Linking habitat complexity with predation of pests through molecular gut-content analyses

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Linking habitat complexity with predation of pests through molecular gut-content analyses

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Improving the diversity of farm systems or landscapes can lead to more effective biological control by providing refuge and alternative resources for colonising natural enemies. Within an experimental cabbage agroecosystem, we examined the effects of habitat management (i.e. herbicide use and cover crops) on pest populations and predator community structure, and report one of the first studies on the trophic links in this system using molecular gut-content analysis. In response to herbicide and cover crop management treatments designed to create different levels of habitat diversity, we quantified the abundance of two pests, \textit{Plutella xylostella} Linnaeus (Lepidoptera: Plutellidae) and \textit{Pieris rapae} Linnaeus (Lepidoptera: Pieridae), and predators. We designed species-specific primers to detect prey DNA in predators’ guts. \textit{Pieris rapae} were significantly more abundant in plots where cover crops were killed early in the season, and habitat management generated unique predator communities in response to weed management treatments. Thirty-five per cent of predators tested positive for prey DNA, and habitat management had interactive effects on predation of \textit{P. xylostella}. Combined we found that habitat management has variable effects on natural enemy–pest interactions.

**Keywords:** cover crop mulch; weed management; primer; DNA; \textit{Pieris rapae}; \textit{Plutella xylostella}; insect; arthropod; predator

**Introduction**

Biodiversity–ecosystem functioning studies suggest that promoting diverse species assemblages leads to increased ecosystem services (Cardinale et al., 2006). In biological control, this can translate into a species-rich natural enemy community providing better prey suppression than a species-poor community (Snyder, Snyder, Finke, & Straub, 2006). Another way to consider the relationship between diversity and biological control is through the diversification of the habitat. Habitat management is a form of agricultural landscape and field diversification that has been proposed as a viable mechanism to build more stable ecosystems where pest outbreaks are less common (Landis, Wratten, & Gurr, 2000; Pickett & Bugg, 1998). Agroecosystem diversification can lead to trophic cascades in which producer diversity can positively impact the third trophic level, the natural enemies...
Therefore, growers can potentially improve biological control through habitat management that increases the habitat complexity and plant diversity of their agroecosystem. Habitat management can take on many forms, but the basic idea is to diversify the system to provide habitat for shelter or alternative food sources for natural enemies (Landis et al., 2000). Diversifying annual crop fields can be achieved by intercropping (Liebman & Dyck, 1993), planting cover crops into cash crops (Brainard & Bellinder, 2004) or planting cash crops into pre-established cover crops (Nicholson & Wien, 1983). The question becomes, How do these strategies improve or influence biological control services in agroecosystems?

Monitoring of pest and natural enemy populations in the field provides information on arthropod diversity and abundance in relation to habitat management. Understanding the mechanisms underlying population reductions requires estimation of predation or parasitism rates (Furlong & Zalucki, 2010). There are multiple ways to estimate predation and parasitism rates (e.g. sentinel insects, Furlong, Shi, Liu, & Zalucki, 2004; direct observation, Costamagna & Landis, 2007; and video surveillance, Grieshop et al., 2012). Recently, molecular gut-content analysis has become a common method to document predation and aid in the identification of species that have the potential to contribute to biological control of pest species (e.g. Chapman, Schmidt, Welch, & Harwood, 2013; Eskelson, Chapman, Archbold, Obrycki, & Harwood, 2011; Fournier, Hagler, Daane, de Leon, & Groves, 2008; Szendrei, Greenstone, Payton, & Weber, 2010). These tools are beginning to be applied to understand the effects of management practices on predation of key pests (e.g. Balmer et al., 2013; Szendrei et al., 2010).

Here, we link the effects of habitat complexity to pest and predator abundance and predation, as measured by molecular gut-content analysis of field-collected predators. Using a cabbage (Brassicaceae: *Brassica oleracea*) experimental agricultural system, we examine the effect of habitat management to diversify agroecosystems on pest populations and biological control (Figure 1). Simplified mono-crop systems encourage tight feeding relations of pests on host plants, the resource concentration hypothesis (Root, 1973; Figure 1a). Habitat management to diversify a cropping system generates competing outcomes. When natural enemies benefit from habitat diversification, this should boost top-down regulation of pest populations, enemies hypothesis (Letourneau, 1987; Root, 1973; Figure 1b). Alternatively, if habitat management benefits pest population growth, then habitat management can have a net negative effect on pest suppression (Figure 1c). To test this hypothesis, we manipulated the presence of weeds and cover crop mulch between rows of cabbage to create different levels of habitat complexity. We assessed whether management treatments influenced: (1) the abundance of two herbivorous pest arthropods: diamondback moth (*Plutella xylostella* L., Lepidoptera: Plutellidae) and imported cabbageworm (*Pieris rapae* L., Lepidoptera: Pieridae), (2) abundance and community dominance of predatory natural enemies and (3) biological control services by estimating the proportion of arthropod predators that had recently consumed *P. xylostella* and *P. rapae* prey items using molecular gut-content analysis with species-specific DNA primers.
Materials and methods

Study location and design

The experiment was performed in a cabbage field at the Horticulture Farm at Michigan State University, East Lansing, MI, USA (42°67′N 84°48′W), in 2011. Cabbage transplants (variety ‘Blue Dynasty’; Siegers Seed Company, Holland, MI) were grown to the 4–6 leaf stage in 98-cell plug trays and hardened off for 14 d before transplanting in the field. Cabbage was transplanted on 31 May 2011 into strip-tilled zones (76 cm between rows) with 36 cm between transplants within the row. Experimental plots measuring 4.6 by 9.1 m were established in six cabbage rows for a total of 24 plots (see Bryant, Brainard, Haramoto, & Szendrei, 2013 for complete plot management details). The experimental design consisted of a randomised complete block with six treatments and four replications. Treatments were a combination of two factors: oat cover crop kill date (early, middle or late season) and herbicide management (low or high intensity). Oat was seeded on the entire field in early spring. The soil in the cabbage rows was then strip tilled just prior to cabbage seedling transplanting, but the rest of the field was left undisturbed. To create different levels of habitat complexity, oat was killed at different times after transplanting, which resulted in treatments that had different amounts of oat mulch and weed biomass between cabbage rows (see Bryant et al., 2013). In addition, our herbicide intensity treatments successfully generated significant differences in the two levels of weed biomass (see Bryant et al., 2013). Thus, we created different habitat diversification treatments from low to high, which differed in the amount of habitat structural complexity composed of oat mulch and weeds.
**Predator community and pest abundance**

The numbers of the two pest species, *P. xylostella* and *P. rapae* larvae, and predators were counted on all the leaves of 10 randomly selected cabbage plants per plot in the centre cabbage rows on 1 July 2011 and 2 August 2011. Leaves were searched thoroughly and all insects on them were counted and identified. In addition, yellow sticky traps (7.5 by 12.3 cm, Great Lakes IPM, Vestaburg, MI, USA) placed in the centre of each plot and changed weekly were used as a second sampling method to monitor predator abundance. These two different methods of sampling were chosen because some predators are active flyers (e.g. Coccinellidae) and are likely to be caught in yellow sticky traps, while others (e.g. Pentatomidae) are less likely to take flight or have no wings (e.g. Araneae) and therefore are more prominent in visual foliar samples.

**Molecular gut-content analysis**

*Primer design for target pests.* *Plutella xylostella* and *P. rapae* larvae were derived from laboratory cultures maintained at the University of Queensland, Australia, which were established from experimental *Brassica* crops grown under standard agronomic practices at Gatton Research Station, Lockyer Valley, Queensland (27°32′ S 152°19′ E). Larvae were homogenised in 0.5 or 1.5 ml mortar-and-pestle centrifuge tubes in 100–400 μl of high-salt extraction buffer (Aljanabi & Martinez, 1997) supplemented with 2% sodium dodecyl sulfate and 400 μg/ml proteinase K and digested at 65°C overnight. DNA precipitates were re-suspended in 50–600 μl (depending upon the size of the specimen) of sterile diH₂O or 0.1×TE pH 8.0. Preliminary polymerase chain reactions (PCRs) (30 μl) for nucleotide sequencing of mitochondrial cytochrome oxidase I (COI) utilised primers C1-J-1751 and C1-N-2191, as well as C1-J-2195 and C1-N-2568 (Simon et al., 1994), with expected amplicon sizes of 488 and 421 bp, respectively. Reactions were run per Promega’s (Madison, WI, USA) protocol except for the addition of 1% polyvinylpyrrolidone (PVP, Fisher Scientific, Pittsburg, PA, USA) and 0.2% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA) (Xin, Velten, Oliver, & Burke, 2003) after the primers and before *Tag* polymerase; and reduced annealing temperatures (38–40°C) and elevated Mg²⁺ (to 3.75 mM) to allow for imperfect matches. Initial denaturation was for 3 min at 94.5°C, followed by 40 cycles of 45 s at 94.5°C, 1 min at 37°C, and 2 min at 72°C; 5 min at 72°C completed the programme. Amplified DNA was visualised by electrophoresis of 6 μl of the PCR/stop reaction in 1.5% agarose. The remainder of the reaction mixture was electrophoresed in 1.5% NuSieve agarose (Cambrex Bio Science Rockland Inc., Rockland, ME, USA) in 1× TAE modified to have a final ethylenediaminetetraacetic acid concentration of 0.1 mM, and the fragments excised for sequencing by BigDye terminator v3.1 kits on an ABI 3100 sequencer (Life Technologies, Foster City, CA, USA). Sequences were uploaded to GenBank, *P. xylostella* (KC986828) and *P. rapae* (KC986829). To design primers specific to these species, we searched GenBank to download relevant data (i.e. Plutellidae and COI, Pieridae and COI) and aligned sequences to our targets using MUSCLE (Edgar, 2004). Editing was performed with Lasergene (DNAStar, Madison, WI, USA) and a forward and reverse primers were designed for each of the two herbivore species: for *P. xylostella*: DBMA 1S '5′-TTAATATAAAAAAGAAATGGGAATGTCA-3′ and DBMA 2A 5′-CTGCAGGATCAAAGAAGGA-3′,
and for *P. rapae*: CPAV 1S 5′-GTAGAAAATGGAGCAGGAACAG-3′ and PRAP 1A 5′-CTGGTAATGATAATAGTAAAAAGTAAAG-3′ (see optimal PCR conditions below). Primer pairs for these species were optimised for specificity and tested with field-collected individuals from Beltsville, MD, USA.

**Predation on target pests.** To determine whether individual predators had consumed *P. xylostella* or *P. rapae*, we performed molecular gut-content analysis on predators collected from the field with the species-specific primers designed above. We hand-collected predators for 15 min per plot (4.6 by 9.1 m) between 8 am and 12 pm on 1 July 2011 and 2 August 2011. To minimise experimental error due to differences among predator activity at different hours of the morning, collection among plots was randomised and three people collected at the same time. All predators were placed directly into 70% ethanol and stored at −20°C. DNA was extracted using the TRIzol®LS Reagent according to the manufacturer’s protocol (Life Technologies, Grand Island, NY, USA). Field-collected predators were analysed for the presence of *P. xylostella* and *P. rapae* DNA. Each PCR reaction included starved negative controls for predators, a *P. xylostella*- or *P. rapae*-positive control and a no-DNA control. Negative controls consisted of predators collected from the Michigan State University Horticulture Farm in 2010 that were starved for 5–10 d depending on the species. The PCR cocktail for *P. xylostella* consisted of 2 μl DNA, 2.5 μl 10× buffer (Life Technologies), 12.7 μl PCR water, 1 μl 25 mM dNTPs mix, 0.5 μl 10 nM forward and reverse primers, 0.05 μl RNase, 10% PVP, 2% BSA, 0.3 μl Taq Polymerase (Life Technologies) and 1.75 μl of 25 mM Mg²⁺ for a total of 23 μl reaction volume. PCR cycling conditions consisted of 94.5°C for 3 min, 94.5°C for 0.45 min, 59°C for 1 min, 72°C for 1 min, with the second through fourth steps repeated 44 times, and the PCR cycling ended with 72°C at 7 min. PCR results were visualised by gel electrophoresis using a 0.75% agarose gel stained with ethidium bromide.

**Statistical analysis**

To explore how predator assemblages changed between habitat treatments, we conducted non-metric multidimensional scaling analyses (NMDS) using the Bray-Curtis (Sorensen) distance measure incorporating predator abundance from the visual surveys of plants and yellow sticky traps (PC-ORD version 5; MjM Software, Glenden Beach, OR, USA). In order to determine whether predator communities were significantly different among habitat treatments, we used a non-parametric multivariate analysis of variance (PerMANOVA) procedure (PC-ORD); this test performs distance-based MANOVA. We also performed an indicator species analysis to evaluate which predator taxonomic groups are associated with our habitat treatments (Dufrêne & Legendre, 1997; PC-ORD). Statistical differences for all analyses were determined at α = 0.05 level.

Analysis of variance was used to assess the effects of habitat treatments: herbicide intensity and timing of cover crop management and interaction on the response variable, natural log transformed pest abundance, and post hoc orthogonal linear comparisons (α = 0.05) were conducted on significant main effects. A generalised linear model with binomial error structure was used to assess the effects of habitat treatments: herbicide intensity and timing of cover crop management and interaction on the binary response variable, incidence of predation (presence/absence of prey
DNA in the guts of predators) for target prey species. Statistical analyses were conducted using SAS statistical software package (SAS version 9.2, SAS Institute, Cary, NC, USA).

Results

Predator community and pest abundance

Predator populations were significantly shifted in response to habitat treatments (Figure 2). The NMDS analyses’ first two axes explained 90% of the variation in the model for the predator field data from visual (Axis 1 = cumulative 61%, Axis 2 = 90%; Figure 2a) and the yellow sticky trap (Axis 1 = cumulative 52%, Axis 2 = 90%; Figure 2b). The stress (the overall fit of the ordination, which is inversely

Figure 2. Non-metric multidimensional scaling for predator abundance collected by visually sampling cabbage foliage (a) or on yellow sticky traps (b) in a cabbage field with six habitat treatments.
related to the match between the dissimilarity indices and the distance in the ordination between pairs of habitat treatments) was 11.49 for visual and 11.60 for the yellow sticky trap sampling data, and the instability was < 0.01 (instability is the variation in the stress measure between subsequent runs of the ordination algorithm; low instability values indicate higher confidence in the stress measure) for both types of sampling. NMDS analysis separated the predator communities by weed management (Figure 2), and the separation was significant by PerMANOVA (visual hand-collected: $F_{5, 306} = 61.05, P < 0.01$; yellow sticky trap: $F_{5, 258} = 81.77, P < 0.01$). Furthermore, the indicator species analysis shows that some predator families represented greater dominance in the community in relation weed treatments. Coccinellidae were associated with the late kill date cover crop treatments under both high and low weed intensity management treatments. Staphylinidae were associated with the intermediate kill date treatment with low intensity herbicide management, and Lampyridae were associated with the late kill date with low intensity management.

At the two sampling dates when predators were collected for gut-content analysis, there were on average 0.37 ± 0.50 (mean ± SEM) $P. xylostella$ and 2.66 ± 2.81 $P. rapae$ larvae per cabbage plant. *Plutella xylostella* abundance was not significantly different among cover crop kill date ($F_{2, 24} = 0.51, P = 0.61$), weed management treatments ($F_{1, 24} = 0.60, P = 0.45$; Figure 3a), and no interactive effects were apparent ($F_{1, 24} = 1.15, P = 0.34$). However, *P. rapae* abundance was significantly different among cover crop treatments ($F_{2, 24} = 13.03, P < 0.01$; Figure 3b). Specifically, post hoc comparisons of the cover crop main effect indicate that *P. rapae* were overall significantly less abundant in early compared to late and intermediate cover crop kill treatments ($t$ value$= 3.94, P < 0.01$; Figure 3b). Weed intensity treatments did not appear to influence abundance ($F_{1, 24} = 1.85, P = 0.19$), and there was no significant interaction between cover crop and herbicide intensity treatments on *P. rapae* abundance ($F_{1, 24} = 0.79, P = 0.47$).

**Molecular gut-content analysis**

**Primer design for target prey.** Target DNA was successfully amplified for *P. xylostella* and *P. rapae* forming amplicon sizes of 165 bp and 250 bp, respectively. Species-specificity was tested against DNA from *P. xylostella*, *P. rapae*, *Coleomegilla maculata* DeGeer, *Coccinella septempunctata* L., *Hippodamia convergence* (Guérin), (Coleoptera: Coccinellidae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), Lampyridae, Phalangida, and spiders from the families Theridiidae, Salticidae, Araneidae, Lycosidae, Linyphiidae and Tetragnathidae. No cross-amplification was observed for any of the non-target taxa, showing that the molecular gut-content analysis indicates the presence of *P. xylostella* or *P. rapae* DNA only.

**Predation on target pests.** Out of the 181 field-collected predators, 35% tested positive for either *P. xylostella* (52/181) or *P. rapae* (47/181) DNA in their guts. The majority of arthropods (88%) collected for gut-content analysis were adults. A diversity of predators tested positive for pest DNA (Table 1). Only 1% was positive for both prey's DNA (16/181) and, although there were significant differences in the abundance of these two prey groups (see above and Figure 3), the proportions of predators positive for either of the two prey species were not significantly different ($\chi^2 = 8.20, df = 13, P = 0.49$). The proportion of predators
Figure 3. The proportion of predators from a cabbage field with six different habitat treatments that tested positive for either *Plutella xylostella* (a), or *Pieris rapae* DNA (b), shown on the left y-axis represented as open or closed bars. Open or closed square symbols represent the mean (±SEM) number of *P. xylostella* (a) or *P. rapae* (b) per cabbage plot on the right y-axis.
positive for *P. xylostella* DNA was significantly influenced by crop kill date ($\chi^2 = 19.86$, df = 2, $P < 0.0007$), but not herbicide treatments ($\chi^2 = 0.01$, df = 1, $P < 0.91$). However, a significant interaction among the habitat treatments indicates that management had interactive effects on predation ($\chi^2 = 18.82$, df = 2, $P = 0.0001$; Figure 3a). In particular, the proportion of predators with gut-content positive for *P. xylostella* was higher in the low herbicide intensity treatment for the late kill date and higher in the high weed intensity treatment in the intermediate kill date (Figure 3a). Conversely, for *P. rapae* results indicate no effect of crop kill date ($\chi^2 = 0.68$, df = 2, $P < 0.71$) or herbicide treatment ($\chi^2 = 0.001$, df = 1, $P < 0.96$; Figure 3b).

### Discussion

The structure of the predatory arthropod community showed distinct changes in response to levels of habitat management, and it could be related to enhanced biological control. In contrast to our predictions, the effects of habitat did not have consistent effects on pests or on predation in this system. While increasing habitat complexity improved biological control for *P. xylostella* (i.e. higher predation), for *P. rapae* habitat treatments reduced abundance, but the mechanism appears to be an indirect effect not explainable via increased predation levels. Our results are potentially encouraging for improving integrated pest management (IPM) strategies in this system for one of the pests, and our data contributes one of the first studies

<table>
<thead>
<tr>
<th>Predator family</th>
<th>No. tested</th>
<th>% <em>Plutella xylostella</em></th>
<th>% <em>Pieris rapae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arachnida</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiliones</td>
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<td></td>
<td></td>
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<tr>
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<td><strong>Araneae</strong></td>
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<td>Araneidae</td>
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<td>33.3</td>
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<td>50.0</td>
<td>50.0</td>
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<td>9</td>
<td>44.4</td>
<td>11.1</td>
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<td>16.5</td>
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</tr>
<tr>
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<td>66.7</td>
</tr>
<tr>
<td><strong>Total/average</strong></td>
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<td>35.7</td>
<td>34.7</td>
</tr>
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</table>
using molecular tools to study the impact of habitat diversification on biological control.

Habitat treatments significantly impacted the abundance of *P. rapae*, but not of *P. xylostella*. This could be the result of changes in adult oviposition behaviour in the more complex habitats, since *P. rapae* tends to be sensitive to characteristics of plants when laying eggs (Root & Karieva, 1984); however, it is not clear why *P. xylostella* did not respond in a similar manner, since this species also changes its host selection behaviour in different plant contexts (George, Collier, & Whitehouse, 2013; Silva & Furlong, 2012). Our analysis also revealed that the predator complex responded to habitat treatments, and that the weedier plots generated higher abundances of *C. maculata* and *H. convergence* (Bryant et al., 2013). This result is consistent with past evidence indicating that increasing weed density generally leads to higher abundance of natural enemies (Andow, 1988; Altieri & Whitcomb, 1979; Pimentel 1961; Schellhorn & Sock, 1997). Mechanisms proposed for natural enemy response include the provision of pollen or nectar for many of the predator species that feed on both plant resources and other arthropods (Lundgren, 2009; Norris & Kogan, 2000).

Interestingly, *P. xylostella* was seven times less abundant on average in our experimental plots than *P. rapae*, yet the numbers of predators that were positive for prey DNA (52 versus 47 out of 181, respectively) were relatively similar for the two species. This is contradictory to predictions where generalist predators are expected to exhibit frequency-dependent prey switching (Sherratt & Harvey, 1993). This may be explained by the differences in larval body size between the two herbivores: *P. xylostella* is only about 1 cm long as a fully grown larva, whereas *P. rapae* is about 3–4 cm long. Therefore, those predators that can feed only on small prey can access *P. xylostella* for a longer period in its lifetime. If this is true, then *P. rapae* may escape some or most predation as it grows. This hypothesis is supported by the fact that neonate *P. xylostella* are the stage most susceptible to predation (Furlong et al., 2004), indicating that predators preferentially consume small larvae, which can take up to 3 hrs to locate a feeding site before mining into the leaf. Other possible explanations are that predators present in this system prefer to feed on *P. xylostella* over *P. rapae* or that *P. xylostella* behaviour exposes them to more predators. This latter hypothesis is intriguing, as although neonate larvae mine in the leaves and are therefore protected from most predators after establishing a feeding site (Silva & Furlong, 2012), newly hatched larvae can move considerable distances within a plant and take several hours to locate a feeding site, thereby increasing the risk of predation. On the other hand, second and fourth instar *P. xylostella* wiggle when touched and therefore may be more apparent to predators (Z. Szendrei personal observations) or parasitoids (see Wang & Keller, 2002) than *P. rapae* larvae, which are more lethargic. Another potential explanation may have to do with the difference in the predator defence of the two herbivores: *P. rapae* larvae are densely covered in hairs (Capinera, 2001) and are able to secrete defensive oil droplets from their dorsal setae (Shiojir & Takabayashi, 2005). Finally, the half-lives of detectability (Chen, Giles, Payton, & Greenstone, 2000; Greenstone, Payton, Weber, & Simmons, 2013) of the DNA of the two species in the gut of the average predator in the complex could just happen to differ several-fold. Our experimental setup was unable to clearly identify predation as the cause for lower *P. xylostella* abundance in the field plots, but our results provide some evidence for this.
According to the indicator species analysis, the most abundant predators in our field were the Coccinellidae that tended to be associated with our more complex, weedy habitat treatments. Some Coccinellidae are known for using weeds as oviposition sites (Griffin & Yeargan, 2002) and therefore may aggregate in parts of the field that have more weeds. On average across all habitat treatments, the gut contents of 11% and 7% of the coccinellids were positive for *P. xylostella* and *P. rapae* DNA, respectively. Since we have not measured the detectability half-life of *P. xylostella* or *P. rapae* DNA in the guts of the predators we tested, we are not able to compare the biocontrol value of coccinellids to other predators in our system (cf. Szendrei et al., 2010), but the detectability of *P. xylostella* DNA in the guts of coccinellids has previously been shown to be shorter than in Lycosidae and Nabidae (Hosseini, Schmidt, & Keller, 2008). Nevertheless, we observed similar results in the case of the predator-positive prey DNA (Figure 3) to our field abundance data, both associating Coccinellidae with our weedy and high cover crop mulch treatments. We expected the spiders to benefit from habitat diversification (Sunderland & Samu, 2000), and on average across our habitat treatments, 10% of spiders were positive for *P. xylostella* or *P. rapae* DNA; however, there was no clear trend for any of the spider families to be associated with the high habitat complexity treatments. This is perhaps because the effects of habitat complexity do not result in higher numbers of these predators directly on cabbage, but are using the areas between rows where the presence of weeds or mulch may have impacted spiders directly (Schmidt & Rypstra, 2010).

The correlation between proportion of prey-positive predators and predator field abundance was positive, indicating that in this system a general increase in predator abundance will lead to more prey consumption. Although our study was not designed to pinpoint the predator group that consumes the most prey, we can still conclude that coccinellids responded positively to our habitat treatments according to both the abundance data and the gut-content analysis. Since this group is ubiquitous in temperate regions of the world, it may well be the target for future conservation biocontrol efforts in this system. Now that we have a working understanding of the biological control species and potential services provided, we can test this hypothesis with further molecular analysis, which will provide estimates of their true value as biocontrol agents of these two pests (Greenstone et al., 2010). Importantly, the detectability half-life (Chen et al., 2000; Greenstone et al., 2013) of *P. xylostella* DNA in the predator complex overall may be longer than that of *P. rapae* DNA.

While there is a significant body of literature on the effects of habitat diversification in brassica fields, starting with the seminal work by Root (1973), the present study is one of the first to apply molecular techniques to biological control in this system. Our results represent new insights into the complexity of managing multiple pests using habitat management, and provide data on the trophic linkages contributing biological control services in this system. Further long-term research will contribute to better understanding of the trophic structure and interactions in this system in response to IPM approaches.
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References


