R. Horwáth. 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical Letters 36:2713-2722.

Fábián, M. 1998. The effects of different methods of preservation on the 15N concentration in Folsomia candida (Collembola). Applied Soil Ecology 9:101-104.

Harmon, M. E., K. J. Nadelhoffer, and J. M. Blair. 1999. Measuring decomposition, nutrient turnover, and stores in plant litter. Standard soil methods for long-term ecological research.Oxford University Press, New York:202-240.

McCune, B., J. B. Grace, and D. L. Urban. 2002. Analysis of ecological communities. MjM software design Gleneden Beach, Oregon.

Michigan State, U. 2013. Gravimetric Soil Moisture.

Nyiraneza, J., M. C. Nolin, N. Ziadi, and A. N. Cambouris. 2011. Short-range variability of nitrate and phosphate desorbed from anionic exchange membranes. Soil Science Society of America Journal 75:2242-2250.

Piekkielek, W., and R. Fox. 1992. Use of a chlorophyll meter to predict sidedress nitrogen. Agronomy Journal 84:59-65.

Wider, R. K., and G. E. Lang. 1982. A Critique of the Analytical Methods Used in Examining Decomposition Data Obtained From Litter Bags. Ecology 63:1636-1642.

Research results and discussion:

Objective 1. Determine to what extent pesticide seed treatments alter the composition of the soil faunal community.

Our data suggest PSTs do not affect soil faunal composition at the community-level, but this management practice can negatively affect detritivore biomass. From our experiment, there was little evidence of community-level differences in soil faunal composition between the control and PST in both 2013 and 2014 (PerMANOVA: 2013 ($p = 0.6646$), 2014 ($p = 0.9212$)). To further probe the community dataset, we organized the taxa into trophic groups and tested for differences in faunal biomass between PST and control (Fig 1.). The amount of detritivore biomass found in the surface residues (litter bags) when both years are combined was significantly reduced with PST (PST: $F_{1,8} = 14.26$, $p = 0.00541$). Interestingly, PST did not significantly affect soil predator biomass or herbivore biomass, which includes pest populations.

We also used Indicator Species Analysis to determine if specific taxa within the soil faunal community strongly associated with either PST or control than would be expected by chance (Fig. 2). Two taxa, Collembola Isotomidae (fungivore) and Symphylan, *Scutigerella immaculate* (Newport), (non-targeted pest), were identified as indicator species for PST. We also identified three taxa that more strongly associated with the control: Carabidae *Bembidion quadrimaculatum* (generalist predator), Coleoptera Cucujoididae larvae (variable trophic groups), and Diplura Japygidae (generalist predator). Collectively, these data suggest non-targeted taxa differentially respond to PST.

Taken together, our results provide a little support for the hypothesis that PSTs alter soil faunal community composition; specifically, there were observable decreases in total detritivore biomass with the use of PSTs. This unintentional reduction in the detritivore community may have consequences on the agroecosystem services these organisms provide, especially nutrient cycling. Moreover, we found evidence to further support PSTs effect on non-targeted soil faunal populations across multiple trophic levels. With the strong association between a non-targeted pest (Symphyla) and the use of PST being particularly surprising. Further studies should investigate the generality of this result because symphyla can be very destructive pests, causing injury to sprouting field crop seeds and roots (Gesell 1983). Furthermore, these pests can be difficult to control with chemical and cultural practices (Natwick 2009), making the association between PSTs and symphlan a cause for concern.

Given the relatively small changes in the soil faunal community in our study, we began to wonder if the previous use of PSTs at our field site changed had legacy effects on the composition of the soil faunal community. In other words, were our control plots acting more as recolonization zones for soil fauna rather than zones where the soil faunal community was being preserved? Because PSTs are so widely used in commodity cropping systems, we are continuing to explore this possibility through additional laboratory experiments.

Objective 2. Determine whether pesticide treated seeds alter the trophic position and/or resource preferences of commonly occurring soil faunal species.

When initially selecting the soil fauna for isotopic analysis, we purposefully selected taxa that were not only common in our samples, but also differed in their trophic positions based on the literature. Based on our data, the diversity of nitrogen isotopic signatures among the taxa (across all sampling times and treatments) indicates the taxa we used differ in their trophic positions (Figure 3). The higher values in $\delta^{15}N$ for Mesostigmata and Japygidae are indicative of their role as higher order predators, whereas the lower $\delta^{15}N$ value for Entomobryidae indicates it

functions more as a microbivore. Resource utilization can be inferred from the $\delta^{13}C$ values with Oribatida consuming more C3 derived plant materials such as soybean and all other taxa either directly consuming less C3 derived inputs or predating on organisms that consume less of these materials. Because of the difficulty isolating soil fauna and accumulating enough biomass for isotopic analyses, these data add valuable insight into the trophic positions and resource utilization of these organisms.

To determine if the soil faunal communities restructure with measurable shifts in their trophic positions and resource utilization following the addition of PST, we compared $\delta^{15}N$ and $\delta^{13}C$ for each taxon, or group of taxa (Figures 4 & 5). For both analyses, samples of Lithobiomorpha, Campodeidae, and Japygidae were grouped because of their similar isotopic positions (Figure 3) and to account for the low replication numbers of Campodeidae (4 reps) and Japygidae (2 reps). Based on differences in $\delta^{15}N$, we found marginal decreases in $\delta^{15}N$ with the use of PSTs for the Lithobiomorpha, Campodeidae, and Japygidae group, while all other taxa were unaffected. This loss in $\delta^{15}N$ suggests that with the use of PST these organisms predate on soil fauna with lower $\delta^{15}N$, i.e. the prey occupy lower trophic positions (Figure 4). We did not observe any differences in resource utilization ($\delta^{13}C$) for each taxon included in our analysis (Figure 5).

Objective 3. Determine the effects of pesticide seed treatments on agriculturally important ecosystem services including decomposition and nitrogen cycling.

Our data suggests both surface residue decomposition and plant available $N\text{-}NO_3^-$ are unaffected by PSTs. There were no differences in aboveground decomposition rates between PST and Control both years (2013: $F_{1,8} = 0.367$, $p = 0.561$; 2014: $F_{1,8} = 0.124$, $p = 0.734$). We also did not detect any differences between plant available $N\text{-}NO_3^-$ between PST and the control both years (2013: PST $F_{1,8} = 1.543$, $p = 0.249$, PST*Time $F_{2,16} = 0.359$, $p = 0.704$; 2014: PST $F_{1,7} = 0.668$, $p = 0.441$, PST*Time $F_{2,15} = 0.895$, $p = 0.429$). Finally, we also did not detect a yield benefit when either corn or soybean was pretreated with pesticides (Corn: $F_{1,8} = 4.0901$, $p = 0.07777$; Soybean: $F_{1,8} = 0.3282$, $p = 0.5825$).

These results, particularly the residue decomposition and plant available nitrogen results, are a little surprising considering we observed changes in detritivore biomass in these systems (Obj. 1 Results). Furthermore, for both corn and soybean crops we did not see a cost benefit of using the pesticide seed treatment in our study. Overall these data did not support our hypothesis that soil community-level performance would decrease because of the PST induced changes to the soil food web.

Figure-1

Figure-2

Figure-3

Figure-4

Figure-5

References

Gesell, S. C., Dennis 1983. Garden Symphylan as a Pest of Field Crops. Insect advice from extension. Department of Entomology, The Pennsylvania State University, http://ento.psu.edu/extension/factsheets/garden-symphylan-as-a-pest-of-field-crops.

Natwick, E. T. 2009. UC IPM Pest Management Guidelines: Cole Crops. UC ANR Publication 3442. UC IPM, University of California Agriculture & Natural Resources, http://ipm.ucanr.edu/PMG/r108500111.html.

Research conclusions:

We foresee this research as contributing to the ongoing debate regarding the environmental consequences of using PSTs with neonicotinoids in our agroecosystems. Our results reveal new unforeseen consequences of using insecticide-fungicide seed treatments and raise further questions about the effectiveness of this management practice in commodity cropping systems. We think farmers and regulatory agencies will be particularly interested in how PSTs with neonicotinoids reduced the detritivore biomass and increased the likelihood of harboring a non-targeted pest. To date we have shared our results with the scientific community, but as we move forward with our publications we plan to share these results with farmers and extension personnel in the mid-Atlantic and New England. It is our hope that these results can help us figure out less deleterious ways of managing pests and ultimately improve the overall sustainability of our agroecosystems

Participation Summary

Education & Outreach Activities and Participation Summary

PARTICIPATION SUMMARY:

Education/outreach description:

To date, we have primarily shared our results with the scientific community through presentations at national and university-level conferences. This includes the Ecological Society of America Conference in 2014 and 2015, the joint Entomological Society of America / Tri-Societies Conference in 2015, and the University of New Hampshire 3MT Competition in 2016. Atwood was awarded First Place and People's Choice Award for her 3MT speech which you can view online. This research was also presented to two undergraduate-level classes, the Soil Ecology and Agroecology courses, at the University of New Hampshire in 2014, 2015, and 2016. Two manuscripts with the data collected from this project are currently in preparation. We also plan to present our data to farmers and agricultural support professionals in the mid-Atlantic and New England this coming spring.

Project Outcomes

Project outcomes:

We did not conduct an economic analysis.

Farmer Adoption

With its widespread use, it is important for farmers and regulatory agencies to better understand the unintended impacts of PSTs on the soil food web and its

ability to function. Thus, we think the agricultural community will be particularly interested in our results suggesting that PSTs with neonicotinoids can reduce soil detritivore biomass and increase the likelihood of harboring a non-targeted pest. These data contribute to public debate regarding the consequences of using PSTs with neonicotinoids in our agroecosystems. Ultimately, we hope that these results can help us identify better ways to manage early season pests and ultimately improve the overall sustainability of our agroecosystems.

Assessment of Project Approach and Areas of Further Study:

Areas needing additional study

1. How do PSTs with neonicotinoids affect soil food webs that have long-term PST exposure compared to those that have no previous exposure?
2. How long does it take for soil food web diversity to recover from exposure to PSTs with neonicotinoids?
3. How are the trophic positions of soil predators not included in our analyses affected by PSTs with neonicotinoids?
4. What affects do PSTs have on rates of belowground decomposition?