# Cover crops have neutral effects on predator communities and biological control services in annual cellulosic bioenergy cropping systems

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ABSTRACT

Maize stover is beginning to be used as a cellulosic biofuel feedstock in the Midwestern United States; however, there are concerns that stover removal could result in increased soil erosion and loss of soil organic matter. Use of a winter cover crop following maize harvest has the potential to mitigate these impacts and may have additional benefits by providing continuous living cover in annual crop habitats leading to changes in insect predator communities and increased biocontrol services. However, cover crops may also be harvested in cellulosic biofuel systems, adding a disturbance event that may negatively affect biocontrol. We contrasted insect predator communities and their impacts in four potential bioenergy cropping systems in Michigan and Wisconsin (USA) during the 2013 and 2014 growing seasons. Two annual maize systems were harvested for grain and stover; one maize system included a cereal rye/Austrian winter pea cover crop. Two perennial systems, switchgrass and a mixed prairie grasses and forbs, were harvested in the fall for biomass. Predatory insect abundance and diversity were lower in both annual cropping systems than in the perennial cropping systems and the inclusion of the cover crop did not significantly alter these responses. Similarly, removal of sentinel insect egg prey was also lower in the annual versus perennial cropping systems, with no significant influence of cover crop. We also explored the potential for cover crops to harbor prey populations in the spring that might encourage oviposition by mobile predators and potentially lead to local population sources or sinks of predators depending on the timing and effect of cover crop harvest. We found that existing predator communities in the cover crop treatments effectively suppressed aphid population growth, limiting their attractiveness to mobile predators. While we found no significant positive or negative impacts of this cover crop system on biocontrol services, bioenergy cover cropping systems could be managed to increase multiple ecosystem services by altering cover crop identity, or timing of planting and harvest.
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## 1. Introduction

The sustainability of biofuel production requires balancing the need to produce bioenergy crops efficiently while enhancing water quality, reducing greenhouse gas emissions, and maintaining biodiversity (Meehan et al., 2013; Werling et al., 2014). Biofuel production in the United States is primarily based on ethanol derived from maize grain (first generation biofuels). However, biofuels derived from lignocellulosic feedstocks (second generation biofuels) are beginning to be produced, with several commercial-scale cellulosic ethanol plants starting production in the United States in 2014. Currently, maize stover, the leaves and stalks typically left after grain harvest, is considered one of the most viable and sustainable feedstocks for lignocellulosic bioethanol because it is widely available (US DOE, 2011), does not compete with food production (Thompson and Meyer, 2013), and existing transportation and refining technologies can be adapted for stover more easily than for novel feedstocks (Hess et al., 2009; Janssen et al., 2013). However, removing stover from the field can

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have varying environmental impacts. Stover harvest is projected to decrease nitrate and phosphorous loadings at the watershed level, while greenhouse gas emissions and sediment loadings are projected to increase (Gramig et al., 2013). Other studies indicate that stover harvest has the potential to increase soil carbon loss (Follett et al., 2012; Lugato and Jones, 2014). A potential way to mitigate the negative impacts of stover harvest is to grow winter annual cover crops following maize. Winter annual cover crops can reduce soil carbon loss, erosion, and nutrient runoff from stover harvest (Lugato and Jones, 2014). They can also be harvested as a forage or biofuel feedstock, and can also create habitat for animals during the otherwise fallow winter-spring period. Thus, the widespread planting of cover crops could improve the environmental sustainability of maize stover bioenergy production (Bonner et al., 2014).

One ecosystem service that may be affected by the wide-scale planting of winter annual cover crops is natural pest control. Natural biological control by predators and parasitoids is an important ecosystem service estimated to be worth $4.5 billion per year to United States agriculture (Losey and Vaughan, 2006). Previously, we have shown that predator biomass and diversity, as well as insect prey removal, are lower in continuous maize compared to perennial feedstocks, such as switchgrass and mixed prairie (Werling et al., 2011b). However, it is possible that the addition of winter cover crops to annual systems may introduce some of the benefits of perennial biomass crops by increasing food resources for predators and improving habitat quality. For example, several legume cover crops are known to harbor pea aphid, Acyrthosiphon pisum (Harris), and some grass cover crops support bird cherry-oat aphid, Rhopalosiphum padi (L.), (Bugg et al., 1991; Tillman et al., 2004); both of which can sustain populations of agronomically important natural enemies (Bugg et al., 1991; Snyder and Ives, 2003). By providing prey and suitable micro-climates, cover crops may serve as temporal habitat bridges that help relay natural enemies into subsequent crops (Ruberson et al., 1999).

Natural enemy life history traits affect the way in which they interact with cover crop systems. For many ground-dwelling predators, cover crops may provide prey and suitable habitat that allow them to overwinter and persist into the subsequent crop. Lundgren and Fergen (2010) found increased abundance of ground-dwelling predators and reduced corn rootworm damage in maize planted after a winter cover crop in contrast to plots without cover crop. Similarly, Carmona and Landis (1999) found significant increases in carabid beetle activity-density in maize plots with cover crops, but this effect was not consistent across years. Cover crops can also impact foliar-dwelling predators. For example, aphidophagous Coccinellidae (lady beetles) typically lay their eggs in dense patches of aphids (Evans 2003). If cover crops support aphid build-up in the spring, they may attract oviposition by coccinellids. However, to prevent competition with the subsequent crop, most cover crops are terminated (i.e., killed) prior to planting via herbicides, mowing, cultivation or a combination of these practices, all of which can reduce the number and effect of predatory invertebrates (Landis et al., 2000). Additionally, cover crops can also be harvested for cellulosic biofuel, adding another disturbance event that may negatively affect predatory invertebrates. Flight-capable adult insects can survive such disturbance, but the non-flying larvae and egg stages of these organisms are more susceptible (Hossain et al., 2000). As such, cover crops could act as either source or sink habitats for foliar predators, depending on the timing of cover crop harvest.

Given the growing use of maize stover for cellulosic biomass and the potential soil conservation benefits of coupling winter cover cropping with stover harvest, we investigated the potential impacts of this cropping system on predator communities and

biocontrol services. The overall goal of our two-year, two-state study was to determine the effects of a winter cover crop system designed to enhance the agronomic performance of a bioenergy cropping system on predatory invertebrate communities and biocontrol services. Our specific objectives were 1) to determine if winter cover crops altered the abundance and diversity of predator communities, and 2) to assess the impact of the resulting predator communities on rates of predation in the subsequent crop. To do so we sampled predatory arthropod communities and compared sentinel prey removal rates, an index of potential biological control activity (Werling et al., 2011b), in maize grown with and without cover crops, and contrasted the results to switchgrass and mixed prairie, two perennial biofuel feedstocks known to support high numbers of natural enemies. Specifically, we hypothesized greater predator abundance and prey removal in the maize following the cover crop compared to maize grown without cover crops. We also anticipated that cover crops would support aphid populations and attract mobile predators potentially serving as either source or sink habitats for insect predators.

## 2. Methods

### 2.1. Study area

Research was conducted during 2013 and 2014 at the two sites of the Great Lakes Bioenergy Research Center (GLBRC) Biofuel Cropping System Experiment, the W.K. Kellogg Biological Station of Michigan State University (MI) (+42°23′42.39″, −85°22′24.77″) and the Arlington Agricultural Research Station of University Wisconsin-Madison (WI) (+43°18′16.00″, −89°19′48.20″). Four of the ten crop treatments in the GLBRC Biofuel Cropping System Experiment (BCSE) were used: continuous no-till maize, continuous no-till maize plus cover crop (hereafter 'cover crop system'), switchgrass (Panicum virgatum L.), and restored prairie. Five replicate plots (40 × 30 m) of each treatment were arranged in a randomized complete block design at both sites.

In the cover crop system, Austrian winter pea (Pisum sativum subsp. arvense (L.) Poir) and cereal rye (Secale cereale L.) (Varieties: MI, 2013: Cougar; MI, 2014: Wheeler; WI, 2013 & 2014: Spooner) were planted in the fall of 2012 and 2013 after maize harvest and stover removal (Table 1). Cover crops emerged in the fall and survived to termination except for those planted in Wisconsin 2013, which did not emerge until the following spring in 2014. In the spring of both experimental years, maize was planted in the continuous maize plots in early May and in the cover crop system in late May to early June (Table 1). Both the continuous maize (glyphosate-tolerant DeKalb 52–59; 102 day maturity) and cover crop system (glyphosate-tolerant Pioneer P8906AM; 89 day maturity) were planted at 75,000 plants ha$^{-1}$, ~4.5 cm deep, with 76 cm row spacing. The cover crop was harvested as a bioenergy feedstock with a John Deere (Deere and Co., Moline, IL) 7450 forage harvester in late May to early June. After harvest, except for MI 2013, cover crop plots were sprayed with glyphosate herbicide (Roundup PowerMax®, Monsanto Company, St. Louis, MO) at 1260 g A.E. ha$^{-1}$ in late May to early June. Due to low productivity, the 2013 MI cover crop was terminated with a flail mower instead of herbicide and biomass was left in place. Maize grain in continuous maize was harvested in late October and in the cover crop system in late October to early November. Maize stover in continuous maize was harvested in late October and in the cover crop system in late October to early November.

Perennial switchgrass and prairie plots were planted in June, 2008 and were harvested annually in late October. The prairie treatment consisted of six native perennial grasses (including big blue stem (Andropogon gerardi), Indiangrass (Sorghastrum nutans), and junegrass (Koeleria cristata)) and twelve native forbs (including

**Table 1**
Calendar of crop management, vacuum sampling, and sentinel egg removal from the two sites of the Great Lakes Bioenergy Research Center Biofuel Cropping System Experiment, 2012–2014.

| | Michigan | | Wisconsin | |
| --- | --- | --- | --- | --- |
| | Year 1 | Year 2 | Year 1 | Year 2 |
| Cover crop planting | Nov. 10, 2012 | Nov. 19, 2013 | Oct. 12, 2012 | Nov. 20, 2013 |
| Pre-maize vacuum sampling 1 | April 16, 2013 | May 5, 2014 | April 22, 2013 | May 2, 2014 |
| Pre-maize vacuum sampling 2 | April 26, 2013 | May 12, 2014 | April 29, 2013 | May 8, 2014 |
| Pre-maize vacuum sampling 3 | April 29, 2013 | May 19, 2014 | May 2, 2013 | May 16, 2014 |
| Sentinel egg removal 1 | April 26, 2013 | May 7, 2014 | April 29, 2013 | May 2, 2014 |
| Cover crop harvest | June 4, 2013 | May 29, 2014 | June 4, 2013 | May 28, 2014 |
| Cover crop termination[a] | June 5, 2013 | May 31, 2014 | June 10, 2013 | June 9, 2014 |
| Maize planting, continuous maize system | May 6, 2013 | May 10, 2014 | May 7, 2013 | May 19, 2014 |
| Maize planting, cover crop system | June 7, 2013 | May 30, 2014 | June 7, 2013 | June 6, 2014 |
| Post-Maize vacuum sampling 4 | June 19, 2013 | June 17, 2014 | July 3, 2013 | July 2, 2013 |
| Sentinel egg removal 2 | June 19, 2013 | June 30, 2014 | July 1, 2013 | June 30, 2014 |
| Mid-season vacuum sampling 5 | July 16, 2013 | July 31, 2014 | July 31, 2013 | July 23, 2014 |
| Sentinel egg removal 3 | July 15, 2013 | Aug. 3, 2014 | July 29, 2013 | July 23, 2014 |
| Maize harvest, continuous maize system | Oct. 28, 2013 | Oct. 29, 2014 | Oct. 25, 2013 | Nov. 7, 2014 |
| Maize harvest, cover crop system | Oct. 28, 2013 | Oct. 29, 2014 | Oct. 25, 2013 | Oct. 29, 2014 |
| Maize stover harvest, continuous maize system | Oct. 29, 2013 | Oct. 30, 2014 | Oct. 28, 2013 | Nov. 9, 2014 |
| Maize stover harvest, cover crop system | Oct. 29, 2013 | Oct. 30, 2014 | Nov. 8, 2013 | Oct. 30, 2014 |

[a] Cover crops were terminated with glyphosate herbicide except Michigan, 2013 terminated with a flail mower.

white wild indigo (*Baptisia leucantha* Torr. & Gray), black-eyed susan (*Rudbeckia hirta* L.), and wild bergamot (*Monarda fistulosa* L.)). Additional details on the GLBRC BCSE are available at (http://lter.kbs.msu.edu/research/long-term-experiments/glbrc-intensive-experiment).

## 2.2. Arthropod sampling

Arthropod predators and aphids were sampled using a modified leaf blower (BG 56 C-E; Stihl, Waiblingen, Germany) to vacuum arthropods from a 0.25 m² area. All foliage and the ground beneath the plants were sampled with the vacuum. Four vacuum samples were randomly taken within each plot. Specimens were sucked into fine mesh collection bags which were closed with rubberbands and placed in a cooler until stored in the laboratory freezer. Lady beetles (Coccinellidae) were identified to species; spiders (Araneae), and harvestmen (Opiliones) were combined; and other predatory insects associated with biological control were identified to family, including beetles (Staphylinidae, Carabidae, Cantharidae, and Lampyridae), flies (Syrphidae and Dolichopodidae), true bugs (Anthocoridae and Nabidae), lacewings (Chrysopidae and Hemerobioidea), and ants (Formicidae). Voucher specimens were deposited in the Albert J. Cook Arthropod Research Collection at Michigan State University. Vacuum sampling occurred on three dates in April/May prior to cover crop termination and maize planting and twice after maize planting; once during the early vegetative stage and again during the early reproductive stage (Table 1).

## 2.3. Measuring biocontrol services

To evaluate biological control services in each cropping system, we measured removal rates of frozen corn earworm (*Helicoverpa zea* [Boddie]) eggs. Two paper index cards with approximately fifty earworm eggs each were attached to the underside of a white 30 × 30 cm platform made from corrugated plastic. The platform was placed horizontally on a plastic pole at canopy height or at 1.5 m in later season maize. One of the cards was covered with a predator exclosure cage fixed with a binder clip. The cage was constructed from a 10 cm diameter Petri dish bottom with its center cut out (5 cm opening) and replaced with a 1 mm wire mesh screen. The exclosure cages were used to determine how many eggs were lost by handling or abiotic factors, so that egg losses on

the card exposed to predators could be adjusted accordingly. Two egg card platforms were set out per plot at the north and south ends of the plot. Egg cards were collected 48 h after deployment and placed in a cooler until they were brought back to the laboratory freezer. The number of remaining eggs on each card was compared to the initial number of eggs to calculate mean percent removal. Egg cards were deployed once prior to cover crop termination and maize planting and twice after maize planting (Table 1).

## 2.4. Measuring aphid suppression in cover crops

To determine if the cereal rye and Austrian winter pea cover crop sustains populations of aphids and to determine the aphid suppression potential of the predator community in the cover crop, we conducted a predator exclosure study in the cover crop system in Michigan 2014. Two tomato cages (1 m tall, 0.36 m diameter) were placed in the north and south sections of each cover crop plot (*n* = 5) on May 15, 2014. Both cages were covered with no-see-um mesh (~625 holes/6.5 cm; Skeeta, Bradenton, FL). The mesh was buried 15 cm into the ground and tied at the top with wire twist-ties to prevent ground-dwelling and flying predators from entering. On May 20, 2014, 25 bird cherry oat aphids, *Rhopalosiphum padi* L., (International Technology Services, Inc., Wayzata, MN) were placed on the vegetation inside each cage. Two days after aphid inoculation (May 22), each cage was randomly assigned to one of two treatments per plot: "open" cages had their mesh completely removed while the mesh on the "closed" cages remained. Aphid numbers inside closed and open cages were counted daily from May 21–24, and again on May 26, 28, and 29.

## 2.5. Statistical analyses

All statistical analyses were completed using R 3.0.3 (R Development Core Team, 2014). To determine whether crop type affected the composition of the predator community, we used non-metric multi-dimensional scaling (NMDS, based on Bray-Curtis dissimilarity) with the *vegan* package (Oksanen, 2011; Oksanen et al., 2013). Our initial examination of the data suggested that the community composition varied between sample dates. Therefore, we divided our data into two groups: samples collected before maize planting (Pre-maize samples 1, 2, and 3), and samples collected after maize planting (Post-maize samples 4 and 5;

Table 1). For each data subset, we performed a separate NMDS analysis on a matrix of the untransformed abundances of each predator group by crop and sample date. Plots with one or fewer insects captured at a sampling date and similarly, taxa with two or fewer individuals captured over the course of the study were excluded from analysis to prevent low sampling success or extremely rare taxa from disproportionately influencing analyses. The explanatory variables were crop type, state, and year. We performed post-hoc pairwise comparisons of community composition between crop types using PERMANOVA.

To determine whether crop type and sample date affected predator abundance (total number of predators), predator diversity (Simpson's Diversity Index (1-D)) and predation rates (proportion of sentinel eggs removed), we used GLM. Models for each response variable (predator abundance, diversity, and predation rates) were then subjected to analysis of deviance using the $\chi^2$ test statistic, followed by pairwise *t*-tests that had been Holm-adjusted for multiple comparisons to determine pairwise differences between crop type and sample date. For each response variable, model specification used the appropriate error structure for each data type: predator abundance was modeled using a negative binomial error structure, predator diversity with a normal error structure, and predation rates with a binomial/logit structure (O'Hara and Kotze, 2010). Models took the form:

$$Response\ variable \sim Year + State + Year \times State + Block$$
$$+ Crop\ Type + Sample\ Date + Crop\ Type$$
$$\times Sample\ Date$$

We performed post-hoc analysis to determine the effects of crop type, sample date, and their interactions using *t*-tests that were Holm-adjusted for multiple comparisons. These tests were applied to residuals produced by applying the full model for each response variable, minus the effect being tested for, to control for variation due to other factors in the means comparisons. Summary statistics were compiled using the *plyr* function (Wickham, 2009).

To determine whether predator exclusion influenced aphid abundances in the cover crop system, data from the predator exclusion experiment were subjected to GLM and model selection using a negative binomial error structure. Number of cover crop rye tillers varied slightly between samples and was included in the global model as an offset variable. The aphid model took the form

$$Aphid\ abundance \sim Date + Cage + Date \times Cage\ Treatment + Block$$
$$+ offset(Tillers)$$

Subsequent analysis of deviance followed an identical procedure to that outline above.

A type I error rate of $\alpha = 0.05$ was used for all relevant statistical tests.

## 3. Results

### 3.1. Predator abundance and community composition

We collected 9596 individual arthropod predators from twenty-six different taxa. Formicidae (*mean* = $4 \pm 2$/sample) and Araneae (*mean* = $2.3 \pm 0.1$/sample) were the most abundant taxa in the vacuum samples, accounting for 55% and 33% of the number of arthropod predators captured, respectively. All other arthropod predators captured averaged less than one individual per sample.

A two-dimensional NMDS plot of the predator community prior to maize planting had a stress of 0.10 (Fig. 1A). PERMANOVA analysis indicated that community composition varied significantly by state, year, and crop type (P = 0.009, P = 0.001, P = 0.001 respectively). Crop type accounted for most of the variation ($R^2$ = 34%) followed by year ($R^2$ = 9%) and state ($R^2$ = 5%). Post-hoc,
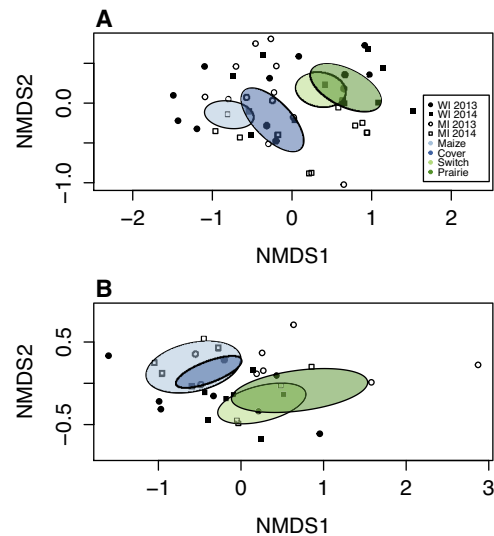


**Fig. 1.** Non-metric multi-dimensional scaling (NMDS) of predator communities in cover crop trials in Michigan and Wisconsin (USA) 2013–2014. Points displayed on the figure represent community composition at each site (Kellogg Biological Station in MI; Arlington Agricultural Station in WI) in each crop type at each sample date. Ellipsoids represent 95% confidence interval of the centroids by cropping system. Ordination of predator community (A) early in the growing season, prior to maize planting and (B) after maize planting. "Maize" is continuous maize, "Cover" is the cover crop system, "Switch" is switchgrass, and "Prairie" is the restored prairie system.

pairwise PERMANOVA indicated that prior to maize planting, predator communities differed between all crop types, although the NMDS visualization suggested the communities shifted on a continuum from continuous maize, to the cover crop system, to switchgrass, to prairie (Fig. 1A). The two-dimensional NMDS analysis on community composition after maize planting resulted in a stress of 0.07 (Fig. 1B). Again, community composition varied significantly by crop type (P = 0.001) accounting for 24% of variation during this sampling period but did not vary significantly by state or year (P = 0.054 and P = 0.094 respectively). Post hoc analysis indicated that communities occurring in continuous maize and the cover crop system did not differ from each other (P = 0.489), nor did communities occurring in switchgrass and prairie (P = 0.470), however, the annual and perennial cropping systems differed from each other (Fig. 1B).

Prairie consistently had higher predator abundance than the other crop types, especially on the last sampling date, and predator abundances were lower in both continuous maize and the cover crop system than in either perennial crop (Fig. 2). Predator abundance varied significantly by crop type ($\chi^2_{(df=3)}$ = 1688.3, P < 0.001), sample date ($\chi^2_{(df=4)}$ = 1612.8, P < 0.001), year ($\chi^2_{(df=1)}$ = 2421.7, P < 0.001), and block ($\chi^2_{(df=9)}$ = 1688.3, P < 0.001). Controlling for variability across years, blocks and stations, post-hoc tests on crop type showed predator abundance was highest in prairie (P < 0.001), followed by switchgrass (P < 0.001), and that there were no differences in predator abundances between continuous maize and the cover crop system (P = 0.18) (Fig. 2).

As the growing season progressed, predator abundances increased. Predator abundances were statistically lower in the first three sample dates conducted prior to maize planting (Dates 1–3) compared to the last sampling date (Date 5) when maize reached early reproductive stage (P ≤ 0.04). However predator abundances taken soon after maize was planted (Date 4) did not differ between the dates prior to maize planting (P = 1.0). Although there was a significant crop type by date interaction according to the analysis of deviance ($\chi^2_{(df=12)}$ = 1445.0, P < 0.001), results from
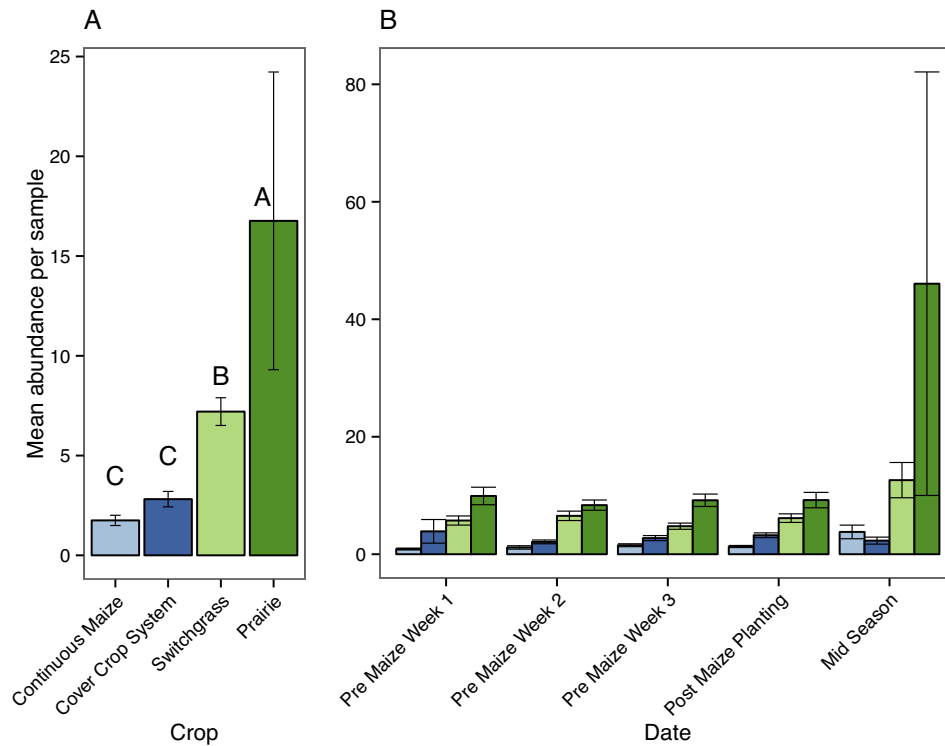
**Fig. 2.** Mean predator abundance per vacuum sample. (A) Mean predator abundances averaged across sample dates. Means with the same letter are not significantly different according to Holm-adjusted pairwise *t*-tests; $\alpha = 0.05$. (B) Mean predator abundances across the growing season. "Pre Maize" samples occurred in April/May prior to maize planting, "Post Maize Planting" during early vegetative maize stages in June/July, and "Mid Season" during early reproductive maize stages in late July. Bars represent the standard error of the mean (SE).

post-hoc tests did not detect any variation from the overall crop type or sample date trends (Fig. 2).

### 3.2. Predator diversity

Predator diversity was lower in the annual systems than in the perennial systems (Fig. 3). Predator diversity varied significantly by crop type ($\chi^2_{(df=3)} = 178.7$, $P < 0.001$), sample date ($\chi^2_{(df=4)} = 165.3$, $P < 0.001$), and state ($\chi^2_{(df=1)} = 194.4$, $P < 0.001$). Controlling for variability between states and sample dates, post-hoc tests showed that predator diversity in the two perennial systems were greater than the two annual systems ($P < 0.001$, Fig. 3) with no difference in predator diversity between the prairie and switchgrass systems ($P = 0.94$), and no difference between the continuous maize and the cover crop system ($P = 0.16$). Post-hoc tests on sampling date showed increasing diversity as the season progressed; except for Dates 1 and 2 ($P = 0.14$), all other dates had higher predator diversity than the previous date ($P \leq 0.04$). Although there was a significant crop type by sample date interaction ($\chi^2_{(df=12)} = 158.9$, $P = 0.002$), as was observed with predator abundance, results from post-hoc tests did not detect any variation from the overall crop type or sample date trends (Fig. 3).

### 3.3. Biocontrol services

Sentinel egg removal was consistently higher in the perennial systems than the annual systems and there were no differences within either of the groups. Prey removal varied significantly by crop type ($\chi^2_{(df=3)} = 6896.3$, $P < 0.001$), sample date ($\chi^2_{(df=2)} = 4939.6$, $P < 0.001$), year ($\chi^2_{(df=1)} = 8140.5$, $P < 0.001$), state ($\chi^2_{(df=1)} = 8205.9$, $P < 0.001$), and block ($\chi^2_{(df=8)} = 8034.0$, $P < 0.001$, Fig. 4). Controlling for variability between years, states, and blocks,

post-hoc tests showed that the two perennial systems had higher removal rates than the two annual systems ($P < 0.001$) and that sentinel prey removal was statistically the same in prairie and switchgrass ($P = 1.0$), and statistically the same in continuous maize and the cover crop system ($P = 1.0$). Post-hoc tests on sample date showed increasing sentinel prey removal rates as the season progressed; each date had a higher rate than the previous date ($P < 0.001$). There was a significant crop type by sample date interaction ($\chi^2_{(df=6)} = 4451.4$, $P < 0.001$), likely because sentinel prey removal rates were uniformly low early in the growing season, prior to maize planting, where the differences between crop types were not detectable (Fig. 4). We also detected a state by year interaction in this analysis: prey removal rates were very low in WI 2014. However, despite these low removal rates within this subset of our data, the same general trend was followed, with the two perennial systems having higher sentinel prey removal rates than the two annual systems.

### 3.4. Aphid suppression in cover crops

In the predator exclusion experiment, aphid densities sharply increased one day after inoculating plants with aphids (Fig. 5). On Day 2 (when mesh was removed on the open cages), aphid densities quickly declined and by Day 4 densities were less than the starting densities of 20 aphids per treatment in both cage treatments. Aphid densities in the closed cages then increase by Day 5 and by the end of the experiment (Day 10) reached nearly four times the starting densities. However, aphid densities in the open cages remained low at densities below the starting densities. When averaged across sampling dates, aphid densities inside the closed cages (*mean* = $37 \pm 5$) were significantly higher ($\chi^2_{(df=1)} = 202.5$, $P < 0.001$) than the open cages (*mean* = $19 \pm 3$).
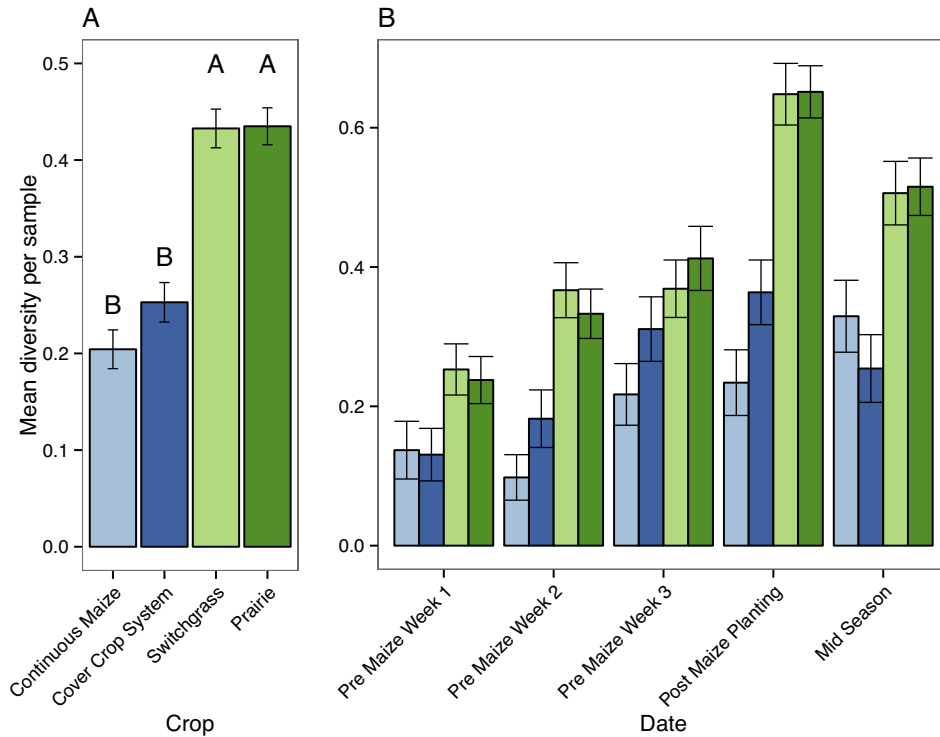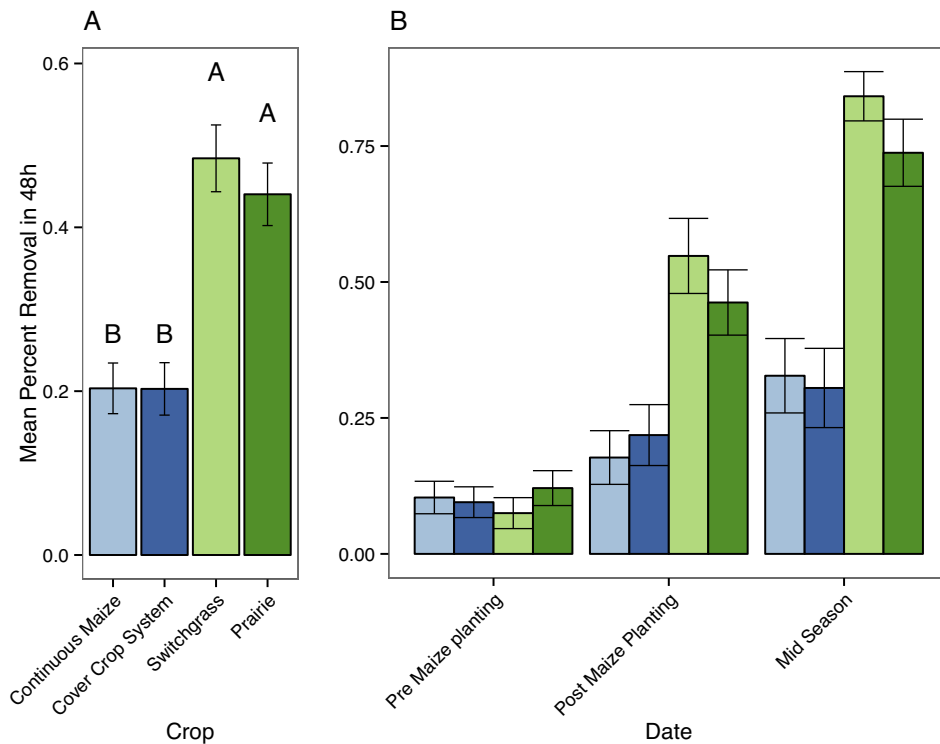
**Fig. 3.** Mean predator diversity (Simpson's 1-D) per vacuum sample. (A) Mean predator diversity averaged across sample dates. Means with the same letter are not significantly different according to Holm-adjusted pairwise *t*-tests; α = 0.05. (B) Mean predator diversity across the growing season. "Pre Maize" samples occurred in April/May prior to maize planting, "Post Maize Planting" during early vegetative maize stages in June/July, and "Mid Season" during early reproductive maize stages in late July. Bars represent the standard error of the mean (SE).



**Fig. 4.** Mean percent sentinel egg removal per 48 h. (A) Mean removal rates averaged across sample dates. Means with the same letter are not significantly different according to Holm-adjusted pairwise *t*-tests; α = 0.05. (B) Mean removal rates across the growing season. "Pre Maize Plant" samples occurred in April/May prior to maize planting, "Post Maize Planting" during early vegetative maize stages in June/July, and "Mid Season" during early reproductive maize stages in late July. Bars represent the standard error of the mean (SE).
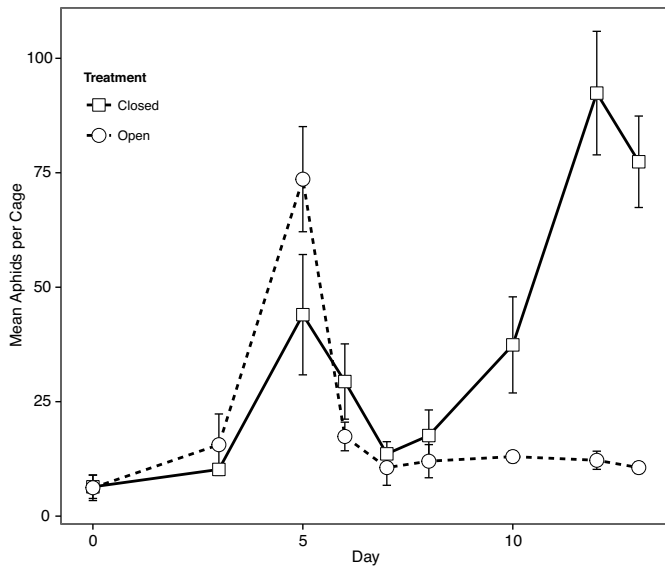
**Fig. 5.** Mean aphid number per cage. Both "open" and "closed" cages had predator excluding mesh until May 22 when mesh was removed from the open cages. On average, aphid numbers in closed cages are significantly higher than open cages after the mesh was removed; $\alpha = 0.05$. Bars represent the standard error of the mean (SE). Error bars on latter dates of open cages too small to display.

## 4. Discussion

The overall impact of cover cropping on natural enemy communities and prey consumption in the annual cropping system was small in comparison to the impact of crop perenniality. Predator abundance and diversity in this study were higher in the perennial biomass feedstocks, switchgrass and prairie, than in the annual maize systems, a pattern that was mirrored by higher rates of sentinel prey removal. This result is consistent with the findings of Werling et al. (2011b) who contrasted maize (without cover cropping) to perennial grass bioenergy crops. In general, perennial systems are thought to support a greater abundance and diversity of predators because of increased resources (food, shelter) and less disturbance compared to annual systems (Webster et al., 2010). We hypothesized that winter annual cover crops could increase biocontrol services in the annual maize system by offering similar resources. However, we found that the inclusion of a winter annual cover crop did not increase the abundance or diversity of the invertebrate predator community or elevate predation rates. Although early in the growing season arthropod communities in cover crop systems differed from those occurring on bare soil in continuous maize, these differences did not continue after maize was planted. These results were consistent across both experimental years in both states. Instead, predator abundance, diversity, and predation rates were similar in magnitude to annual maize without a cover crop, suggesting that adding a winter crop to our maize system did not provide any additional benefits to biocontrol.

A number of factors may have precluded the cover crop system from enhancing biocontrol services in the maize system. Herbicides and mowing used to terminate the cover crops in late spring may have had negative effects on important invertebrate predators via habitat loss or direct mortality in the case of mowing (Brust, 1990; Birkhofer et al., 2007; Thomas and Jepson, 1997). Furthermore, while most winter annual cover crops are terminated prior to subsequent crop planting, we may have compounded this disturbance by also harvesting the cover crop to use as a biofuel feedstock. In prior cover crop research, the microclimate created by cover crop residue left in the field has been cited as the mechanism

behind increased predator abundance and suppressed prey populations (Lundgren and Fergen, 2010). In our system, the above ground cover crop vegetation was harvested, likely reducing the microclimate benefits of the cover crop on biocontrol in the subsequent maize. Finally, the harvesting of maize stover at the end of the growing season is an additional disturbance event unique to our cellulosic ethanol system. Maize residue is an overwintering site for a number of invertebrate predators (Plagens and Whitcomb, 1986), and leaving crop residue in the field may increase predator density and activity the following spring (Halaj et al., 2000).

In addition to disturbances, the rye/pea cover crop may have had limited capacity to influence predators in our region due to the relatively late planting date of the cover crop followed by cold winters typical of the Upper Midwest. Ryelage, rye grown for animal feed, is typically planted by mid-October and harvested by mid-May in our area, but it supplemented with nitrogen fertilizer in the spring to boost biomass. Other cover crop studies that have reported increased predator densities in cover crop treatments either planted their cover crops earlier in the fall (e.g., Lundgren and Fergen, 2010) or were conducted in warmer growing zones (e.g. Bugg et al., 1991). The greater cover crop biomass may have produced greater opportunities for predators and their prey resources to colonize cover crop fields. Vegetative density has been correlated with predator abundance (Rebek et al., 2005), and hay height and density has been positively correlated with spider diversity (Schwab et al., 2002). The average plant height in our cover crop system at termination was 57 cm in MI, 2014 prior to harvest and the average cover crop yield was 430 kg DM/ha. In comparison, a North Carolina cover crop experiment reported ten to twenty times greater rye yields and four times greater stand heights (Smith et al., 2011). Therefore, implementing practices to plant cover crops earlier in the fall through interseeding or aerial seeding may increase cover crop biomass and potentially increase biocontrol services in our study region.

Along with crop management, the cover crop may not have altered biocontrol services in continuous maize because it did not attract early season colonizing predators from the surrounding landscape. While resident predators, such as ants and spiders, were prevalent in our cropping systems, predators colonizing from outside of the system (e.g., lady beetles, lacewings) were not. Previous studies have reported aphid populations in cover crops that attract coccinellid predators (Bugg et al., 1991; Smith et al., 1994). We expected early season aphid populations in the cover crop system to attract Coccinellidae and other colonizing predators, providing either a source of predators that would enhance biocontrol in the subsequent maize crop (or elsewhere in the landscape), or conversely a predator sink if the cover crop was terminated when these colonizing predators are vulnerable to disturbance (i.e. the egg through pupal stages). The predator exclosure experiment demonstrated that aphids can thrive on a cereal rye cover crop if they are protected from predators. Aphid populations in the open cages were low, likely due to effective biological control by resident natural enemies, although we did not control for aphids lost to wandering. The lack of Coccinellidae in vacuum samples during the early season of our experiment is likely a result of these low aphid numbers. Coccinellidae exhibit a numerical response to aphid prey, especially in the early season (Evans and Youssef, 1992). Aphid populations in our cover crop system never reached critical levels to attract coccinellids (Donaldson et al., 2007) and support oviposition. Although we expected cover crops to act as either a source or sink of predators depending on the timing of cover crop termination, neither occurred in our experiment.

Finally, our results may have been influenced by sampling method. We relied on vacuum sampling while many previous

studies used pitfall traps (e.g. Lundgren and Fergen, 2010; Carmona and Landis, 1999). Vacuum sampling efficiently collects some resident invertebrate predators, such as spiders and rove beetles, but may be inefficient at sampling other predator groups, particularly mobile predators (Elliott et al., 2006). Therefore we might not have captured the full effect of the cover crop treatment on the predator communities because we were overestimating the resident predators compared to colonizing predators such as ladybeetles. However, visual estimates of Coccinellidae were also taken throughout the experiment (data not shown) and few ladybeetles were detected with this method as well.

In conclusion, our results show that planting a rye/pea cover crop in continuous maize systems grown for grain and stover did not alter biocontrol services under the conditions we studied. Additional research is needed to determine if earlier establishment of the cover crop (e.g. by interseeding into standing maize) would change the aphid—natural enemy balance in the spring sufficiently to result in the anticipated source-sink dynamics. In addition, reducing the amount of cover crop biomass removed as a bioenergy feedstock in the spring may allow the resident natural enemies to be relayed into the subsequent crop and potentially enhance biocontrol services. However, adding cover crops to continuous maize rotations can enhance other ecosystem services such as biomass productivity while maintaining soil organic matter, improving the environmental and economic factors associated with harvesting maize stover for cellulosic ethanol (Kim and Dale, 2005). These enhanced components of sustainability coupled with the neutral effects of cover crops on biological control services reported in this study, suggest their suitability for our region. Our study also confirms prior results showing that perennial biomass crops including switchgrass and restored prairie do enhance biocontrol (Werling et al., 2011a, 2011b), while also enhancing multiple other ecosystem services such as pollination and methane consumption (Werling et al., 2014).

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