Running title: Harvest effects on insect biodiversity and biocontrol services

Title: Harvesting biofuel grasslands has mixed effects on natural enemy communities and no effects on biocontrol services

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Summary

1. Perennial bioenergy systems, such as switchgrass and restored prairies, are alternatives to commonly used annual monocultures such as maize. Perennial systems require lower chemical input, provide greater ecosystem services such as carbon storage, greenhouse gas mitigation, and support greater biodiversity of beneficial insects. However, biomass harvest will be necessary in managing these perennial systems for bioenergy production, and it is unclear how repeated harvesting might affect ecosystem services.
2. In this study, we examined how repeated production-scale harvesting of diverse perennial grasslands influences vegetation structure, natural enemy communities (arthropod predators and parasitoids), and natural biocontrol services in two states (Wisconsin and Michigan, USA) over multiple years.
3. We found that repeated biomass harvest reduced litter biomass and increased bare ground cover. Some natural enemy groups, such as ground-dwelling arthropods, decreased in abundance with harvest whereas others, such as foliar-dwelling arthropods increased in abundance. The disparity in responses are likely due to how different taxonomic groups utilize vegetation and differences in dispersal abilities.
4. At the community level, biomass harvest altered community composition, increased total arthropod abundance, and decreased evenness but did not influence species richness, diversity, or biocontrol services. Harvest effects varied with time, diminishing in strength both within the season (for total abundance and evenness), across seasons (for evenness), or were consistent throughout the duration of the study (for community composition). Greater functional redundancy and compensatory responses of the different taxonomic groups may have buffered against the potentially negative effects

of harvest on biocontrol services.

5. *Synthesis and applications*: Our results show that in the short-term, repeated harvesting of perennial grasslands (when insect activity is low) has mixed effects on natural enemy communities and no discernable effects on biocontrol services. However, the long-term effects of repeated harvesting on natural enemies and other arthropod-derived ecosystem services such as pollination and decomposition remain largely unknown.

6. Keywords: Bioenergy, prairies, beneficial insect biodiversity, ecosystem services, perennial systems, landscape composition, ecosystem function.

Introduction

Agricultural simplification has been linked to reduced soil carbon storage (Robertson, Paul & Harwood 2000; Fargione *et al.* 2008), habitat degradation for animals (Gardiner *et al.* 2010; Robertson *et al.* 2011), and reduced water quality (Donner & Kucharik 2008). In recent years, the use of perennial systems for bioenergy production as alternatives to annual monocultures, such as soybean and corn, has received much attention as these systems provide numerous environmental benefits (Ceotto 2008; Webster *et al.* 2010; Werling *et al.* 2014). For example, perennial systems, such as switchgrass and restored prairies, require lower chemical input and reduced management efforts compared to conventional bioenergy crops resulting in increased carbon storage (Tilman, Hill & Lehman 2006), improved water quality (Costello *et al.* 2009), and reduced greenhouse gas emissions (Gelfand *et al.* 2013). Perennial systems also generally support a higher abundance and diversity of many taxa including plants, beneficial insects, and methanotrophic bacteria, leading to greater provisioning of ecosystem services such as pollination, natural biological control, and methane consumption (Werling *et al.* 2014). Furthermore, perennial systems can be grown on marginal lands (Dauber *et al.* 2012) reducing competition with food production (Tilman *et al.* 2009). As such, there is a great potential for perennial systems to be sustainable long-term alternatives to conventional bioenergy crops.

While the use of low-input, high-diversity perennial systems is promising for sustainable bioenergy production (Tilman, Hill & Lehman 2006), repeated harvesting will be necessary for management, which could have negative consequences for biodiversity and ecosystem services (BES). For example, biomass harvest in perennial systems could negatively affect plants and insects as large machinery needed to remove biomass could result in soil compaction, thus affecting plant establishment and growth (Carrow 1980; Schrama *et al.* 2012), and ground nesting insect activity (Sardiñas & Kremen 2014). Furthermore, the removal of aboveground biomass could eliminate important nesting and food resources for insects, potentially serving as ecological sinks if eggs and larvae are also removed with biomass and there is no immigration from outside sources (Kruess & Tscharntke 2002; Steffan-Dewenter & Leschke 2003). In contrast, harvesting could enhance BES by mimicking the beneficial effects of fire (Swengel 2001). For example, removing large amounts of aboveground biomass can reduce competition for space and light, fostering increase plant diversity (Williams, Jackson & Smith 2007; Jungers *et al.* 2015). Similarly, harvesting could alter microclimate factors, such as soil temperature and moisture, which could increase ground dwelling insect activity and potentially alter plant and insect phenologies (Ewing & Engle 1988; D'Aniello *et al.* 2011). These harvest-mediated changes in the microhabitat and plant community could strengthen bottom-up effects resulting in greater abundance and diversity of beneficial insects.

The effects of biomass harvest might vary with the foraging and dispersal ranges of arthropods and spatial context (Dempster 1991; Swengel 2001). For example, biomass harvest might have little effect on the abundance and diversity of highly mobile insects such as lady beetles and pollinators because they can easily move to adjacent, undisturbed habitats during harvest and recolonize afterwards. Thus, harvest effects might be transient if recolonization is quick and if the area of harvest is small relative to the dispersal ranges of the organisms (Humbert *et al.* 2012). Alternatively, for less mobile arthropods and life-stages such as eggs, larvae, and flightless adults, biomass harvest might have detrimental effects. In these cases, the surrounding landscape context might thus mediate these negative harvest effects. For example, perennial systems surrounded by natural habitat might minimize the detrimental effects of harvest because these natural habitats are sources for beneficial insects that can rescue declining local populations (Tscharntke *et al.* 2012; Gámez-Virués *et al.* 2015).

The objective of this study was to examine the effects of biomass harvest on vegetation structure, biodiversity of beneficial predatory and parasitic arthropods (hereafter "natural enemies"), and natural biocontrol services in mixed prairie-grasslands. Specifically, we asked the following questions: (1) Does repeated, annual fall harvesting affect grassland vegetation structure the following year?; (2) Does repeated biomass harvest affect the abundance and diversity of natural enemies and biocontrol services?; (3) How do harvest effects vary with time (both within and across years)?; and (4) Does landscape context mediate harvest effects on natural enemies and biocontrol? We hypothesized that repeated harvesting in the fall would have negative effects for arthropods the following growing season by removing important nesting habitats, killing eggs, or other non-mobile forms thus serving as ecological sinks. We also hypothesized that sites surrounded by a greater proportion of natural habitat could mitigate the negative effects of biomass harvest.

Material and methods

Experimental Design

We sampled arthropods in mixed prairie-grasslands in Wisconsin (WI) and Michigan (MI) from 2013 to 2015. We identified a list of 90 potential candidate sites that could be used for this study. Minimum site sizes and years since establishment were 0.30 km^2 and 5 years, respectively. Sites were mapped using GIS and we used the US Department of Agriculture (USDA) National Agriculture Statistics Service Cropland Data Layer (USDA 2013) to extract land cover information surrounding each site within a 1.5 km radius from site centers. The proportion of natural and semi-natural habitat in the landscape was calculated as the proportion of the total area covered by grasslands (perennial pasture, hay, and unmanaged/natural grasslands) and forest (deciduous, evergreen, mixed, shrub land, and woody wetland). These habitats (hereafter “natural habitats” for simplicity) are predominately perennial with very little management and disturbance. From the full list, a subset of 34 sites were selected such that they spanned a gradient of low to high proportion of natural habitats -- previously shown to influence biocontrol services (Werling et al. 2011). Next we randomly the harvest treatment (hereafter “harvest”) to half of the sites while the others were unmanipulated “control”, ensuring that the harvest and control treatments had similar landscape gradients. The final sites used for the study were largely public lands managed by US Fish and Wildlife Services (N = 19) and Department of Natural Resources (N = 8) but also included some private lands (N = 7). Sites spanned a gradient of natural habitat from 5.12 to 72.71% and ranged from 0.31-1.20 km^2 in size. While we do not have detailed management history information for all sites, we know that prior to the start of the experiment, sites were maintained as grasslands via burning, chemical, or mechanical removal of woody material for a number of years. However, the sites were not managed 3-7 years prior to the start of the experiment.

Biomass harvest was conducted at the end of the growing season with standard commercial equipment at the entire site level leaving approximately 30 cm of standing plant residue with all harvestable biomass removed from the field. All harvest events occurred in the fall of each year of the study (September to October), except for three sites in one year which were harvested in early spring (2014) rather than the previous fall (2013) because of unforeseen circumstances (e.g., wet weather, federal government shut-down). Harvesting at these sites was conducted soon after snow melt when no new growth would have been impacted by the spring harvest thus making these three sites comparable to the fall harvested sites. The duration of the experiments varied between the two states (3 years in WI; 2 years in MI). In WI, the first of three annual harvest treatments was applied in fall 2012 and post-harvest sampling occurred in 2013 (number of sites, N = 18), 2014 (N = 20), and 2015 (N = 18). In MI, the first of two harvest treatments was fall 2013 and post-harvest sampling occurred in 2014 (N = 12) and 2015 (N = 6).

Vegetation structure

We confined our vegetation and arthropod sampling to a 50 m x 100 m field area (0.005 km^2) at each site. To minimize edge effects, the sampling area was placed at least 50 m away from the site edge. To determine whether harvest influenced vegetation structure, plant cover was assessed monthly at each site in June, July, and August (hereafter "sampling round") at three permanent sampling stations (separated by 50 m). Plant cover was measured visually by estimating % bare ground (to the nearest 5%), % dead (litter) cover, % live forb cover, and % live grasses in quadrats (30 cm x 30 cm) placed randomly on the ground near the sampling station (~5 m apart, N = 3 quadrats per sampling station). Quadrats were placed in new locations around each sampling station for each sampling round. We also recorded the mean height of the litter, forb and grass layers by placing a meter stick at the center of the quadrat (N = 9 quadrats per sampling round per site).

To estimate plant biomass (g dry mass m^{-2}) at a site, we first multiplied the cover and mean height measurements of each of the vegetation categories to get an index of plant "volume" in a quadrat. Non-destructive biomass estimates were necessary to preserve the integrity of the fields from repeated sampling within- and across the growing seasons. We determined the relationships between each of the estimated plant volume index and plant biomass collected off-site (R^2 = 0.40 to 0.73, see Appendix S1 in Supporting Information).

Natural enemy diversity and biocontrol

Arthropod natural enemies were sampled at the same time vegetation was surveyed (June, July, and August). At each of the three permanent sampling stations per site, we placed one double-sided sticky trap (to capture flying insects), one pitfall trap (to capture ground-dwelling arthropods), one sweep transect (to capture foliar-dwelling arthropods). Each type of trap was separated by 5 m and each sampling station was separated by 50 m (N = 9 traps per site). To measure biocontrol potential, we placed one sentinel prey stand at each sampling station (N = 3 stands per site).

Yellow, sticky traps (15 cm x 30 cm, unbaited, Trecé Pherocon ®, Adair, Oklahoma, USA) were placed just above the vegetation and left out for two weeks continuously during each sampling round. Pitfall traps consisted of 1 L deli containers (10 cm diameter opening, Dart Conex®, Mason, Michigan, USA) filled ¾ full with 50:50 propylene glycol:water solution, placed flush with the ground, and covered with a 6 mm wire mesh to prevent small mammals and herpetofauna from falling into the traps. Plastic covers (30 cm diameter) were staked 10 cm above the traps to prevent rainfall from entering the cups. Pitfalls were also placed out for two weeks continuously during each sampling round. Sweep net sampling occurred along 1 m x 50 m belt transects (50 back and forth sweeps per transect) using a 38 cm diameter sweep net on sunny days with little wind (< 5 km per hour). All arthropods classified as natural enemies (predators or parasitoids known to attack arthropod herbivores) were counted and identified to the family level, superfamily levels for parasitic Hymenoptera, and order level for Arachnida (see Table S1, Borror, Triplehorn & Johnson 1992).

To measure biocontol potential, we used the approach of Werling et al. (2011), where we measured the removal rates of sentinel corn earworm (*Helicoverpa zea*) eggs. Eggs were obtained from a commercial rearing facility (Benzon Research Inc., Carlisle, Pennsylvania, USA) and were frozen prior to use to prevent hatching. Cluster of eggs (30 to 50 eggs) were placed on 2.5 cm x 2.5 cm index cards (hereafter "egg cards"). At each sampling station, there was a single egg card stand (1 m PVC pipe with a 30 cm x 30 cm white corrugated plastic platform affixed on top) with two egg cards placed underneath the platform. One egg card was exposed to natural enemies (“open” treatment) and the other “caged” treatment was protected from natural enemies using a 10 cm petri-dish cage to account for egg loss not due to predation (e.g., handling, desiccation). Eggs were placed in the field for 48 h (N = 3 paired egg card per site per sampling round). The percent egg removal rate, R was calculated separately for eggs in the open and caged treatments as R (open or closed) = 100 – [(initial – final number of eggs) / initial number of eggs]. To account for egg loss due to factors other than predation (e.g., desiccation, wind, and handling), R was then adjusted by using egg loss from the caged treatments for each pair. Therefore, the final removal rate, R_{final} was calculated as R (open cages) – R (closed cages). Rates of egg loss in closed cages were generally low (6.8% ± 0.81; mean ± SE).

Statistical analyses

We were interested in how vegetation structure and natural enemy communities at the field scale responded to the harvest treatment therefore each site was treated as an independent replicate. All vegetation and arthropod measurements were therefore averaged across the sampling stations within a site for a given sampling round. We combined the WI and MI datasets in order determine how biomass harvest affected biodiversity and ecosystem services within grasslands across a broad geographic region. Analyses were performed using R v3.03 (R Development Core Team 2014) unless otherwise stated.

To determine how vegetation structure was influenced by harvest, we used linear mixed-effects models (LMM) with harvest treatment (harvest, control) as a fixed effect and site as a random (intercept) effect. We included sampling round, year, and state, as fixed covariates in the model. The response variables were percent bare ground (arc-sine square-root transformed) and vegetation biomass (litter, forb, and grass analyzed separately, all ln-transformed). We evaluated all possible three- and two-way interactions with the harvest treatment. None of the three-way interactions were significant ($P \leq 0.05$), therefore they were dropped and we re-ran LMMs with only two-way interactions with harvest. We tested whether data met LMM assumptions (e.g., residuals normally distributed, homogeneity of variance) and assumed Gaussian distributions. We used the *nlme* package (Pinheiro *et al.* 2016) for LMM, and models were fit using maximum likelihood methods. Significance levels were assessed using Wald χ^2 tests with the *car* package (Fox & Weisberg 2011). We used (and report) Type 3 SS to test for significant interaction terms; if none were significant, we used (and report) Type 2 SS to test for significance of the main effects. Post-hoc comparisons of significant interactions terms were analyzed using the *lsmeans* package in R (Lenth 2016).

To determine how the natural enemy community was influenced by harvest, we used LMMs with the same predictors listed above and the response variables were total predator abundance (ln-transformed), family-level richness, predator diversity (Simpson's, 1-D), evenness (Pielou's), and predation rates, R_{final} (arc-sine square-root transformed). We also included the proportion of natural habitat (within 1.5 km radius from site centers) as a fixed covariate because some natural enemy groups have large foraging and dispersal ranges. We used a permutational MANOVA (PERMANOVA, Bray-Curtis dissimilarity) to examine how harvest treatment and covariates influenced natural enemy community composition. Models were fit and significance levels were assessed using the same procedures as above. We also used a similarity percentage analysis (SIMPER) to examine which taxonomic groups contributed to differences in community composition between the harvest and control natural enemy communities (Clarke 1993). Community composition was visualized using non-metric multidimensional scaling (NMDS, Bray-Curtis dissimilarity), specifying a two-axis solution. We determined the contributions of different taxonomic groups *within* the harvest and control treatments using the *envfit* function in the vegan package (Oksanen *et al.* 2015). We used the *RVAideMemoire* package in R (Hervé 2015) for the PERMANOVAs and PRIMER v7 (Clarke & Gorley 2015) for SIMPER.

Finally, we were interested in how different arthropod taxonomic groups responded to the harvest treatment. We used LMMs to examine how harvest treatment, sampling round, proportion of natural habitat, year, and state (all fixed effects) influenced the abundances of each of the following taxonomic groups separately; predatory foliar-dwelling beetles, parasitoids, flies, lace wings, true bugs, ground-dwelling beetles, ants, earwigs, and arachnids. Site was a random effect in the model. We ln-transformed all abundance data and assumed Gaussian distributions. All two- and three-way interactions with harvest treatment were evaluated (three-way interactions were eventually dropped), and we used the same procedures as above for model fitting and assessing levels of significance.

Results

Harvest effects on vegetation structure

Harvesting increased bare ground cover almost three fold from 3.2 % ± 1.2 (mean ± 1 SE) in control sites to 11.6% ± 1.6 in harvest sites. Harvest effects were stronger in 2015 compared to earlier years (Harvest x Year interaction: $\chi^2 = 6.05$, $df = 1$, $P = 0.01$, Fig. 1, see Table S1). Harvest also reduced litter biomass by half from 650 g dry wt m^{-2} (± 59.9) in control sites to 259 g dry wt m^{-2} (± 30.9) in harvest sites ($\chi^2 = 10.66$, $df = 1$, $P < 0.01$). Harvesting did not influence forb ($\chi^2 = 0.81$, $df = 1$, $P = 0.37$) or grass biomass ($\chi^2 < 0.01$, $df = 1$, $P = 0.99$). Instead, forb and grass biomass were affected by sampling round (forb: $\chi^2 = 27.19$, $df = 1$, $P < 0.01$; grass: $\chi^2 = 59.29$, $df = 1$, $P < 0.01$) and year (forb: $\chi^2 = 3.92$, $df = 1$, $P = 0.05$).

Harvest effects on natural enemy community metrics and biocontrol responses

Natural enemy communities were dominated by ants, parasitoids, crickets, and spiders making up 83.4% of all captured individuals (see Table S2). Although there were significant differences in natural enemy community structure within and across years and between states, there was nevertheless significant effects of harvest on the arthropod community metrics (Table 1). For example, harvest increased the total abundance of natural enemies but the effects were stronger at the start of the season in June compared to later in the season in August (33% increase in abundance in June in harvest sites versus no effect in August, Harvest x Sampling round: $\chi^2 = 3.69$, $df = 1$, $P = 0.05$, Table 1). Harvest also interacted with time to negatively influence evenness with the strongest negative effects occurring in June (Harvest x Sampling Round: $\chi^2 = 4.19$, $df = 1$, $P = 0.04$) and in 2013 (Harvest x Year: $\chi^2 = 4.77$, $df = 1$, $P = 0.03$). Harvest also altered community composition ($F_{1,212}$ = 5.71, P < 0.01). Community composition in both harvest and control sites were correlated with variation in ants and parasitoids (Fig. 2), however, control sites were also correlated with variation in spiders whereas harvest sites were correlated with variation in flies, true bugs, and ground beetles. Harvest and control sites were ~24% dissimilar in community composition; spiders, true bugs, ground beetles, and flies contributing to >53% of the variation between the two community types. Harvest did not affect family-level richness (χ^2 = 0.15, df = 1, P = 0.69, Table 1), diversity (χ^2 = 1.57, df = 1, P = 0.21), or predation rates (χ^2 = 0.10, df = 1, P = 0.75) nor did it interact with the proportion of natural habitat in the landscape to influence any of the community metrics.

Taxon-specific responses to harvesting

Biomass harvest affected each taxonomic group differently (Fig. 3, Tables S3 & S4). For foliar-dwelling insects, harvest generally increased their average abundances with the strongest effects in 2015 (Harvest x Year interaction: χ^2 = 4.03, df = 1, P = 0.05, see Table S3). Alternatively, harvest had generally negative main effects on average abundance of ground-dwelling insects, with the strongest effects in 2013 (Harvest x Year interaction: χ^2 = 4.65, df = 1, P = 0.03, see Table S4). Biomass harvest had consistent effects across years for some taxonomic groups. For example, there were positive main effect of harvest for true bugs (χ^2 = 4.55, df = 1, P = 0.03) and negative harvest effects for spiders (χ^2 = 31.41, df = 1, P < 0.01) across all treatment years. In contrast, harvest effects interacted with year for other groups. For example, harvesting affected some taxa in later years (e.g., 2.5 fold increase in fly abundance in harvested sites in 2015 only), while for other taxon (e.g., ants) harvest effects were only seen in the first year (75% increase in ant abundance in 2013 only). Biomass harvest interacted with the proportion of natural habitat to affect the abundances of spiders ($\chi^2 = 3.90$, $df = 1$, $P = 0.05$) and foliar-dwelling insects ($\chi^2 = 3.85$, $df = 1$, $P = 0.05$, Fig. 4). In particular, the proportion of natural habitats positively influenced average foliar insect abundance and negatively influenced spider abundances in harvest sites only; there were no relationships with landscape composition in control sites. Biomass harvest did not interact with the proportion of natural habitat in the landscape to influence most taxonomic groups or any of the community metrics (Tables S3 & S4).

Discussion

The use of perennial grasslands for bioenergy production may provide a sustainable alternative to annual biomass crops such as corn and soybean; however, it is unclear how management of such grasslands, in particular repeated harvesting, affects the biodiversity of natural enemies and biocontrol. In our study, conducted across two states and over multiple years, we found that harvesting grasslands affected vegetation structure resulting in generally negative effects on some ground-dwelling arthropods and positive effects on foliar dwelling arthropods. At the community level, biomass harvest increased total arthropod abundance, decreased evenness, and altered community composition but did not affect family-level richness, diversity, or predation rates. All together, these results suggest that harvesting grasslands for bioenergy production appears to have mixed and temporally-variable effects on natural enemy communities and no discernable impact on biocontrol services.

Harvest effects varied with taxonomic groups

Harvesting grasslands altered vegetation structure by removing litter biomass and increasing bare ground cover which subsequently can alter soil conditions such as pH, moisture, and temperature. These changes to the abiotic environment influenced natural enemies, but the magnitude and direction of harvest effects varied with taxonomic group. These varying responses may be due to the different ways in which natural enemies utilize the habitat and how harvesting impacts those habitat features (Warren, Scifres & Teel 1987; Debinski *et al.* 2011). For example, ground-dwelling predators were generally negatively affected by harvest; similar results were found in other haying studies (Cizek *et al.* 2011; Mazalova *et al.* 2015). Reduced abundances may be due to reduced litter biomass which provides cover, associated prey resources, pupation and nesting habitat for these ground-dwelling arthropods. Furthermore, ground-dwelling predators have relatively limited dispersal abilities compared to more mobile insects that could have escaped harvesting by utilizing adjacent undisturbed habitats and recolonizing after the harvest event (Morris & Rispin 1988; Baines *et al.* 1998). Ants, on the other hand, responded positively to harvest. Unlike the other litter-dwelling arthropods which were negatively affected by biomass removal, most of the ant species observed in this study nest underground (e.g., *Formica*, *Lasius, Aphaeogaster,* and *Myrmica* species) and were therefore unaffected by aboveground biomass removal *per se*. Instead, disturbance-mediated changes in soil temperature and moisture (Boulton, Davies & Ward 2005; Moranz et al. 2013) may have increased ant foraging activity compared to control sites resulting in greater ant abundances over the season.

In contrast, foliar-dwelling arthropod predators with greater dispersal capabilities and resource requirements were positively affected by harvest but only in later years. Foliar arthropods could have escaped the negative impacts of harvest by escaping to neighboring undisturbed areas and recolonized after the disturbance had passed (Swengel 2001). Harvest-mediated differences in plant community composition and productivity could also explain the positive arthropod responses. While we did not observe differences in forb and grass biomass between the control and harvest sites (though positive trends were observed), in a separate study conducted in the same experimental fields at the same time (Spiesman et al. 2016), harvest sites had greater plant diversity and different plant species composition compared to control sites. Greater plant diversity and productivity may have been due to increased availability of resources such as light and bare ground following harvest to allow subordinate plant species to colonize and/or persist (Antonsen & Olsson 2005; Foster et al. 2009; Questad et al. 2011). These harvest-mediated changes in plant community composition may have influenced natural enemies directly by providing additional food (e.g. nectar, pollen) and nesting resources, or indirectly by increasing insect herbivore abundances.

Harvest effects on community structure and biocontrol function

While biomass harvest influenced individual taxonomic groups differently, harvesting had mixed effects on the overall natural enemy community and no effect on predation rates despite repeated harvesting at large-production scales. These relatively weak harvest effects at the community level could be due to several reasons. First, insects within these grasslands have evolved a range of functional and numerical responses to disturbance with some taxonomic groups increasing or decreasing in abundances (Vogl 1974; Arenz & Joern 1996). Compensatory responses of the different taxonomic groups to harvest was observed in our study system which averaged out and resulted in weak overall effects at the community level (i.e., short-term effects on abundance and evenness and no effect on richness, diversity, and predation rates). We did, however, observe consistent differences in the composition of the natural enemy community with harvest suggesting that biomass harvest influenced species turnover and identity. While quantifying the extent to which harvest influences the degree of turnover or similarity (i.e., beta diversity) is beyond the scope of this paper, such analyses could reveal whether harvest increases or decreases diversity at larger spatial scales. This information could be useful to land managers and conservation biologists interested in understanding the role of disturbance in preserving biodiversity at regional scales (Vellend *et al.* 2007; Matthews & Spyreas 2010; Burkle, Myers & Belote 2016).

Second, harvest effects on some community metrics were short-lived or varied with time. For example, there were significant harvest effects on overall abundance (positive) and evenness (negative) in early summer (June) but those effects dissipated as the growing season progressed. There were also negative effects of harvest on evenness in the first year of the study (2013) but not in later years. High diversity of arthropods and greater functional redundancy in these perennial grasslands may have buffered against the potentially negative effects of harvest (the insurance hypothesis, Yachi & Loreau 1999) and/or may have allowed this system to recover faster to pre-disturbance (or control) levels. Compensation and redundancy in plant and arthropod responses have been observed in other diverse systems following disturbance such as fire, grazing, and haying (Daubenmire 1968; Walker, Kinzig & Langridge 1999; Swengel 2001; Debinski *et al.* 2011) leading to greater ecosystem stability. To determine whether resilience is unique to diverse systems such as prairies (the focal habitat of this study), a similar study in low diversity grasslands such as switchgrass or *Miscanthus* would help elucidate whether our findings were generalizable to all perennial grasslands or limited to diverse prairie systems.

Lastly, annual fall harvest may not represent a strong disturbance event in these grasslands, compared to fire which completely remove above-ground biomass and potentially harms below ground propagules (Bulan & Barrett 1971; Swengel 1996, 2001). While biomass harvest in this study was conducted at a large spatial scale (production-scale) and repeated annually, harvest occurred once per year during a period of low insect activity (late fall). Both ground-dwelling and foliar-feeding insects may have escaped the impacts of harvest by seeking refuge or overwintering in areas protected from the disturbance event (e.g. underground, underneath rocks, habitat edges, Swengel 2001). A higher frequency of harvest or during a period when insect activity is at its peak may elicit stronger community-level responses (Swengel 2001). Next, this study was conducted over a relatively brief period (3 years), therefore the long-term consequences of harvest for insect communities are not yet known. Lag times in insect responses to harvest may exist where repeated removal of above-ground biomass (along with eggs and larvae) over the long term might eventually suppress insect populations directly (Swengel

2001) or indirectly through their effects on plant communities (Foster *et al.* 2010). Lastly, this study was conducted in grasslands that were managed for a number of years prior to the start of the experiment. The arthropod community may have already been composed of disturbance-resistance species (i.e., species with ability to escape or tolerate disturbance) at the start of the study and therefore resistant to subsequent disturbance events. Arthropod communities in unmanaged or natural grasslands may be more sensitive to environmental change and therefore more susceptible to annual harvesting, especially if they are largely composed of low-dispersing species. Detailed management history data or conducting this study in previously unmanaged grasslands would help elucidate the extent to which management history plays a role in arthropod community recovery following disturbance.

Landscape effects on natural enemy communities

Previous work has demonstrated that local natural enemy communities are generally positively affected by the amount of natural habitat in the surrounding landscape (reviewed by Chaplin-Kramer *et al.* 2011); however, many of these studies were conducted in low-diversity habitats such as monoculture corn and soybean. We predicted that natural habitats surrounding our harvest sites could mitigate the potentially negative effects of harvest by increasing the likelihood of rescue effects therefore we predicted stronger landscape effects in harvest sites. In this study, we observed significant positive effects of the proportion of natural habitat on mean foliar insect abundances and negative effects on spider abundances in the harvest sites only. These relationships could be due to rescue effects from the surrounding source habitats (natural habitats for foliar insects and cropland for the spiders). We did not see any relationships between the proportion of natural habitat and arthropod abundances in the control sites, for any community metric, and for most taxonomic groups. For a diverse grassland community such as prairies, the amount of natural habitat surrounding a local area might not be as important at the community level compared to landscape configuration features such as connectivity, spatial arrangement, and fragment size (Wiens 1976; Stoner & Joern 2004; Tscharntke *et al.* 2012; Rösch *et al.* 2013). For example, grasslands isolated from other grasslands might show a stronger negative response to harvesting as recolonization from the surrounding area following disturbance might be slow, particularly for weak dispersers (Rösch *et al.* 2013). Therefore, understanding how harvest interacts with landscape configuration (rather than amount of natural habitat *per se*) might provide a more complete picture of harvest impacts in bioenergy landscapes.

Conclusions

In this study, late-season harvest affected vegetation structure and natural enemy communities. Populations of specific taxa were affected differently likely in part due to variation in natural history and ways in which they utilize the habitat. We did not see any harvest effects on biocontrol services which we hypothesize is due to compensatory responses of the different taxonomic groups and functional redundancy within natural enemy communities. Landscape complexity, measured as the amount of natural habitat surrounding the crop field, did not affect local arthropod community structure. Other landscape features such as isolation, amount of edges, and fragment size which were not evaluated in this study, might interact with harvest to affect local arthropod communities.

While this study spanned multiple years and across a large geographic area, an additional caveat is long-term effects of harvest on vegetation structure, arthropod communities, and biocontrol services remains unknown. Nevertheless, grassland communities may be resilient to annual disturbances such as harvesting and their use for bioenergy production may have relatively small negative consequences for BES.

Although BES appear not to be significantly affected by harvesting over the short-term, other responses are worth considering. For example, the removal of aboveground biomass could negatively affect stem-nesting pollinators and associated pollination services (Buri, Humbert & Arlettaz 2014). Furthermore, increased bare ground cover associated with biomass removal could increase soil erosion and surface runoff thus affecting water quality (Kort, Collins & Ditsch 1998), increase invasion of weeds and non-native plants (Zedler 2009), or alter soil microbe communities thus affecting decomposition and nutrient availabilities (Xue *et al.* 2016). Therefore, measuring biodiversity responses of other taxonomic groups and ecosystems services will allow us to broaden our understanding of how biomass harvest might impact grassland ecosystems. Expanding our understanding of the various community and ecosystem responses in grasslands to various biomass cropping system management options, such as harvesting, will allow us to determine whether perennial grassland systems used for bioenergy production are a significant improvement over traditional annual monoculture systems that are widely used today.

Author Contributions: This study was planned and designed by Tania N. Kim, Timothy

Meehan, Claudio Gratton and Doug Landis. The experiments were performed by Tania Kim, Aaron Fox and Bill Wills. Data analysis and main writing of the manuscript were carried out by Tania Kim.

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Data accessibility

Data used in the linear mixed effects model and site locations will be uploaded to DRYAD upon acceptance of the manuscript.

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Table 1. Biomass harvest effects on (a) natural enemy abundance (ln-transformed), (b) family-level richness, (c) Simpson's diversity, (d) Pielou's evenness, (e) community composition, and (f) predation rates (arcsine square-root transformed) in perennial grasslands in WI and MI. Parameter estimates and 1 SE (in parentheses) estimated from linear mixed effects models. Significance was determined using a Wald Chi-square statistic for all tests except community composition where a *F*-statistic was used in the PERMANOVA. Bold font represents significant effects ($P \leq 0.05$).

	A. Abundance			B. Richness			C. Diversity		
Variables	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 3 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS
Harvest	176.66 (196.98)	0.89	0.34	403.85 (438.56)	0.15	0.69	-86.40 (50.87)	1.57	0.21
Sampling Round	-0.05 (0.06)	9.17	**<0.01**	0.20 (0.13)	0.74	0.38	0.029 (0.02)	17.33	**<0.01**
Year	0.25 (0.06)	18.92	**<0.01**	0.52 (0.15)	15.68	**<0.01**	0.022 (0.02)	11.99	**<0.01**
State	0.23 (0.18)	2.81	0.09	0.02 (0.42)	0.08	0.76	-0.05 (0.06)	1.83	0.17
Proportion Natural Habitat	0.01 (0.01)	0.65	0.42	<0.01 (0.02)	0.07	0.78	< -0.01 (< 0.01)	<0.01	0.97
Harvest : Sampling Round	-0.16 (0.08)	3.69	**0.05**	-0.24 (0.19)	1.61	0.20	0.03 (0.02)	2.40	0.12
Harvest : Year	-0.08 (0.09)	1.07	0.30	-0.20(0.21)	0.95	0.32	0.04 (0.03)	3.14	0.07
Harvest : State	0.20 (0.37)	0.53	0.46	-0.14 (0.85)	1.08	0.29	-0.07 (0.12)	0.40	0.52
Harvest : Prop. Natural Habitat	-0.01 (0.01)	0.99	0.31	<0.01 (0.04)	1.29	0.25	< 0.01 (< 0.01)	0.18	0.66

Table 1 (continued)

	D. Evenness			E. Community composition			F. Predation rate		
Variables	Estimate (SE)	χ2 *df* = 1	*P* Type 3 SS	SS	*F* *df* = 1,212	*P* Type 2 SS	Estimate (SE)	χ2 *df* = 1	*P* Type 2 SS
Harvest	-112.24 (52.51)	4.78	**0.04**	0.13	5.71	**<0.01**	-44.87 (138.41)	0.10	0.75
Sampling Round	0.02 (0.02)	2.09	0.15	0.13	5.65	**<0.01**	0.19 (0.04)	58.65	**<0.01**
Year	< 0.01 (0.01)	0.09	0.76	0.49	20.59	**<0.01**	0.07 (0.04)	5.95	**0.02**
State	-0.05 (0.06)	0.52	0.48	0.18	7.69	0.74	-0.39 (0.12)	10.25	**<0.01**
Proportion Natural Habitat	<-0.01 (<0.01)	0.28	0.60	0.05	2.07	0.48	<0.01 (<0.01)	0.63	0.43
Harvest : Sampling Round	0.05 (0.02)	4.19	**0.04**	0.02	0.85	0.40	0.06 (0.06)	1.07	0.30
Harvest : Year	0.06 (0.02)	4.77	**0.03**	0.03	1.51	0.12	0.02 (0.06)	0.11	0.74
Harvest : State	-0.06 (0.12)	0.24	0.63	0.03	1.18	0.08	0.19 (0.25)	0.56	0.45
Harvest : Prop. Natural Habitat	<0.01 (<0.01)	0.09	0.76	0.04	1.78	0.23	-0.01 (0.01)	1.29	0.26

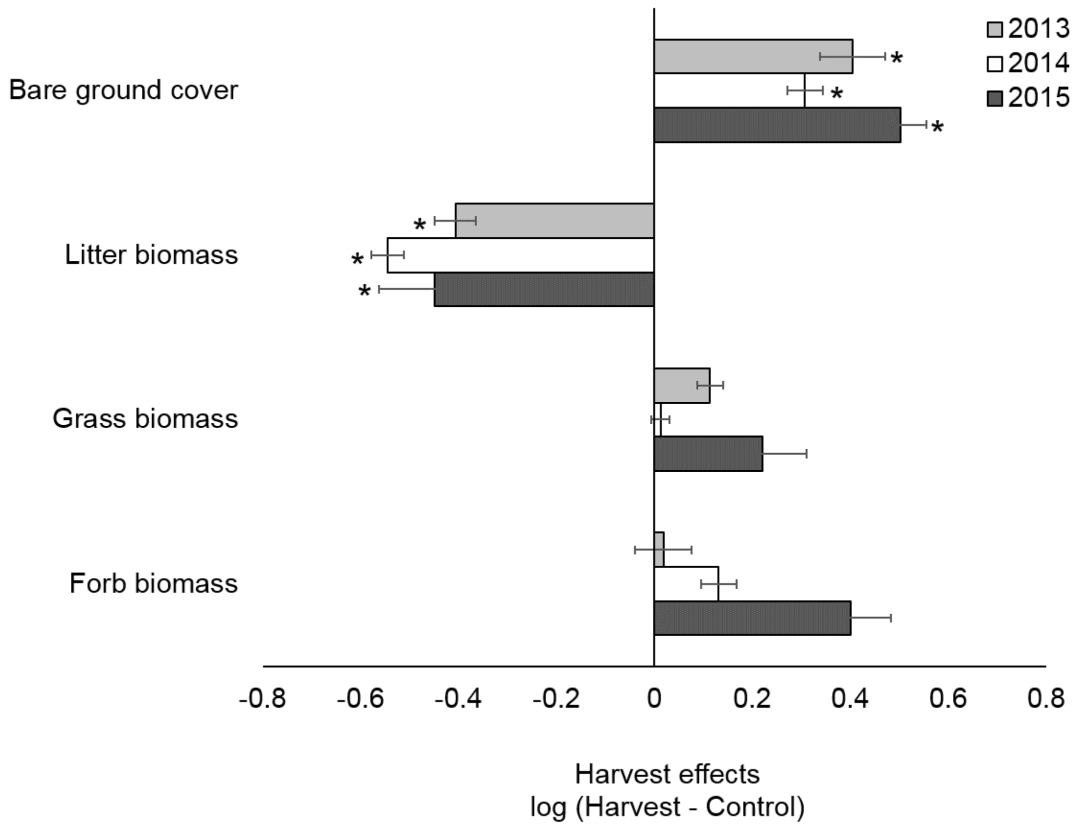

Figure 1. Biomass harvest effects on bare ground cover, litter biomass, forb biomass, and grass biomass in perennial grasslands in Michigan and Wisconsin. Cover and biomasses were averaged across the growing season and states for any given year. Harvest effects (x-axis) determined as the log-transformed difference between the mean harvest and mean control responses (log (harvest – control)). Asterisks indicate significant harvest treatment effects on each of the response variables in a particular year from the linear mixed effects models and post-hoc comparisons ($P \leq 0.05$, see Table S1). Error bars represent ± 1 SE from all pairwise differences between all the harvest and control sites.

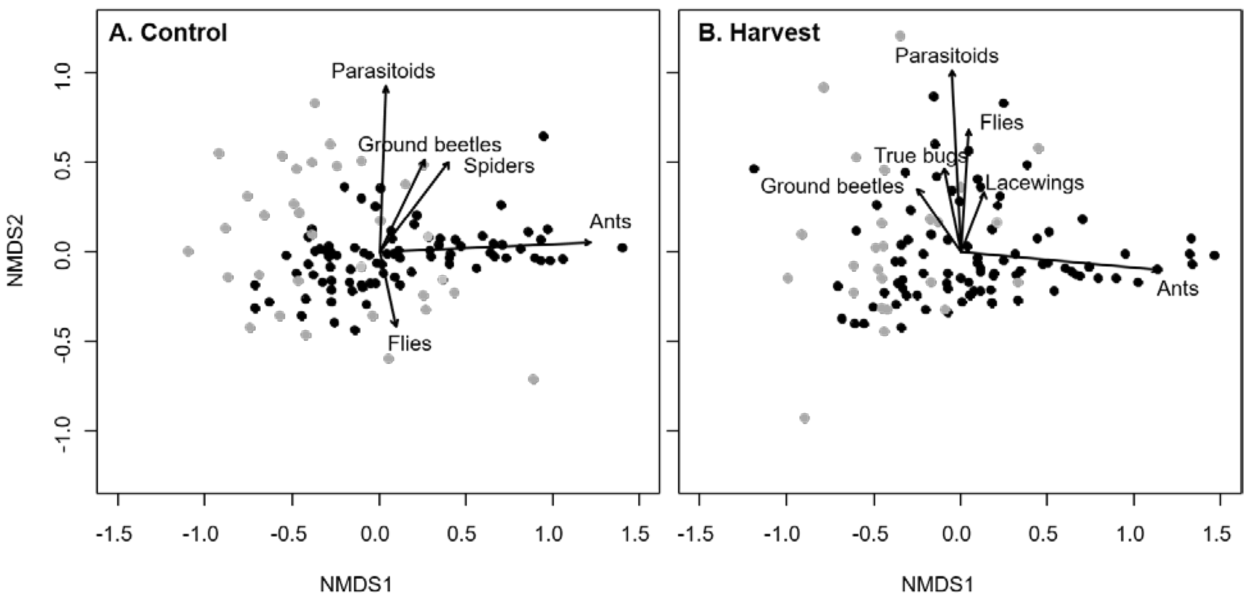

Figure 2. Ordination of natural enemy community composition using non-metric multidimensional scaling (NMDS, Bray-Curtis dissimilarity) of natural enemy abundance data (2013-2015) at (a) control and (b) harvest sites. Vectors represent taxa that significantly correlate to variation in community composition ($P \leq 0.05$). Points represent natural enemy communities at each site per sampling round per year. Grey points are sites in Michigan; black points are sites in Wisconsin.

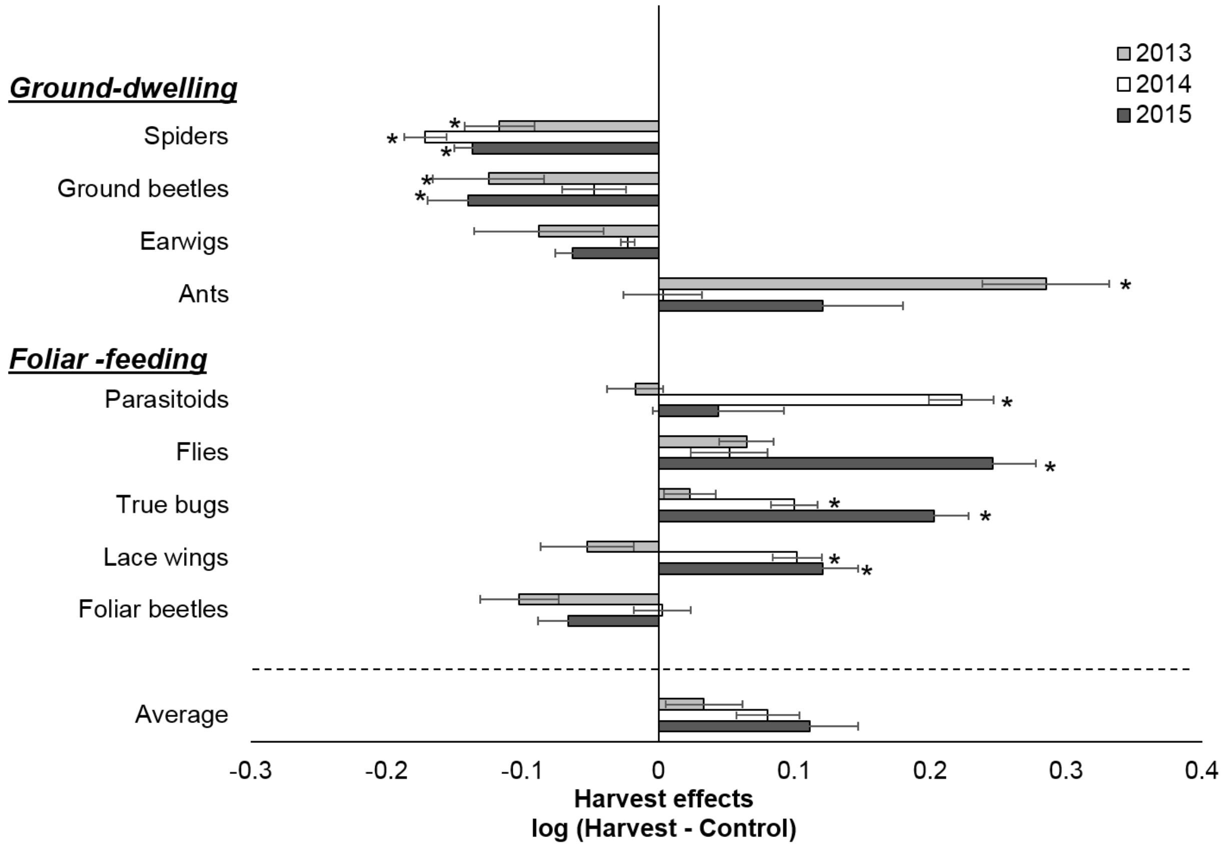

Figure 3. Average and taxon specific abundance responses of natural enemies to biomass harvest in Michigan and Wisconsin. Abundances were averaged across the growing season and state in a given year. Harvest effects (x-axis) determined as the log-transformed difference between the mean harvest and mean control responses (log (harvest – control)). Asterisks denote significant difference between control and harvest treatments in a particular year from linear mixed effects models and post-hoc comparisons ($P \leq 0.05$, Tables S3 & S4). Error bars represent ± 1 SE from all pairwise differences between the harvest and control sites.

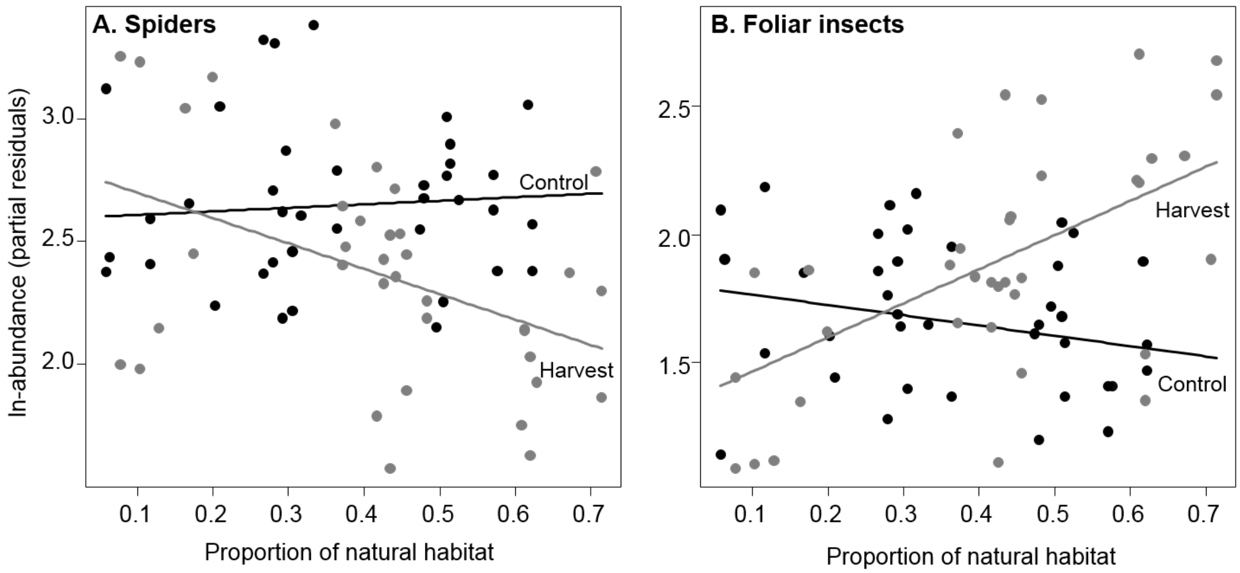

Figure 4. Relationships between the proportion of natural habitat and the abundances of (A) spiders and (B) foliar-dwelling natural enemies in Michigan and Wisconsin. Each point represents mean abundances per site per sampling year. All abundances were ln-transformed and partial residuals are shown on the y-axes. Grey points and lines are harvest sites; black points and lines are control sites. Significance determined from linear mixed effects models and post-hoc comparisons (Tables S3 & S4). Both plots show significant harvest treatment x proportion of natural habitat interactions ($P \leq 0.05$).

Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1. Relationships between plant volume index and actual biomass.

Table S1. Biomass harvest effects on bare ground cover and plant biomass.

Table S2. Specimen list of captured individuals identified to the family or super family levels.

Table S3. Foliar dwelling arthropod responses to biomass harvest.

Table S4. Ground dwelling arthropod responses to biomass harvest.

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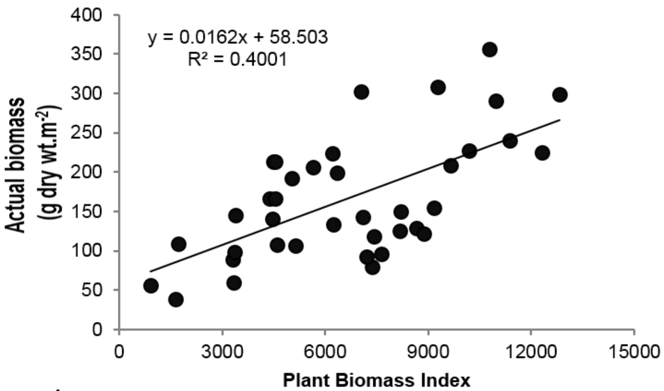
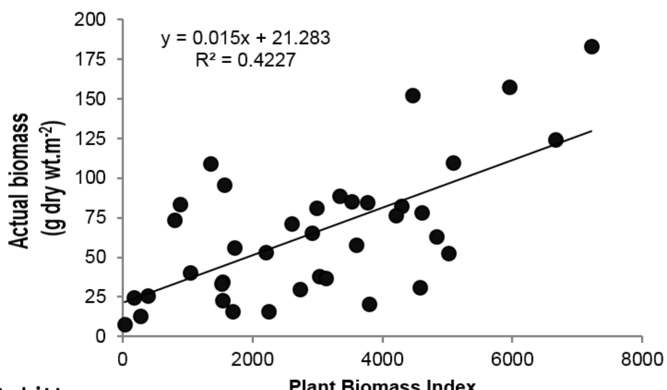
Appendix S1. Relationships between plant volume index (% cover x height) and actual biomass.

In 2015, we determined the relationships between biomass estimates and actual biomass in a subset of sites in WI (N = 10 sites). At each site, we estimated biomass cover (%) and measured the height of each vegetation category (litter, forb, and grass) in four quadrats (30 cm x 30 cm) located off site (> 50 m from site edge) in June and August when plant biomass is relatively low and high, respectively. We harvested biomass from these quadrats and placed them in a 60 ºC drying oven for at least 48 h. Biomass was separated into the three vegetation categories and weighed. The relationships between estimated biomass (plant volume index, % cover x height) and actual dry biomass (g dry wt.m^{-2}) of grasses (A), forb (B), and litter or dead biomass (C) are shown below.

A. Grass



B. Forb



C. Litter

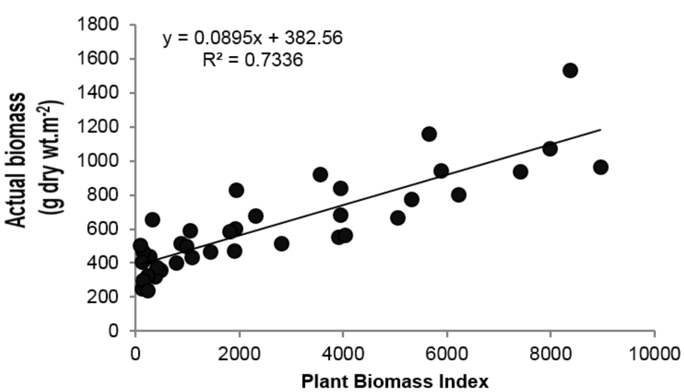


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Table S1. Biomass harvest effects on (A) bare ground cover (arcsine square-root transformed), (B) litter biomass (ln-transformed), (C) forb biomass (ln-transformed), and (D) grass biomass (ln-transformed) in perennial grasslands in Michigan and Wisconsin. Parameter estimates and 1 SE (in parentheses) estimated from linear mixed effects models. Significance was determined using Wald Chi-square statistics. Bold font represents interpretable significant effects ($P \leq 0.05$).

	A. Bare Ground			B. Litter biomass			C. Forb biomass			D. Grass biomass		
Variable	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 3 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS
Harvest	-119.29 (49.42)	6.05	0.01	435.75 (337.5)	10.66	**<0.01**	-163.83 (393.02)	0.81	0.37	295.45 (208.01)	<0.01	0.99
Sampling Round	<0.01 (0.01)	0.42	0.52	-0.01 (0.16)	1.21	0.27	0.50 (0.12)	27.19	**<0.01**	0.33 (0.06)	59.29	**<0.01**
Year	<0.01 (0.01)	0.23	0.63	-0.31 (0.19)	10.33	**<0.01**	-0.23 (0.13)	3.92	**0.05**	0.02 (0.07)	1.00	0.32
State	-0.12 (0.07)	2.97	0.09	0.81 (0.55)	4.30	**0.04**	-0.27 (0.47)	0.51	0.48	-0.04 (0.24)	0.15	0.70
Harvest : Sampling Round	0.02 (0.02)	0.82	0.37	0.28 (0.23)	1.48	0.22	-0.13 (0.17)	0.68	0.41	0.03 (0.09)	0.14	0.71
Harvest : Year	0.06 (0.02)	6.05	**0.01**	-0.22 (0.26)	0.69	0.41	0.08 (0.19)	0.18	0.67	-0.15 (0.10)	2.10	0.15
Harvest : State	0.12 (0.10)	1.62	0.20	0.01 (0.80)	<0.01	0.99	0.07 (0.68)	0.01	0.91	0.23 (0.35)	0.45	0.50

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Table S2. Total abundances of natural enemies captured from pitfall, sticky, and sweep net traps in Wisconsin (2013-2015) and Michigan (2014-2015). Most specimen were identified to the family level. Parasitic wasps were identified to the family and superfamily level. Arachnids were identified to the order level.

Beetles	Total
Coccinellidae	684
Lampyridae	727
Cantharidae	1081
Carabidae	4660
Staphylinidae	3380

Flies	Total
Syrphidae	3889
Dolichopodidae	8972

Parasitic wasps	Total
Braconidae	1037
Ichneumonidae	999
Ceraphronoidea	292
Platygastroidea	2054
Cynipoidea	197
Prototrupoidea	88
Chalcidoidea	9967
Mymarommatoidea	7

True bugs	Total
Anthocoridae	639
Nabidae	577

Lace wings	Total
Chrysopidae	261
Hemerobiidae	52

Arachnids	Total
Opiliones	6328
Araneae	26172

Others	Total
Formicidae	74238
Gryllidae	43056
Forficulidae	241

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Table S3. Average and taxon-specific responses of foliar dwelling/feeding arthropods to the harvest treatment in Michigan and Wisconsin. All abundances were ln-transformed to meet GLM assumptions. Parameter estimates and 1 SE (in parentheses) estimated from linear mixed effects models. Significance was determined using Wald Chi-square statistics (df = 1). Bold font represents significant effects ($P \leq 0.05$).

	Average			Foliar beetles			Flies		
Variable	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 3 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 3 SS
Harvest	-389.14 (198.36)	4.03	**0.05**	-192.48 (250.5)	1.48	0.22	-539.53 (283.89)	1.19	0.28
Sampling Round	0.141 (0.06)	5.59	**0.02**	0.11 (0.07)	1.18	0.28	0.11 (0.08)	0.60	0.44
Year	0.31 (0.07)	20.50	**<0.01**	0.17 (0.08)	13.72	**<0.01**	0.24 (0.10)	30.19	**<0.01**
State	-0.34 (0.22)	2.45	0.12	-0.45 (0.19)	7.61	**0.01**	-0.33 (0.35)	1.31	0.25
Proportion Natural Area	<-0.01 (0.01)	0.75	0.39	0.01 (<0.01)	2.15	0.14	-0.01 (0.01)	0.03	0.85
Harvest : Sampling Round	-0.03 (0.09)	0.15	0.70	-0.12 (0.11)	1.26	0.26	-0.13 (0.12)	1.10	0.29
Harvest : Year	0.19 (0.10)	4.03	**0.05**	0.09 (0.12)	0.62	0.43	0.28 (0.14)	3.77	**0.05**
Harvest : State	-0.18 (0.37)	0.25	0.62	0.03 (0.40)	0.01	0.93	<0.01 (0.68)	<0.01	0.99
Harvest : Prop. Natural	0.02 (0.01)	3.85	**0.05**	<-0.01 (0.02)	0.21	0.65	0.04 (0.03)	1.84	0.18

	Parasitoids			True bugs			Lace wings		
Variable	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 2 SS	Estimate (SE)	$\chi2$ *df* = 1	*P* Type 3 SS
Harvest	-206.65 (305.08)	3.407	0.07	327.5 (81.95)	4.55	**0.03**	-331.88 (166.19)	4.17	0.04
Sampling Round	0.22 (0.09)	13.88	**<0.01**	0.05 (0.05)	1.03	0.31	0.07 (0.05)	2.08	0.15
Year	0.45 (0.10)	46.85	**<0.01**	0.06 (0.02)	5.52	**0.02**	-0.06 (0.05)	1.25	0.26
State	-0.3 (0.29)	0.11	0.74	-0.14 (0.22)	0.09	0.76	0.28 (0.23)	1.56	0.21
Proportion Natural Area	<0.01 (0.01)	0.43	0.51	0.01 (0.01)	0.61	0.43	<0.01 (0.01)	0.14	0.71
Harvest : Sampling Round	0.05 (0.13)	0.13	0.72	-0.03 (0.07)	0.18	0.67	-0.09 (0.07)	1.59	0.21
Harvest : Year	0.10 (0.15)	0.48	0.49	0.16 (0.09)	3.40	0.07	0.16 (0.08)	4.17	**0.04**
Harvest : State	0.87 (0.59)	2.26	0.13	0.34 (0.42)	0.67	0.42	-0.21 (0.44)	0.24	0.63
Harvest : Prop. Natural	-0.04 (0.03)	2.43	0.12	-0.01 (0.02)	0.89	0.35	0.02 (0.02)	0.99	0.32

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Table S4. Average and taxon-specific responses of ground dwelling/feeding arthropods to the harvest treatment in Michigan and Wisconsin. All abundances were ln-transformed to meet GLM assumptions. Parameter estimates and 1 SE (in parentheses) estimated from linear mixed effects models. Significance was determined using Wald Chi-square statistics. Bold font represents significant effects ($P \leq 0.05$).

	Average			Spiders			Ground beetles		
Variable	Estimate (SE)	$\chi2$ $df = 1$	*P* Type 3 SS	Estimate (SE)	$\chi2$ $df = 1$	*P* Type 3 SS	Estimate (SE)	$\chi2$ $df = 1$	*P* Type 2 SS
Harvest	451.57 (214.15)	4.66	**0.03**	131.00 (89.43)	31.41	**<0.01**	157.70 (252.59)	0.49	0.49
Sampling Round	-0.10 (0.07)	2.18	0.14	-0.06 (0.05)	2.76	0.10	0.01 (0.07)	1.11	0.29
Year	0.21 (0.07)	8.59	**<0.01**	0.16 (0.06)	7.79	**0.01**	0.47 (0.08)	48.88	**<0.01**
State	0.70 (0.23)	9.39	**<0.01**	0.45 (0.15)	16.84	**<0.01**	0.07 (0.35)	0.20	0.65
Proportion Natural Area	<-0.01 (0.01)	<0.01	0.99	0.01 (<0.01)	0.49	0.49	0.02 (0.02)	2.21	0.14
Harvest : Sampling Round	-0.20 (0.09)	4.73	**0.03**	<-0.01 (0.08)	<0.01	0.97	-0.13 (0.10)	1.63	0.20
Harvest : Year	-0.22 (0.11)	4.65	**0.03**	-0.06 (0.09)	0.50	0.48	-0.08 (0.12)	0.41	0.52
Harvest : State	-0.25 (0.38)	0.45	0.50	0.27 (0.30)	0.86	0.35	-0.73 (0.67)	1.22	0.27
Harvest : Prop. Natural	<0.01 (0.01)	0.12	0.74	-0.03 (0.01)	3.90	**0.05**	<0.01 (0.03)	0.01	0.94

	Ants			Earwigs		
Variable	Estimate (SE)	$\chi2$ $df = 1$	*P* Type 3 SS	Estimate (SE)	$\chi2$ $df = 1$	*P* Type 2 SS
Harvest	587.29 (286.55)	4.40	**0.04**	-57.83 (116.95)	0.92	0.34
Sampling Round	-0.18 (0.08)	4.84	**0.03**	<-0.01 (0.03)	0.07	0.79
Year	0.20 (0.10)	4.30	**0.04**	-0.08 (0.04)	6.82	**0.01**
State	0.96 (0.39)	6.23	**0.01**	0.21 (0.17)	1.29	0.26
Proportion Natural Area	0 (0.01)	<0.01	0.99	<-0.01 (<0.01)	0.26	0.61
Harvest : Sampling Round	-0.16 (0.12)	1.91	0.17	-0.01 (0.05)	0.07	0.79
Harvest : Year	-0.29 (0.14)	4.38	**0.04**	0.03 (0.05)	0.26	0.61
Harvest : State	1.33 (0.75)	3.28	0.07	-0.18 (0.32)	0.31	0.58
Harvest : Prop. Natural	-0.06 (0.03)	3.33	0.07	<0.01 (0.01)	0.08	0.78