Earthworm Cover Crop Habitat Preference

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by

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Abstract

Forage radish (*Raphanus sativus var. Longipinnatus*), a member of the brassicacea family, is becoming increasingly popular in conservation tillage practices. Brassica cover crops have been observed to have allelopathic effects on soil organisms due to the glucosinolates in their tissues. A pilot study examining the effects of different tillage methods on earthworm populations found a significant lack of earthworms in forage radish roll-down tillage when sampling with mustard solution compared to roll-down tillage without forage radish. This study seeks to explain these results by exploring two alternative hypotheses. The first hypothesis is that the lack of earthworms observed in the pilot study was due to negative response of earthworms to brassica cover crops. The second hypothesis is that the results were a sampling artifact because of an interaction between the brassica-derived, mustard sampling solution and the brassica cover crops.

Field trials and habitat choice chambers were used to assess earthworm preference for different cover crops, including forage radish. Fall and spring field trial sampling was conducted with mustard and onion solutions to identify any interaction between brassica cover crops and the sampling method. Results of field trials and choice chamber trials suggested no significant earthworm avoidance of forage radish. Results of the field trials also suggested that cover crop habitats did not significantly influence the effectiveness of mustard sampling solution. This study can assist farmers deciding whether to use forage radish in their agricultural systems.

Introduction

Conventional agricultural systems often practice mechanical tillage to manage weeds and loosen soils for planting. However, mechanical tillage can lead to undesirable environmental changes including erosion and soil compaction long-term (Chan 2001). Conservation tillage is a practice that minimizes the frequency or intensity of tillage to improve soil health, reduce runoff, and limit erosion (UC Sustainable Agriculture Research and Education Program 2017). A major tool in conservation tillage is cover crops, which can be used with roller-crimping as an alternative to mechanical tillage. Cover crops managed through roller-crimping are pushed down and crimped to create a weed suppressing layer of dead organic matter that main crops are planted directly into (Rodale 2020). Cover crops also help mitigate soil degradation in agroecosystems by providing soil cover when main crops are not planted.

Farmers can tailor their cover crop selections to the needs of the farm. For example, legume cover crops support bacteria that specialize in fixing atmospheric nitrogen into soil for plants to use while brassica cover crops help manage nematodes, weeds, and disease (Clark 2012). Cover crops also slow erosion and enhance nutrient and moisture availability (Clark 2012). Cover crops that loosen soils and smother weeds are well suited for use in conservation tillage.

The conservation tillage method of planting forage radish (*Raphanus sativus* var. *Longipinnatus),* a member of the brassica family, is becoming increasingly popular for its weed management and soil loosening properties (Weil et al. 2009). When planted in early fall, forage radish develops large above-ground biomass and deep taproots before dying overwinter. The large above ground biomass produces a virtually weed free seedbed in early spring by limiting weed emergence in the fall and winter (Weil et al. 2009). Then, seeds or transplants can be placed directly in the holes left behind by the decomposed taproots. Forage radish also assists in pest control, a primary use of many other brassica family cover crops (Clark 2012).

Brassica cover crops are an effective form of pest control against nematodes due to the glucosinolates in their tissues. Glucosinolates act as a vermifuge, a worm expelling agent, and may reduce the need for synthetic pesticides (Brown & Morra, 1997). However, their effect on non-target beneficial soil organisms, including earthworms, is not well known. Literature regarding the effects of brassica cover crops on earthworms is contradictory (Valckx et al. 2011, Roarty et al. 2017, Brown & Morra 1997). A field study found that mustard cover crop, *Brassica juncea,* supported lower earthworm abundance than other, non-brassica cover crops (Roarty et al. 2017). However, earthworms did not avoid brassica crops compared to other non-brassica crops in laboratory food and habitat preference tests (Valckx et al. 2011).

Cover cropping, tillage, and other agricultural management practices directly influence earthworm presence and function in agroecosystems (Brown & Morra 1997, Chan 2001). Earthworms are important contributors to healthy agroecosystems because they help make soil less acidic while increasing nutrient availability for plants. As engineers that loosen the soil to allow for greater root penetration, earthworms work together with cover crops to create improved soil conditions for main crops. *Lumbricus terrestis* is the earthworm species most commonly found in agroecosystems (Kim et al. 2015).

A pilot study, conducted in fall 2018, examining the effects of different tillage methods on earthworm populations found zero earthworms in forage radish roll-down tillage which was significantly less than the earthworm abundance found in roll-down tillage without forage radish (Macpherson et al. 2018). Because forage radish is a member of the brassica family, this suggest allelopathic effects of the forage radish on earthworms. Allelopathy is any direct or indirect effect of plants on other organisms through production of chemical compounds that escape into the environment (Halbrendt 1996). This is consistent with lower earthworm abundance under brassica cover crops observed in the field study (Roarty et al. 2017). However, a complete lack of earthworms is more drastic than any observations in existing literature. Furthermore, direct comparisons cannot be made because earthworm response specifically to forage radish has not been studied previously.

Additionally, the extraction method or mustard solution used in the pilot study may have been a confounding variable. Mustard solution was selected because it is a commonly used, eco-friendly method to extract earthworms from the soil (Macpherson et al. 2018, Singh et al. 2016). However, mustard is in the brassica family, so the presence of similar glucosinolates in brassica family cover crops, such as forage radish, may influence the effectiveness of mustard sampling. The interaction between mustard sampling solution and brassica cover crop habitats has not been explored in the existing literature because many studies examining the interactions between cover crops and earthworms employed hand sampling (Roarty et al. 2017, Valckx et al. 2011). These gaps in the literature and the unique results of the pilot study informed the development of this study.

This study aims to determine whether cover crop species, specifically forage radish, affect earthworm abundance. Earthworm habitat preference was tested with the cover crops field pea (*Pisum sativum*), oats (*Avena sativa*), mustard (*Brassica juncea*), and forage radish (*Raphanus sativus var. Longipinnatus*). Winter pea was selected because of observed earthworm preference for field pea habitat (Roarty et al. 2017). Mustard was selected because it is in the brassica family with forage radish and because earthworm preference for mustard has been tested in previous studies (Brown & Morra 1997, Roarty et al. 2017, Valckx et al. 2011).

Based on previous earthworm habitat preference studies (Brown & Morra 1997, Roarty et al. 2017) and earthworm behavior (Mathieu et al. 2010), I developed two alternative hypotheses to explain the lack of earthworms observed in the forage radish roll-down tillage fields in the pilot study (Macpherson et al. 2018). I hypothesized that (i) allelopathic effects of brassica cover crops negatively impact earthworms. From this, I predicted that there would be a lower abundance of earthworms in mustard and forage radish plots than field pea and oat plots. Alternatively, I hypothesized that (ii) mustard solution is less effective as a vermifuge in areas previously planted in brassica cover crops because the glucosinolates in mustard solution are already present in this environment so additional glucosinolates would be less noticeable or earthworms would be less sensitive to them. From this, I predicted that earthworms sampled from brassica family cover crops would be less responsive to extraction by mustard solution than earthworms sampled from non-brassica cover crops.

Methods

Habitat Preference: Greenhouse Trials

I designed the greenhouse trials to test earthworm preference for different crops in a controlled setting. Earthworms are more likely to follow existing burrows than create new ones and distance can be a limiting factor for earthworms moving out of unfavorable habitats (Mathieu et al. 2010). The greenhouse trials removed the variable of existing burrows and decreased the distance earthworms needed to travel to move across habitats present in the outdoor trials. The earthworm species was also controlled as *Lumbricus terrestris*. I tested earthworm habitat preference in choice chambers with two rounds of four blocks.

I constructed the choice chambers in a plus sign formation, modelled after those used in an earthworm soil preference study (Kim et al. 2015), with one plot of each species (field pea, oat, mustard, and forage radish) surrounding a center bare soil plot (Fig. 1 and Fig. 2). The configuration of crop species around the bare center plot varied across blocks. I sowed the crops into grow-bags (21.6cm x 25.4cm x 35.6cm) filled with commercially purchased topsoil (Fig. 2). To allow earthworms to move between plots, I cut openings (30cm x 18-21cm) between the bags and sealed them with duct tape (Fig. 3). The duct tape also connected the bags. Drainage holes in the bottom of the grow bags were covered with mesh to prevent earthworms from escaping while still allowing for drainage.

I planted crops planted in two rounds, once on 16 September 2019 and again, two weeks later, on 1 October 2019. Testing occurred in November 2019 with the same two-week separation as planting. For the first round of testing, I placed the earthworms in the choice chambers on 7 November and collected on 17 November. For the second round, I placed the earthworms in the choice chambers on 14 November and collected on 24 November.

At the beginning of each trial, I placed eight commercially purchased (EvergreenNightcrawlers), large *Lumbricus terrestris* earthworms into the central bare soil container of each block. After ten days, I destructively hand-sampled the choice chambers to determine the locations of the earthworms (Fig. 3). The ten-day time frame was selected based on other earthworm choice chamber studies and earthworm dispersal rates (Valckx et al. 2011, Kim et al. 2010, Mathieu et al. 2010). The openings between chambers were blocked prior to sampling to limit earthworm movement between chambers in response to disturbance. One block in trial one was lost to aphid damage. Additionally, I recovered less than eight earthworms from two blocks in each trial.

Habitat Preference: Field Trials

Study Site

I designed the field trials to test the effect of different crops on earthworm abundance and whether the effectiveness of different sampling solutions varied with crop type. I conducted the field trials in raised garden beds in the Kauffman garden at Dickinson College in Carlisle, Pennsylvania (40.204778, -77.199489*)*. The climate conditions of the area are typically warm, humid summers and cold winters. Summer 2019 was an uncharacteristically dry season. The beds were fallow in the spring of 2019 and previously grew vegetables, mainly tomatoes and herbs. The soil was periodically supplemented with compost.

Four beds were planted in a randomized block design (2.6m x 1m) with four plots (1.3m x 0.5m) in each bed. I sowed field pea, oats, mustard, and forage radish into the raised garden beds in plots in late August 2019 (Fig. 4). Seeding rates were consistent with recommendations from a regional extension source: field peas 4oz/100ft^2, oats 4-6oz/100ft^2, mustard 1oz/100ft^2, forage radish 1oz/100ft^2 (Clark 2012). I mistakenly planted the field pea plots as a pea-buckwheat mixture. I pulled the buckwheat plants out after four weeks and spot supplemented bare sections with additional pea seeds.

I sampled earthworms on 9 November 2019 and 14 March 2020. I sampled earthworms twice in each plot in 25cm x 25cm quadrats, once with mustard solution and once with onion solution (Fig. 4 and 5). Quadrats sampled on each sampling date were 25cm apart and sampling quadrats did not overlap between sampling dates. Mustard solution was created by mixing 40g mustard powder in 4L of water (Hale 2007). Onion solution was created by mixing 90g onion powder in 4L of water (Singh et al. 2016). I poured the solutions over the sampling area at a rate that allowed the solution to seep into the ground with limited runoff outside of the sampling quadrat. Every earthworm that surfaced in the quadrat within five minutes of pouring the solution was collected. Then, earthworms were placed in 10% formalin for 24 hours and then transferred to 70% isopropanol solution for preservation and later identification. I measured the biomass of earthworms collected from each plot after preservation. I intended to identify the earthworms to the species level, but COVID-19 disrupted these plans.

Data Analysis

Data analysis was conducted in the R statistical language (R Core Team 2017). Data for the choice chambers, fall field trial, and spring field trial were analyzed separately with analysis of variance (ANOVA). I checked ANOVA assumptions of normality and homogeneity of variance using residual plots. I visualized the data in separate bar graphs depicting earthworm abundance in each crop species and by sampling method.

For the choice chamber data, the proportion of earthworms in each crop species was calculated from the total number of earthworms recovered from each chamber. Then, I analyzed the data with ANOVA using the following formula:

Proportion of Earthworms ~ Round + Crop Species

A significant effect of crop species would indicate that there was a significant difference in the proportion of earthworms recovered from different crop species. Hypothesis one would be supported by lower abundance of earthworms in mustard and forage radish plots than field pea and oat plots.

The field trials were analyzed with ANOVA using the following formula:

Earthworm abundance ~ Block + Sampling Method * Crop Species

A significant effect of crop species would indicate a significant difference in the abundance of earthworms collected among crop species. Hypothesis one would be supported by lower abundance of earthworms in mustard and forage radish than field pea and oat. A significant interaction between sampling method and crop species would suggest that the effect of sampling method differs among crop species. Hypothesis two would be supported if there was a significant difference in earthworm abundance between sampling methods in the brassica cover crops only. This would indicate lower effectiveness of mustard sampling in brassica cover crop habitats compared to field pea and oat.

Figure 1. Image of choice chamber with openings displayed.

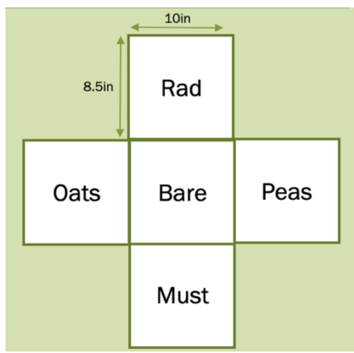

Figure 2. Greenhouse choice chamber Rad=forage radish, Must=mustard, peas=field peas.

Figure 3. Destructive sampling of choice chambers.

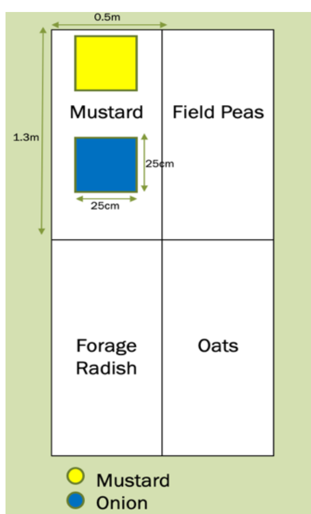

Figure 4. Field trial example bed with sampling quadrats.

Figure 5. Field trial spring sampling.

Results

In the greenhouse choice chambers, no significant difference in average proportion of earthworms was observed among any cover crop species (Main effect of crop, $F_{4, 29}$=1.75, p=0.17) (Fig. 6). There was a trend toward lower proportion of earthworms in forage radish than other cover crops.

Results from the fall field trials indicated no significant difference in earthworm abundance among crop species (Main effect of crop, $F_{3, 21}$ =1.43 p=0.26). However, there was a trend toward greater earthworm abundance in brassica cover crops in which forage radish supported the highest abundance of earthworms and field pea supported the lowest abundance of earthworms (Fig. 7). Additionally, there was no significant difference in average mass of earthworms among crop species (Main effect of crop, $F_{3, 21}$=1.02, p=0.41). However, there was a trend toward greater earthworm mass in forage radish (Fig. 8).

In the fall field trials, a marginally significant difference in abundance of earthworms extracted by each sampling method indicates an overall effect of sampling method (Effect of sampling method, $F_{1, 21}$=3.20, p=0.09) with mustard sampling extracting 48% more earthworms than onion sampling (Fig. 9). In the brassica cover crops, mustard and forage radish, there was a trend toward greater extraction of earthworms by mustard solution than by onion solution. This trend was less distinct in oat and reversed in field pea plots. However, the interaction between sampling method and crop species was not significant (Sampling method x crop species interaction, $F_{3, 21}$=2.14, p=0.13) (Fig. 9).

Results from the spring field trials indicated no significant difference between average abundance of earthworms extracted from each crop species (Main effect of crop, $F_{3, 21}$=1.60, p=0.22) despite a trend toward lower abundance of earthworms in oat plots (Fig.

10). There was a trend toward greater earthworm mass in mustard, but there was no significant difference between average mass of earthworms extracted among crop species (Main effect of crop, $F_{3, 18}$=1.40, p=0.27). (Fig. 11).

In the spring field trials, there was no significant interaction between sampling method and crop species (Sampling method x crop species interaction, $F_{3, 21}$=1.18, p=0.34) (Fig. 12). However, earthworm abundance was significantly different between each sampling method indicating an overall effect of sampling method (Effect of Sampling method, $F_{1, 21}$=8.3120, p=0.01) with mustard sampling extracting 46% more earthworms than onion sampling (Fig. 12).

In the spring field trials, a review of the ANOVA diagnostics revealed an outlier in earthworm abundance in the mustard cover crop. Repeating the analysis without the outlier still indicated no significant different between average abundance of earthworms extracted from each crop species (Main effect of crop, $F_{3, 20}$=1.61, p=0.22) (Fig. 13). There continued to be no significant difference in abundance of earthworms extracted by each sampling method between crop species (Effect of sampling method and crop species interaction, $F_{3, 20}$=1.25, p=0.32) (Fig. 14). There was still a significant difference in abundance of earthworms extracted by each sampling method among crop species with mustard sampling extracting a greater abundance of earthworms (Effect of sampling method, $F_{1, 20}$=8.98, p=0.01) (Fig. 14).

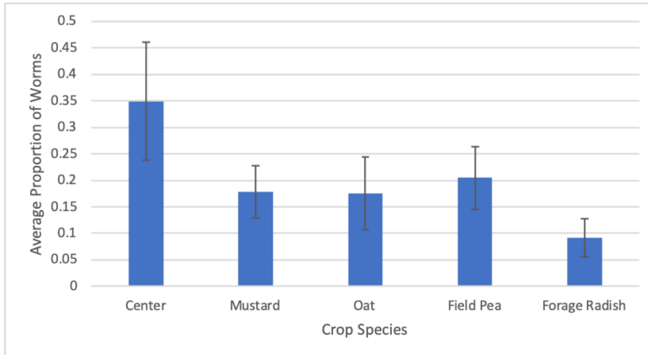

Figure 6. Average proportion of earthworms (+/- one standard error) in each plot of the greenhouse choice chambers (n = 7 blocks).

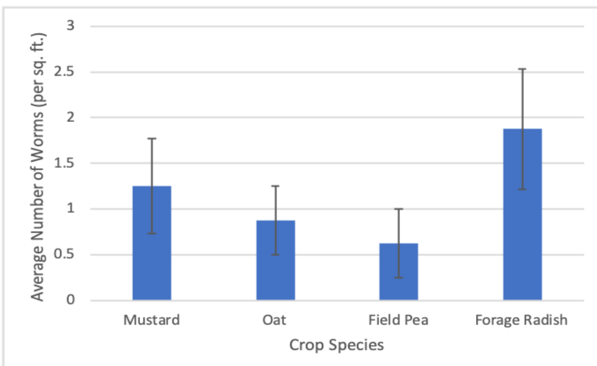

Figure 7. Average density of earthworms (+/- one standard error) in each plot in the fall field trials (n=4 blocks).

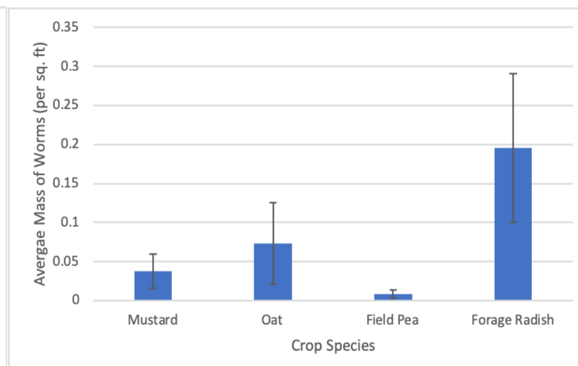

Figure 8. Average mass of earthworms (+/- one standard error) in each plot in the fall field trials (n=4 blocks).

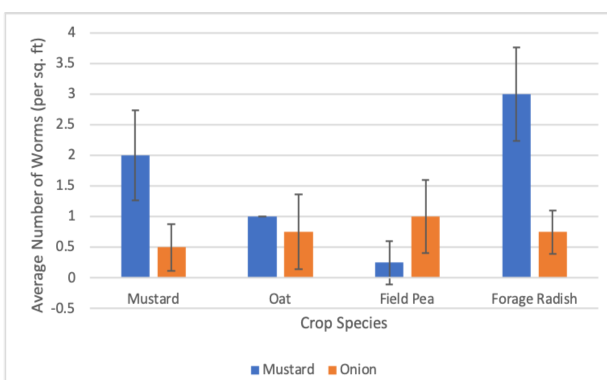

Figure 9. Average density of earthworms (+/- one standard error) extracted by mustard and onion sampling in each plot in the fall field trials (n=4 blocks).

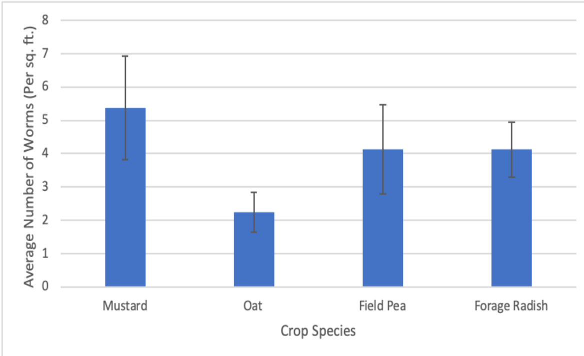

Figure 10. Average density of earthworms (+/- one standard error) in each plot in the spring field trials (n=4 blocks).

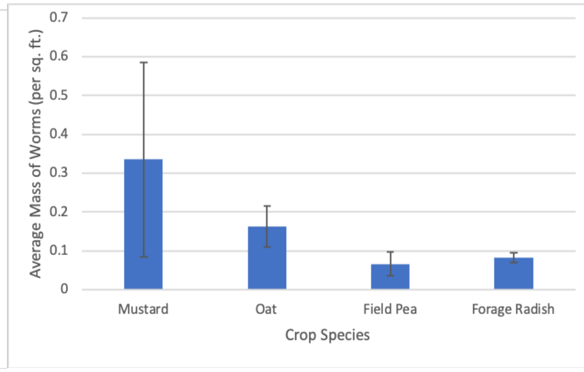

Figure 11. Average mass of earthworms (+/- one standard error) in each plot in the spring field trials (n=4 blocks).

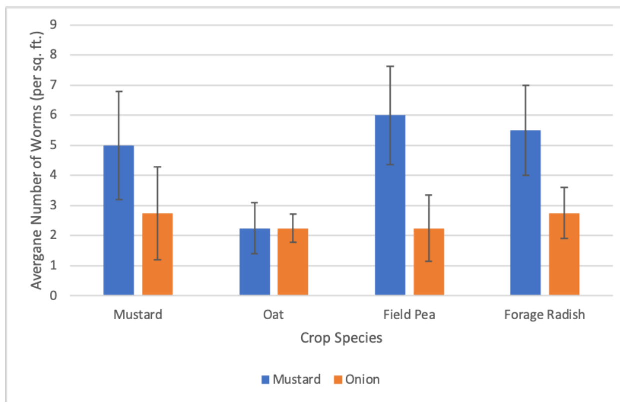

Figure 12. Average density of earthworms (+/- one standard error) extracted by mustard and onion sampling in each plot in the spring field trials (n=4 blocks).

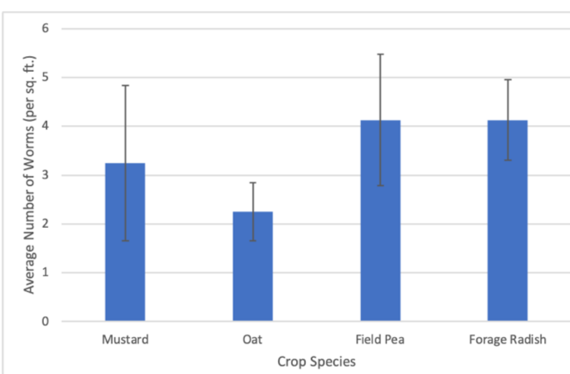

Figure 13. Average density of earthworms (+/- one standard error) in each plot in the spring field trials with outlier removed (n=4).

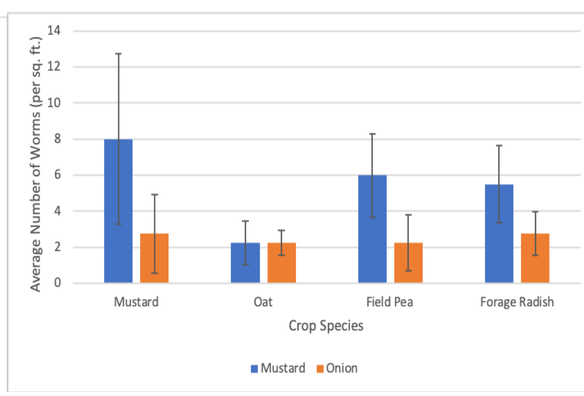

Figure 14. Average density of earthworms (+/- one standard error) extracted by mustard and onion sampling in each plot in the spring field trials with outlier removed. (n=4).

Discussion

In this study, I found that earthworms did not respond negatively to brassica cover crops and did not exhibit a significant preference for any of the four cover crops (field pes, oat, mustard, and forage radish) I tested. I also found no significant interaction between cover crops and the effectiveness of different sampling methods. Mustard sampling was more effective than onion sampling across all cover crops.

The field trial data was consistent with my prediction that earthworms sampled from brassica family cover crops would be less responsive to extraction by mustard solution than by onion solution. The lack of significant interaction between sampling method and crop species suggests that crop species did not significantly influence the effectiveness of each sampling method. The greater effectiveness of mustard sampling was evident by the significantly greater earthworm abundance extracted by mustard solution in the field trials. This is supported by studies comparing the effectiveness of different sampling methods, which recognize allyl isothiocyanate, a glucosinolate derivative, as the active ingredient in mustard solution (Singh et al. 2016, Singh et al. 2018). Isothiocyanate is activated when the glucosinolates in brassica plants are hydrolyzed (Gimsing & Kirkegaard 2006).

Hydrolysis of glucosinolates influences their allelopathic effects and is favored by maximum plant cell disruption and the addition of water (Gimsing & Kirkegaard 2006). The allelopathic effects of glucosinolates are strongest within the first few hours after incorporation of plant material into the soil (Bangarwa et al. 2011). This suggests strong allelopathic effects of the powdered mustard and water solution in the five-minute sampling window for extracting earthworms in this study. The mustard sampling solution intensified the allelopathic effects of brassica cover crops in agroecosystems.

Agricultural management practices influence the allelopathic effects of glucosinolates. The allelopathic effects of brassica cover crops in agroecosystems can be increased through tillage and glucosinolates are detectable in the soil for a week after incorporation (Bangarwa et al. 2011, Gimsing & Kirkegaard 2006). Glucosinolate concentrations in soils do not accumulate over successive brassica plantings and any increased effects of glucosinolates overtime may be due to legacy effects of the cover crops (Crotty et al. 2016). The relationship between cover crop management and allelopathic effects is evident in this study and other studies on earthworm habitat preference.

In this study, earthworms did not exhibit a preference for a specific cover crop in the greenhouse choice chamber trials and both field trials. Though earthworm abundance was statistically similar in all of the trials, different trends in abundance were visible in each trail. In the greenhouse choice chamber trials, there was a trend toward a lower proportion of earthworms in the forage radish. In the field trials, there was a trend toward greater abundance of earthworms in the forage radish and mustard than in the field pea and oat cover crops. This trend was more pronounced in the fall than in the spring. Patterns in earthworm mass were statistically similar to earthworm abundance, reinforcing the lack of earthworm preference for a specific cover crop. The lack of earthworm preference for a specific cover crop was inconsistent with prediction that there would be a lower abundance of earthworms in the brassica cover crops. The trends in the data suggest that glucosinolates in brassica cover crops may not be responsible for earthworm habitat preference in this study.

Other earthworm habitat preference studies reflect the relationship between cover crop management and allelopathic effects. The results from the choice chambers were similar to another lab experiment that found that earthworms did not avoid brassica cover crops compared to non-brassica cover crops (Valckx et al. 2011). That study was used to support the claim that glucosinolates were not responsible for lower earthworm abundance under mustard cover crops in a larger field study (Roarty et al. 2017). However, cover crops in the larger field study were incorporated into the soil through tillage while the lab study used live cover crops and superficially administered, chopped cover crop residues (Roarty et al. 2017, Valckx et al. 2011). This suggests that there was greater hydrolysis, and therefore greater allelopathic effects, of glucosinolates in the larger field study than in the lab experiment, so glucosinolates may still be responsible for earthworm habitat preference in the larger field study (Gimsing & Kirkegaard 2006, Roarty et al. 2017, Valckx et al. 2011). The lack of tillage in this study and the pilot study accurately represented typical management of forage radish because forage radish can replace spring tillage (Macpherson et al. 2018. Weil et al. 2009). However, the allelopathic effects of other cover crops, specifically mustard, in this study, may have been limited by the lack of tillage. Other mechanisms for earthworm habitat preference may explain the results in this study, lab experiment, and the pilot study (Valckx et al. 2011, Macpherson et al. 2018).

The different timelines influence the relevance of short-term and long-tern effects of cover crops on earthworm populations. The larger field study was conducted over five years with three successive cover crop plantings before earthworms were sampled (Roarty et al 2017). This timeline captured the legacy effects of the cover crops and reduced the impact of previous land use history on earthworm habitat preference. In this study and the pilot study, only one succession of cover crops was planted in the field trials, so previous land use may have influence earthworm abundances (Macpherson et al. 2018).

The interaction between earthworm behavior and the spatial scales of these studies may also explain some of the differences in results (Baisley 2020, Macpherson et al. 2018, Roarty et al. 2017, Valckx et al. 2011). Earthworms live in spatially distinct clusters, with *L. terrestris* adults organizing in distinct patches ~15m in diameter (Valckx t al. 2008). The authors of earthworm behavior studies suggested that earthworms migrate about 1.1m to 14m annually (Nuutinen et al. 2006, Mathieu et al. 2010). The expansive research plots in the larger field study accommodated earthworms' organization patterns and migration abilities (Roarty et al. 2017, Mathieu et al. 2010, Nuutinen et al. 2006, Valckx et al. 2008). Sampling replicates in the pilot study were within the same row, and, therefore, were not independent of one another (Macpherson et al. 2018). The lack of independent replicates increases the possibility that the presence of earthworms in the sample areas was influenced by the organization habits of earthworms and did not accurately represent habitat preference. The migration abilities of earthworms suggest that the size of the field trial blocks in this study was conducive to earthworm movement between plots (Mathieu et al. 2010, Nuutinen et al. 2006). However, it is unclear how the confined choice chambers in the lab trials and the raised beds in the field trials influenced earthworm behavior.

Conclusion

Mustard sampling solution was more effective than onion sampling solution for extracting earthworms. Cover crop habitats did not significantly influence the effectiveness of mustard sampling. This suggests that mustard sampling can be continued to be used in research examining earthworm response to cover crop habitats without concern for biased extraction of earthworms.

Additionally, the results of this study suggest that the allelopathic effects of glucosinolates in brassica cover crops did not significantly influence earthworm habitat preference. The allelopathic effects of glucosinolates in forage radish cannot explain the lack of earthworms found in the forage radish cover crops in the pilot study (Macpherson et al. 2018). Other mechanisms, such as tillage practices and previous land use history, may have a greater impact on earthworm habitat preference than cover crops. Tillage may be especially influential because of the relationship between plant cell disruption, such as that caused by tillage, and glucosinolate activation. More research should be conducted to examine how no-till practices associated with forage radish influence its biofumigation potential. Forage radish can be continued to be used by farmers to control weeds and reduce tillage without concern for damaging earthworm populations.

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