

Landscape composition influences pollinators and pollination services in perennial biofuel plantings



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ARTICLE INFO

Article history:

Received 14 August 2013

Received in revised form 23 March 2014

Accepted 14 April 2014

Available online 27 May 2014

Keywords:

Pollination
Ecosystem services
Sunflower
Native bees
Grassland
Landscape context

ABSTRACT

Biofuel cropping systems are considered a potential source of renewable energy and an integral component of a sustainable energy policy. The type of biofuel crop selected for production has the capacity to substantially alter landscape composition, affecting biodiversity conservation and ecosystem services. To understand how increasing production of perennial grasses for biofuels may affect pollinators and pollination services, we identified 20 agricultural fields that varied in their proportion of surrounding grassland cover. Bees and pollination services were measured at each site to determine how bee abundance, diversity, and community composition responded to increasing proportions of grassland, and to quantify how pollination services changed as the proportion of grassland increased in the landscape. Bees were collected from sentinel sunflowers, and pollination services were measured by comparing seed set from open and closed sunflowers at each site. Landscape composition had a significant effect on bee abundance, diversity, and community composition with a greater abundance of bees and a more diverse bee community found visiting flowers at sites with more of the surrounding landscape in perennial grassland. In contrast, the bee community in low grassland sites was dominated by *Apis mellifera*, suggesting that pollination in these landscapes may be more sensitive to declines in this species. Despite these differences, the level of sunflower pollination was similar across sites, even though the bee community responded to changes in landscape composition. Increasing grassland cover through the addition of perennial biofuel plantings would be expected to support a more diverse bee community and a greater abundance of bees, yielding reliable pollination services.

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1. Introduction

Biofuel cropping systems are one alternative to non-renewable energy sources and are seen as an integral component of a sustainable energy policy. In the United States, an annual target of 35 billion gallons of ethanol by 2022 has been proposed, including a mandate that 57% of this capacity come from renewable fuels such as bioenergy crops (Tyner, 2008). Annual crops such as corn and perennial crops such as mixed grasslands are being investigated as potential bioenergy crops. Large scale changes in land cover are a potential consequence of future expansion of bioenergy crop production, which may have significant impacts on biodiversity. As a result, biofuel production systems are being compared for their ability to produce biomass while also conserving biodiversity and the provision of multiple ecosystem services (James et al., 2010;

Landis and Werling, 2010; Wu et al., 2012; Jarchow and Liebman, 2012).

Incorporating perennial biofuel crops such as native grasslands into agricultural landscapes has the potential for significant effects on biodiversity and the ecosystem services provided to agricultural systems. Empirical studies have consistently found that increasing plant diversity positively impacts arthropod diversity (Siemann et al., 1998; Knops et al., 1999; Koricheva et al., 2000; Scherber et al., 2010), and recent research on biofuel cropping systems also revealed that annual and perennial biofuel crops differentially support beneficial arthropods (Werling et al., 2011). In particular, the abundance and diversity of pollinators were higher in perennial biofuel crops (e.g. prairie and switchgrass) compared to the annual bioenergy crop corn (Gardiner et al., 2010). Developing bioenergy cropping systems that are productive yet support diverse pollinator communities may offer a strategy for energy production that concurrently conserves biodiversity and promotes ecosystem services that are valuable to agricultural and semi-natural landscapes. In agricultural landscapes across the United States, crop pollination is valued at \$3 billion per year (Losey and Vaughan, 2006), while

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globally approximately 87% of flowering plants rely on pollination for fruit and seed set (Ollerton et al., 2011). Although the value of pollination services provided to natural systems has yet to be quantified, it is expected to exceed the value provided to agricultural systems (LeBuhn et al., 2013).

The selection of bioenergy crops for expanded production has the potential to affect pollinators by modifying the diversity and composition of agricultural landscapes. At the local (field) scale, bioenergy crops in the Upper Midwest region of the United States will range from monocultures of annual crops, such as corn, to diverse mixtures of perennial grasses and forbs. The selection of bioenergy crops will substantially affect plant diversity at local scales, influencing the availability of pollen, nectar, and nesting habitat which are primary drivers of pollinator abundance and diversity (Holzschuh et al., 2011; Stanley and Stout, 2013; Nicholls and Altieri, 2013). At larger spatial scales, the addition of bioenergy crops will also change landscape composition. Rising corn prices have recently spurred the conversion of marginal lands into annual bioenergy crops such as corn, which has expanded monoculture plantings and lowered landscape diversity (Wright and Wimberly, 2013). In contrast, adding diverse perennial grassland mixtures could increase landscape diversity and augment the amount of semi-natural habitat needed to support beneficial organisms. Agricultural systems with higher proportions of semi-natural habitat and greater diversity of land use are positively correlated with bee abundance and diversity (Holzschuh et al., 2007; Klein et al., 2012; Kennedy et al., 2013). Furthermore, the proportion of semi-natural habitat in the landscape surrounding pollinator-dependent crops seems integral to maximizing pollination due to the food and nesting resources available to support native bee populations (Winfree et al., 2007; Klein et al., 2012; Holzschuh et al., 2012). These results suggest that diverse perennial bioenergy cropping systems have the potential to facilitate pollinator conservation and increase pollination services across broad spatial scales.

Understanding how the expansion of different biofuel cropping systems will shape landscape composition and subsequently affect biodiversity and ecosystem services will be critical to developing a sustainable bioenergy policy. Our aim in this research was to determine whether pollinators and pollination services vary in response to the amount of perennial grasslands in the surrounding landscape. We selected 20 agricultural fields along a gradient of low to high amounts of perennial grassland in the surrounding landscape to sample bees and measure pollination services. We predicted that (1) bee abundance and diversity would be positively correlated with the amount of perennial grassland, (2) bee community composition would change across the gradient of low to high grassland cover, and (3) pollination services would increase as the proportion of grassland increased in the landscape surrounding fields.

2. Methods

2.1. Study sites

To measure the effect of landscape composition on bees and pollination services, we identified 20 soybean fields in Michigan, USA that varied in their proportions of surrounding perennial grassland and were at least 3 km apart (Fig. 1). Soybean fields were used as study sites because they occur in various landscape contexts, and the soybean crop, a monoculture intensively managed for weeds, limits variability (e.g. plant diversity, management practices) at the field scale. In addition, the soybean plant has self-pollinating flowers, meaning it does not require insect pollination but visitation by bees can improve yield (Free, 1993; Milfont et al., 2013). A preliminary analysis of landscape variables was performed at 500 m, 1000 m, 1500 m, and 2000 m, and the strongest response

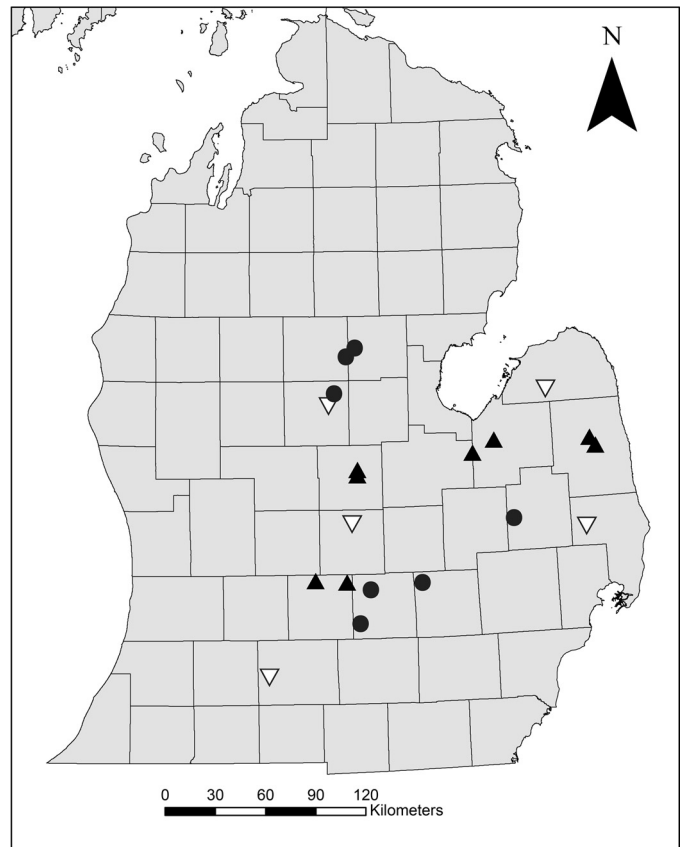


Fig. 1. Location of the 20 Michigan soybean fields used to sample bees and measure pollination services. Symbols represent the proportion of grassland measured in the 1500 m surround study sites (circles—high grassland cover; hollow triangles—intermediate grassland cover; solid triangles—low grassland cover).

to landscape variables was found for the 1500 m scale. The 2012 cropland data layer (CDL, 30 m resolution) was used to characterize the area of annual and perennial habitats within a 1500 m radius of each site (USDA, 2013). Research examining the effect of landscape composition on bees often combines semi-natural area into one variable (Kennedy et al., 2013, but see Woodcock et al., 2013; Le Feon et al., 2010, 2013). Because we were interested in the effect of increasing perennial grassland biofuel crops on bees, natural area was classified separately as either grassland or forest, allowing the relative importance of these variables to be determined. The CDL was reclassified prior to analysis to include the original cropland classes in addition to classes for grassland (combined areas of clover, pasture, hayfields, shrubland, and perennial grasslands), forest (deciduous, coniferous, and mixed forest combined) and urban (open, low, moderate, and high intensity urban combined). The classes included in the grassland category were not under biofuel production but served as a surrogate for what would be expected if perennial biofuel production was expanded across Michigan. The classes included in the grassland variable were combined because each represents a perennial habitat that contains floral resources and experiences few agricultural disturbances, making the nesting and food resources similar across classes. After reclassification, we used ArcGIS 10.0 to create 1500 m buffers around the center of each site. The “Calculate Area” tool was then used to determine the proportion of grassland, forest, urban, and crops within each buffer. In addition to calculating land cover percentages, we also quantified landscape diversity represented by Simpson’s diversity for the 1500 m surrounding sites using the reclassified layer (McGarigal et al., 2012).

2.2. Sentinel plant production

The effect of grassland cover on pollination services was measured using sentinel sunflower plants deployed to soybean fields. Sunflowers, *Helianthus annuus*, variety Sunspot, were grown in 6" pots in the greenhouse under 24 h light and a temperature of $26.7 \pm 2^\circ\text{C}$. Sunflowers were fertilized every 7–14 days using Peters Professional® water soluble fertilizer (20–20–20). Different sunflowers were used to measure bee visitation and pollination services (see methods below). Once disk flowers began to bloom, plants were transported to field sites.

2.3. Bee visitation

The abundance and diversity of bees at each site was determined by performing timed collections on sentinel sunflowers. For timed collections, we established two sampling stations that were at least 50 m from field edges and 20 m apart. At each sampling station, we placed two sunflower plants with open ray and disk flowers. Bee sampling was conducted simultaneously at each station with one observer per station using a hand-held vacuum (Bioquip, Rancho Dominguez, CA) to collect pollinators visiting the flower head. Bees at each station were collected over a 30 min period and only flower visitors that made contact with disk flowers (i.e. anthers and/or stigmas) were collected. Each field ($n = 20$) was surveyed 3–5 times in the weeks preceding or following a pollination trial (see methods on measuring pollination services). Agronomic practices used to control weed and insect pests prevented access to fields on some dates, resulting in unequal numbers of samples collected across sites. Each field was surveyed at least once in the morning and once in the afternoon with sampling occurring on sunny days with temperatures above 24°C . All collected bees were returned to the lab for species-level identification, using the online key to Bees of Eastern North America at www.discoverlife.org in addition to published species-level keys (Rehan and Sheffield, 2011; Gibbs, 2011). Bee abundances were averaged across all collection periods for each field and square root transformed before analysis to improve normality. Bee diversity was quantified among sites using Simpson's diversity index (Clarke and Warwick, 2001).

2.4. Measuring pollination services

At each field, sunflowers were deployed at the two sampling stations located a minimum of 50 m from a field edge and 20 m apart. Each station received a total of four sunflowers: two covered sunflowers, (hereafter referred to as closed) which prevented visitation by pollinators, and two open sunflowers that allowed access by pollinators (hereafter referred to as open). The heads of closed sunflowers were covered with a 1 m \times 1 m exclusion bag made from nylon mesh (white no-see-um netting, Quest Outfitters, Sarasota, FL). A Styrofoam cage ($\sim 0.03 \times 0.03 \times 0.03$ m), which was attached to a plant stake, was built to fit around the head of the sunflower to prevent the flower from moving pollen by inadvertently touching the exclusion bag. Sunflowers were deployed to field sites over the course of 2–3 days and remained in the field for seven days. Cotton wicks running from each potted sunflower to a water source were used to water sunflowers while in the field. After seven days, sunflowers were returned the greenhouse where plants were treated with *Bacillus thuringiensis* (Dipel, Valent BioSciences, Libertyville, IL) to prevent feeding damage by Lepidopterans and then monitored weekly for insect pest and disease problems. Sunflowers remained in the greenhouse until seeds matured, and sunflower heads could be collected and dried. Three pollination trials were conducted during the 2012 field season, with one occurring in early July, early August, and mid-September. The soybean crop was in

flower during both the July and August pollination trial but was not in flower during the September trial.

Pollination services were quantified across fields by measuring sunflower seed set, determined by weighing fully developed seeds removed from heads. Seed set was averaged across open ($n = 4$) and closed ($n = 4$) plants to obtain the average seed set for open and closed sunflowers per field for each sampling date. For each site, the averaged open seed weight was then subtracted from the averaged closed seed weight, which accounted for non-insect sources of pollination such as pollination due to handling or transportation.

2.5. Data analysis: Community composition

Bee community composition was compared among sites using non-metric multidimensional scaling (NMDS). The abundance of each bee species collected from each site was averaged across the 2012 season. The similarity between sites was then quantified using the zero-adjusted, Bray–Curtis coefficient (Clarke et al., 2006), which alleviates multivariate heteroskedasticity when zeros are present for many of the species. The resulting similarity matrix is the basis for creating a NMDS ordination, in which sites are ranked based on their similarity to each other. Sites with similar bee communities are placed closer together in ordination space, and as the distance between sites increases, sites become more dissimilar in composition. In order to statistically test for differences in bee composition between sites, a categorical variable was required, and sites were classified based on their proportion of surrounding grassland. Sites surrounded by a proportion of grassland less than 0.05 were classified as low grassland, sites with intermediate proportions of grassland (0.06–0.29) were medium grassland sites, and sites with greater than 0.3 were categorized as high grassland. Statistical significance was determined between sites (i.e. low, medium, high grassland) using analysis of similarity (ANOSIM), which performs pairwise comparison between classes (Clarke and Warwick, 2001). ANOSIM cannot detect differences in both the composition and variability of communities between treatments. To determine whether treatment differences were due to composition or variability, we performed a permutation test to determine differences in multivariate dispersion across treatments (PERMISP module for dispersion about the median in PRIMER; Anderson et al., 2008), which indicated variability in composition was not different across treatments ($P = 0.11$). As a result, any differences in the bee communities across treatments were due to differences in composition. Finally, the percent contribution each species made to differences in community composition was quantified using "SIMPER" analysis in PRIMER (Clarke and Warwick, 2001). This analysis is performed using the standard Bray–Curtis coefficient, which is slightly different than the zero-adjusted coefficient used in the ANOSIM. As a result, we determined the level of correlation between the zero-adjusted and standard similarity matrices using RELATE in PRIMER (Spearman's $\rho = 0.82$, $P = 0.001$; Clarke and Gorley, 2006), which indicated the two similarity matrices were correlated and permitted the use of the SIMPER analysis. All community composition analyses were performed using the PRIMER-E software (Clarke and Gorley, 2006).

2.6. Data analysis: Modeling bee abundance, bee diversity, and seed set

The relationships between landscape variables and bee abundance, bee diversity, and seed set were evaluated using a model-selection approach. First, a principal components analysis (PCA) was used to reduce the number of landscape variables used in model selection (Supplementary data A), by identifying variables that were correlated with each other (Legendre and Legendre, 1998). PC1 had high positive factor loadings of grassland and

Table 1
Model selection statistics for $i = 7$ models predicting bee abundance and bee diversity as a function of proportion grassland (grass), proportion forest, and Simpson's landscape diversity (land diversity) in the 1500 m surrounding sites. The overall best model and competing models ($\text{AICc} \leq 2$) are bolded.

i	Explanatory variables	Bee abundance			Bee diversity		
		ΔAICc	W_i	R^2	ΔAICc	W_i	R^2
1	Grass + forest + land diversity	1.98	0.20	0.62	3.77	0.069	0.27
2	Grass + forest	0	0.54	0.63	1.77	0.18	0.27
3	Grass + land diversity	4.17	0.067	0.54	5.60	0.027	0.21
4	Forest + land diversity	7.57	0.012	0.46	1.80	0.18	0.27
5	Grass	2.62	0.14	0.54	3.97	0.062	0.11
6	Forest	5.87	0.029	0.46	0	0.45	0.27
7	Land diversity	18.00	<0.001	<0.001	6.27	0.020	0.004

forest and negative factor loadings of crop, while PC2 had a positive loading of landscape diversity and a negative loading for urban cover (Supplementary data A, Fig. A1). From the PCA analysis, the landscape variables used in model evaluation included: grassland, forest, and landscape diversity. Although some correlation existed between grassland and forest, we included both variables in the model set because both foraging and nesting habitat are important to bees and are differentially provided by grassland and forest habitats. In evaluating seed set, we also wanted to determine the effect of bee abundance and diversity. A preliminary analysis using backward selection indicated that landscape diversity and bee diversity produced models with high AICc values and were consistently the first variables dropped during model selection. As a result of this preliminary analysis, we constructed a second set of models using the proportion grassland, proportion forest, and bee abundance as explanatory variables (seven possible models; Table 2). Available degrees of freedom prevented the inclusion of interaction terms in our model sets. As a result, all variable combinations

except interactions were used to construct our model set (seven possible models; Tables 1 and 2).

Using a model-selection approach, AICc-values were calculated for each model from which we quantified AICc differences, ΔAICc (Burnham and Anderson, 2002). Models with $\Delta\text{AICc} < 2$ are considered competing models and strongly supported by the data. From AICc values, we also calculated model weights, i , and variable weights (Burnham and Anderson, 2002). Model weights are used to indicate the importance of a model with increasing weights indicating the likelihood a particular model as the overall best model. By summing the weights of all models containing a particular variable, the relative importance of each model variable was determined, with higher weights indicating increased importance of the variable (Anderson et al., 2000). We also examined R^2 values to evaluate which models explained the most variation in the data. AICc and R^2 values were determined in R version 3.0 (R Development Core Team, 2013).

3. Results

3.1. Community composition

Overall, 248 bee pollinators of sunflower including 37 bee species were collected during 75 h of observation (Supplementary data B, Table B1). Bee community composition was significantly different across the gradient of low to high grassland cover. A NMDS ordination indicated that bee communities were significantly different between sites with high and low grassland cover (Fig. 2, ANOSIM, $r = 0.55$, $P = 0.001$) but were similar between sites with intermediate and low and between intermediate and high grassland cover (intermediate vs low: $r = 0.22$, $P = 0.057$; intermediate vs high $r = 0.076$, $P = 0.26$). A total of 15 bee species contributed to the assemblage similarity among high grassland sites with *Halictus ligatus* Say, *Apis mellifera* Linnaeus, *Agapostemon virescens* (Fabricius), and *Augochlorella aurata* (Smith) contributing to over 80% of the similarity in assemblage composition. In contrast, only six bee species contributed to community composition among low grassland sites with *A. mellifera* contributing to ~80% of the similarity in assemblage composition (Supplementary data C, Table C1).

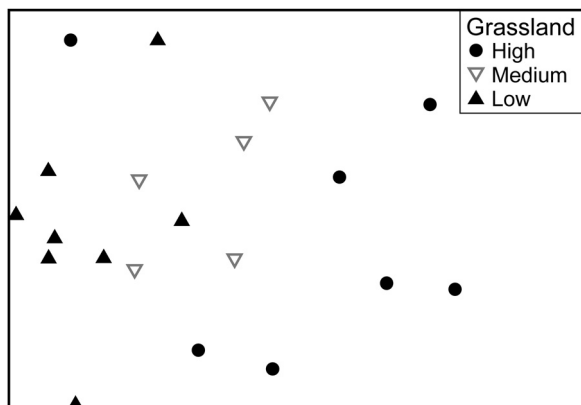


Fig. 2. Ordination of bee community composition using non-metric multidimensional scaling (NMDS) shows that communities are different between sites with high and low grassland in the 1500 m surrounding sites (stress = 0.17). Each symbol represents a site and sites close together in ordination space have similar community composition.

Table 2
Model selection statistics for $i = 7$ models predicting seed set for the months of July, August, and September 2012 as a function of proportion grassland (grass), proportion forest, and bee abundance (beeN) in the 1500 m surrounding sites. The overall best model and competing models ($\text{AICc} \leq 2$) are bolded.

i	Explanatory variables	July seed set			August seed set			September seed set		
		ΔAICc	W_i	R^2	ΔAICc	W_i	R^2	ΔAICc	W_i	R^2
1	Grass + forest + beeN	1.12	0.22	0.28	1.92	0.15	0.36	1.41	0.29	0.49
2	Grass + forest	4.67	0.031	0.02	0.0	0.38	0.36	10.92	0.002	0.03
3	Grass + beeN	0.0	0.41	0.25	7.91	0.007	0.05	0.0	0.59	0.47
4	Forest + beeN	3.21	0.17	0.01	1.39	0.19	0.32	5.14	0.045	0.29
5	Grass	2.79	0.076	0.02	7.12	0.011	0.003	9.52	0.005	0.002
6	Forest	3.12	0.063	0.004	0.86	0.25	0.27	9.12	0.006	0.02
7	BeeN	2.61	0.083	0.03	6.41	0.016	0.04	4.68	0.057	0.24

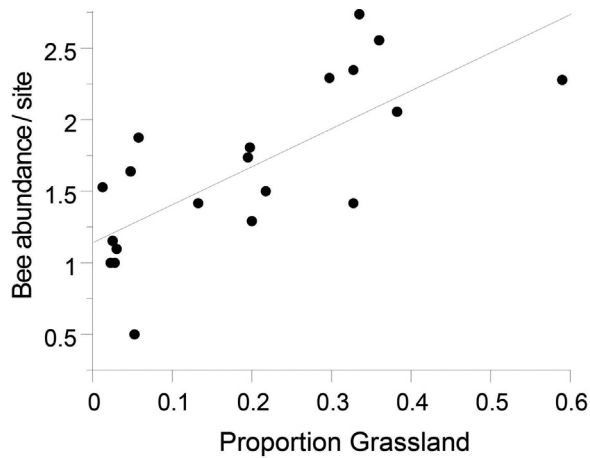


Fig. 3. Bee abundance averaged across the 2012 season increased with the proportion of grassland within the 1500 m surrounding sites ($r=0.54$, $P=0.002$).

3.2. Bee abundance, bee diversity, and seed set

Among all possible models, bee abundance was best explained by the amount of grassland and forest in the surrounding landscape (Table 1). The overall best model, which included grassland and forest, accounted for 54% of the model weights and explained 59% of the variation in the data. Variable weights identified grassland, with a weight of 0.96, as the most influential variable explaining bee abundance and having a positive relationship with abundance (Fig. 3; $r=0.54$, $P=0.002$). Forest had the second highest variable weight at 0.79 followed by landscape diversity with a weight of 0.28. In contrast to abundance, bee diversity was best explained by the amount of forest in the surrounding landscape. The overall best model included only forest, accounted for 45% of the model weights, and explained 23% of the variation in bee diversity (Table 1). In all competing models, forest was positively correlated with bee diversity (Fig. 4; $r=0.27$, $P=0.018$). Variable weights for bee diversity were 0.89 for forest, 0.35 for grassland, and 0.30 for landscape diversity.

Model results explaining seed set were variable across months. In July, the overall best model explaining seed set, which included the proportion of grassland and bee abundance, accounted for 39% of the model weights and explained 15% of the variation in July seed set (Table 2). The variable weights indicated forest had the lowest variable weight of 0.42 while bee abundance and the

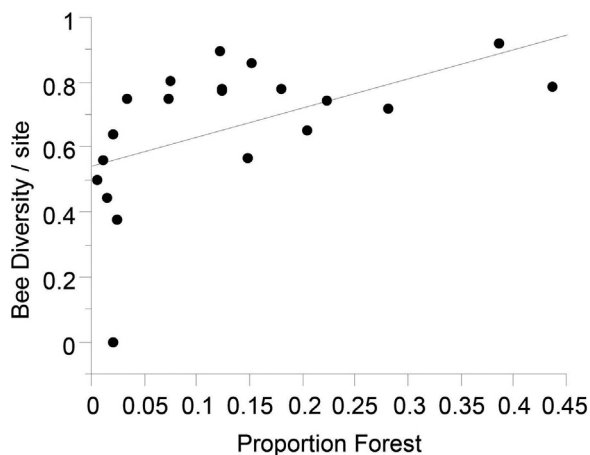


Fig. 4. Bee diversity calculated using the Simpson's diversity index using bees collected during the 2012 season increased with the proportion of forest in the 1500 m surrounding sites ($r=0.27$, $P=0.018$).

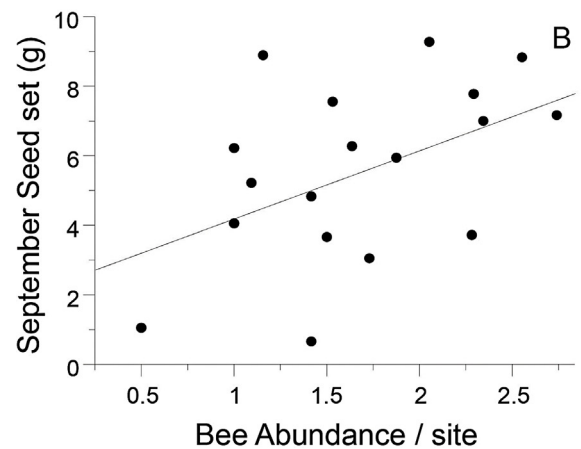
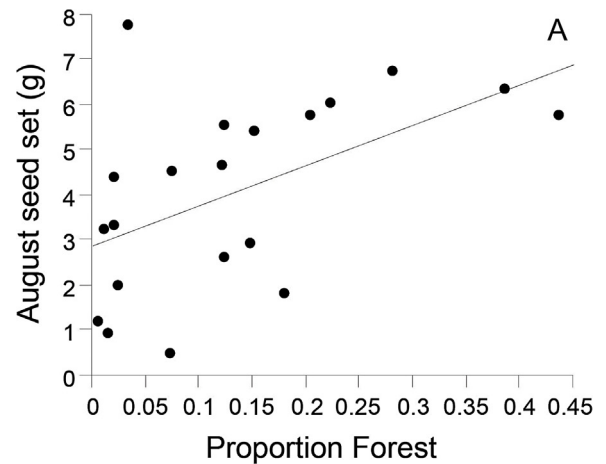


Fig. 5. August seed set (measured in grams, g) showed a positive correlation with the proportion of forest in the 1500 m surrounding study sites ((A) $r=0.27$, $P=0.018$). In contrast, seed set in the month of September was positively correlated with the average number of bees (square root transformed) collected at each site over the course of the 2012 season ((B) $r=0.23$, $P=0.04$).

proportion of grassland had similar variable weights of 0.79 and 0.74, respectively. Interestingly, when bee abundance, the variable with the highest weight, was the only variable in the model it was not significantly correlated with July seed set ($r=0.03$; $P>0.05$). However, July seed set was negatively correlated with bee abundance but only when grassland was also in the model (Supplementary data D, Fig. D1). In August, the overall best model explaining seed set included the proportions of grassland and forest. The overall best model accounted for 38% of the model weights and explained 29% of the variation in August seed set (Table 2). Several competing models were present and in each model seed set was positively correlated with the proportion of forest (Fig. 5A; $r=0.27$, $P=0.018$). The proportion of forest had the highest variable weight at 0.96 followed by the proportion of grassland with a weight of 0.55 and bee abundance with a weight of 0.36. The model results explaining seed set in September were similar to the results found in July. Again, the overall best model included the proportion of grassland and bee abundance with only one competing model, the full model (Table 2). The overall best model accounted for 59% of the model weights and explained 40% of the variation in September seed set. Variable weights identified bee abundance with a weight of 0.98 as the most influential variable explaining September seed set followed by the proportions of grassland and forest (weights 0.89 and 0.34, respectively). Seed set was significantly positively correlated with bee abundance in September (Fig. 5B, $r=0.23$, $P=0.04$). Seed set was also compared in sites surrounded by high and low proportions of grassland

(hereafter, high grassland, $n=7$ and low grassland, $n=8$) with ANOVA, but no significant difference in seed set was found between these different landscape contexts in July, August, or September ($F_{1,14} \leq 0.24$, $P > 0.05$, across all months).

4. Discussion

This study highlights the role of grasslands in supporting bees and pollination services across agricultural landscapes and their potential importance within a sustainable biofuels policy. Here, we find that (1) bee abundance was positively correlated with the amount of grassland in the surrounding landscape, (2) bee diversity increased as the amount of forest increased in the landscape surrounding sites, (3) community composition was different in landscapes with high and low proportions of grassland, and (4) pollination services were similar between high and low grassland sites, and best explained late in the season by higher bee abundance. A biofuels policy that promotes expanding perennial biofuel production would be expected to increase bee abundance, support a more diverse pollinator community, and perhaps improve the reliability of pollination services.

Bee abundance was positively correlated with grassland, the most influential variable explaining abundance. Because bees require both food and nesting resources, grassland habitats may provide resources across the season that are not available in other natural habitats or that are complementary to the resources provided by other habitats. Complementary habitat use has been documented for foraging bees, where bees follow the spatiotemporal patterns in floral resources across the landscape (Mandelik et al., 2012). In this system, diverse grassland habitats may offer a reliable source of pollen and nectar that locally enhances bee abundance by attracting and retaining pollinators transitioning from depleted foraging habitats, such as flowering crops once bloom is completed. Bees transitioning into grassland habitats to forage as resources in neighboring habitats decline may account for the higher abundance of bees observed in sites surrounded by more grassland.

In contrast, forest habitats may compliment grassland habitats by offering bees opportunities for nesting, contributing to the positive correlation between bee diversity and forest cover. Increased bee diversity is often positively correlated with forest cover (Priess et al., 2007; Brosi, 2009; Jha and Vandermeer, 2010), because forests provide nesting resources for stem and cavity nesting bees (Brosi et al., 2007). In this study system, forest habitat may provide nesting sites for stem- and cavity-nesting bees not available in grassland habitats. Furthermore, forests may also offer soil nesting bees easier access to nesting sites as grassland habitats are often covered with thick thatch layers that may discourage nesting. For example, in this study *Bombus*, *Megachild*, and *Ceratina* species are all stem and cavity nesting bees likely utilizing forest habitats for nesting resources (Supplementary material B, Table B1). As a result, the availability of nesting sites in forests may locally increase bee diversity as well as contribute to the regional pool of species across the landscape. As a consequence, forests may serve as the source habitat for overwintering and nesting bees that once emerged disperse into nearby grasslands to forage. Thus, grassland habitats provide floral resources that attract bees from neighboring nesting habitats building bee abundances within a season, while forests offer nesting resources that maintain bee diversity across the landscape. While our study adds to the growing body of research that demonstrates the importance of grassland and forest habitat for bees, increasing perennial grasslands as part of a sustainable biofuels policy may specifically benefit bee abundances in resource-limited agricultural landscapes.

The proportion of grassland in the surrounding landscape also affected bee community composition, with distinct communities

found in sites surrounded by low and high proportions of grassland. We found bee community composition in low grassland sites contained fewer species compared to high grassland sites, and these communities were dominated by the honey bee, *A. mellifera*. This non-native species contributed to 80% of the similarity in community composition among low grassland sites, supporting previous research that found agricultural intensification and the associated loss of resource rich habitat can shift pollinator communities to ones dominated by common bee taxa (Brosi et al., 2007; Carre et al., 2009). Furthermore, recent meta-analyses found managed honey bee visitation to flowers is less affected by isolation from semi-natural habitat than that of wild bees (Winfree et al., 2009; Garibaldi et al., 2011). Honey bees may be less affected by declines in semi-natural area because they have a larger body size than many solitary bees species allowing larger foraging ranges (Greenleaf et al., 2007; Gathmann and Tschardt, 2002; Steffan-Dewenter and Kuhn, 2003). Honey bees may also be more common in agricultural landscapes because farmers are actively placing and managing hives in these landscapes (Garibaldi et al., 2011). Although stocking soybean fields with *A. mellifera* is not practiced in Michigan, two study sites were adjacent to neighboring properties with managed colonies of *A. mellifera* and located in a region of Michigan where *A. mellifera* are used to pollinate crops (personal observation A. Bennett). The decline of solitary bee species in low grassland sites is likely affected by the loss of food and nesting resources and increased foraging distances between resource rich habitats, while the abundance of *A. mellifera* in these sites is potentially attributable to their larger foraging ranges and presence of managed hives.

Although bee community composition was significantly different between sites surrounded by low and high proportions of grassland, no differences were found for seed set. The lack of statistical difference in seed set between sites surrounded by high and low proportions of grassland suggests both bee communities were providing similar levels of pollination. While not directly tested, several mechanisms may explain the pattern we observed. First, communities in high and low grassland sites shared relatively high abundances of bee species that were effective pollinators of sunflower such as *A. mellifera* and *H. ligatus*, both generalist bees that visit a wide variety of flowers (Moure and Hurd, 1987; Cane and Sipes, 2006). Second, native bees have higher visitation rates to native plants compared to cultivars (Comba et al., 1999; Corbet et al., 2001; Morandin and Kremen, 2013). The use of a cultivar as our sentinel plant may have lowered visitation rates especially in high grassland sites where community composition was more diverse producing similar pollination rates. Third, while sites surrounded by higher proportions of grassland were positively correlated with bee abundance, pollinators that prefer Asteraceae may not have increased, producing the similar rates of seed set observed across sites. Finally, the proportion of grassland, approximately 30% of the land cover in high grassland sites, may not have been high enough to produce significant differences between high and low grassland sites.

Although seed set was not different between sites with high and low proportion of grassland, results from the linear regression models indicated bee abundance was the most important variable explaining seed set in both July and September. However, we found seed set increased with bee abundance in September, but was negatively correlated with bee abundance in July. In July, the soybean (*Glycine max*) crop was in flower, and the mass flowering may have increased competition between the sunflowers and the soybean flowers for pollination services, modifying the relationship between bee abundance and seed set. Despite the fact soybean flowers are not very showy, these flowers are one of the few resources available in agricultural dominated landscapes in mid-summer and a number of wild bees do visit their flowers (Milfont et al., 2013). Research has found mass flowering crops, such as

canola, can increase wild bee abundance (Hanley et al., 2011; Holzschuh et al., 2013), but the effect of seed set on nearby plants is mixed. In some examples, mass flowering crops facilitate pollination of nearby plants, whereas in other cases reduce pollination through increased competition (Cussans et al., 2010; Holzschuh et al., 2011). Because July seed set was negatively correlated with bee abundance and positively correlated with grassland, this suggests that the mass flowering of the soybean crop may be locally increasing bee abundance but concurrently having a negative effect on seed set through increased competition or modification of pollinator behavior. For example, *Apis* and *Bombus* species exhibit a foraging behavior, floral constancy, where they preferentially visit the same flower species (Hill et al., 1997; Gegeer and Laverty, 2005). This behavior could locally increase bee abundance while reducing visitation and seed set to sentinel sunflowers. Because local factors such as flower abundance and diversity are known to influence pollinators and pollination services (Klein et al., 2003; Potts et al., 2003; Ghazoul, 2006; Frund et al., 2010), the relative importance of local and landscape variables may change over the course of the season, highlighting the need to consider the temporal dynamic between scales.

In contrast to July and September, seed set in August was best explained by the proportion of forest in the surrounding landscape. Although the positive correlation between seed set and forest cover in August was unexpected, shifts in the bee community over the course of the season may explain the increased importance of forest habitat in August. The activity period of many bee species changes across the season (Wolf and Ascher, 2008), and Richards et al. (2011) observed that bee abundance in grasslands of the same region peaked in July followed by a sharp decline in August and a rebound of some bee groups, such as Halictidae by September. In our study, the bee community present in August may have shifted to a community composed of bees accessing forest habitat for nesting resources, leading to the increased importance of forest cover. Unfortunately, the number of bees collected did not provide enough data to allow an analysis of community composition across months, which could have detected changes in the bee community and their associated nesting habitats across the season.

5. Conclusions

As demand for renewable sources of energy increase, biofuel crop production is expected to expand, providing a unique opportunity to reshape the composition of agricultural landscapes. Our results demonstrated increasing proportions of grassland habitat were positively correlated with higher bee abundance and that increased bee abundance positively affected pollination services delivered to a pollination-dependent plant. Policy to encourage perennial grass production could significantly increase the proportion of grassland cover across the landscape, and our results suggest that increasing grassland cover positively impacted bee abundance and community composition. Programs designed to benefit native bee conservation will need to consider existing regional land use while providing incentives to expand the production of perennial grassland crops. A multifaceted biofuels policy that includes practices to encourage pollinators may ultimately help offset declining bee populations and ensure pollination services to nearby agricultural crops and native plant communities.

Acknowledgments

A special thanks to the participating landowners and Ben Werling who established our network of sites. Thanks to Ashley McNamara, Lindsey Pudlo, Jon Roney, and Laura Maihofer who provided invaluable field assistance and Jason Gibbs who provided bee

identifications. This research was funded by the U.S. DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02-07ER64494) and by USDA-NIFA (grant 2012-67009-20146).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2014.04.016>.

References

- Anderson, D.R., Burnham, K.P., Thompson, W.L., 2000. Null hypothesis testing: problems, prevalence, and an alternative. *J. Wildl. Manage.* 64, 912–923.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd., Plymouth.
- Brosi, B.J., Daily, G.C., Ehrlich, P.R., 2007. Bee community shifts with landscape context in a tropical countryside. *Ecol. Appl.* 17, 418–430.
- Brosi, B.J., 2009. The complex responses of social stingless bees (Apidae: Meliponini) to tropical deforestation. *For. Ecol. Manage.* 258, 1830–1837.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York, NY.
- Cane, J.H., Sipes, S., 2006. Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. In: Waser, N.M., Ollerton, J. (Eds.), *Plant-Pollinator Interactions from Specialization to Generalization*. The University of Chicago Press, Chicago, IL.
- Carre, G., Roche, P., Chifflet, R., Morison, N., Bommarco, R., Harrison-Cripps, J., Krewenka, K., Potts, S.G., Roberts, S.P.M., Rodet, G., Settele, J., Steffan-Dewenter, I., Szentgyorgyi, H., Tscheulin, T., Westphal, C., Woyciechowski, M., Vaissiere, B.E., 2009. Landscape context and habitat type as drivers of bee diversity in European annual crops. *Agric. Ecosyst. Environ.* 133, 40–47.
- Clarke, K.R., Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.
- Clarke, K.R., Somerfield, P.J., Chapman, M.G., 2006. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denude assemblages. *J. Exp. Mar. Biol. Ecol.* 130, 55–80.
- Clarke, K.R., Warwick, R.M., 2001. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, second ed. PRIMER-E, Plymouth.
- Comba, L., Corbet, S.A., Barron, A., Bird, A., Collinge, S., Miyazaki, N., Powell, M., 1999. Garden flowers: insect visits and the floral reward of horticulturally-modified variants. *Ann. Bot.* 83, 73–86.
- Corbet, S.A., Bee, J., Dasmahapatra, K., Gale, S., Gorrington, E., La Ferla, B., Moorhouse, T., Trevail, A., Van Bergen, Y., Vorontsova, M., 2001. Native or exotic? Double or single? Evaluating plants for pollinator-friendly gardens. *Ann. Bot.* 87, 219–232.
- Cussans, J., Goulson, D., Sanderson, R., Goffe, L., Darvill, B., Osborne, J.L., 2010. Two bee-pollinated plant species show higher seed production when grown in gardens compared to Arable Farmland. *PLoS One*, 5.
- Free, J.B., 1993. *Insect Pollination of Crops*, second ed. Academic Press, London.
- Frund, J., Linsenmair, K.E., Bluthgen, N., 2010. Pollinator diversity and specialization in relation to flower diversity. *Oikos* 119, 1581–1590.
- Gardiner, M.A., Tuell, J.K., Isaacs, R., Gibbs, J., Ascher, J.S., Landis, D.A., 2010. Implications of three biofuel crops for beneficial arthropods in agricultural landscapes. *Bioenergy Res.* 3, 6–19.
- Garibaldi, L.A., Steffan-Dewenter, I., Kremen, C., Morales, J.M., Bommarco, R., Cunningham, S.A., Carvalheiro, L.G., Chacoff, N.P., Dudenhofer, J.H., Greenleaf, S.S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L.A., Potts, S.G., Ricketts, T.H., Szentgyorgyi, H., Viana, B.F., Westphal, C., Winfree, R., Klein, A.M., 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecol. Lett.* 14, 1062–1072.
- Gathmann, A., Tschardt, T., 2002. Foraging ranges of solitary bees. *J. Anim. Ecol.* 71, 757–764.
- Gegeer, R.J., Laverty, T.M., 2005. Flower constancy in bumblebees: a test of the trait variability hypothesis. *Anim. Behav.* 69, 939–949.
- Ghazoul, J., 2006. Floral diversity and the facilitation of pollination. *J. Ecol.* 94, 295–304.
- Gibbs, J., 2011. Revision of the metallic *Lasioglossum* (*Dialictus*) of eastern North America (Hymenoptera: Halictidae: Halictini). *Zootaxa* 3073, 1–216.
- Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153, 589–596.
- Hanley, M.E., Franco, M., Dean, C.E., Franklin, E.L., Harris, H.R., Haynes, A.G., Rapson, S.R., Rowse, G., Thomas, K.C., Waterhouse, B.R., Knight, M.E., 2011. Increased bumblebee abundance along the margins of a mass flowering crop: evidence for pollinator spill-over. *Oikos* 120, 1618–1624.
- Hill, P.S.M., Wells, P.H., Wells, H., 1997. Spontaneous flower constancy and learning in honey bees as a function of colour. *Anim. Behav.* 54, 615–627.
- Holzschuh, A., Steffan-Dewenter, I., Kleijn, D., Tschardt, T., 2007. Diversity of flower-visiting bees in cereal fields: effects of farming system, landscape composition and regional context. *J. Appl. Ecol.* 44, 41–49.
- Holzschuh, A., Dormann, C.F., Tschardt, T., Steffan-Dewenter, I., 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. *Proc. R. Soc. B-Biol. Sci.* 278, 3444–3451.

- Holzschuh, A., Dudenhoffer, J.H., Tschamtkke, T., 2012. Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biol. Conserv.* 153, 101–107.
- Holzschuh, A., Dormann, C.F., Tschamtkke, T., Steffan-Dewenter, I., 2013. Mass-flowering crops enhance wild bee abundance. *Oecologia* 172, 477–484.
- James, L.K., Swinton, S.M., Thelen, K.D., 2010. Profitability analysis of cellulosic energy crops compared with corn. *Agron. J.* 102, 675–687.
- Jarchow, M.E., Liebman, M., 2012. Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production. *Crop Sci.* 52, 1330–1342.
- Jha, S., Vandermeer, J.H., 2010. Impacts of coffee agroforestry management on tropical bee communities. *Biol. Conserv.* 143, 1423–1431.
- Kennedy, C.M., Lonsdorf, E., Neel, M.C., Williams, N.M., Ricketts, T.H., Winfree, R., Bommarco, R., Brittain, C., Burley, A.L., Cariveau, D., Carvalheiro, L.G., Chacoff, N.P., Cunningham, S.A., Danforth, B.N., Dudenhoffer, J.H., Elle, E., Gaines, H.R., Garibaldi, L.A., Gratton, C., Holzschuh, A., Isaacs, R., Javorek, S.K., Jha, S., Klein, A.M., Krewenka, K., Mandelik, Y., Mayfield, M.M., Morandin, L., Neame, L.A., Otieno, M., Park, M., Potts, S.G., Rundlof, M., Saez, A., Steffan-Dewenter, I., Taki, H., Viana, B.F., Westphal, C., Wilson, J.K., Greenleaf, S.S., Kremen, C., 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecol. Lett.* 16, 584–599.
- Klein, A.M., Steffan-Dewenter, I., Tschamtkke, T., 2003. Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *J. Appl. Ecol.* 40, 837–845.
- Klein, A.M., Brittain, C., Hendrix, S.D., Thorp, R., Williams, N., Kremen, C., 2012. Wild pollination services to California almond rely on semi-natural habitat. *J. Appl. Ecol.* 49, 723–732.
- Knops, J.M.H., Tilman, D., Haddad, N.M., Naeem, S., Mitchell, C.E., Haarstad, J., Ritchie, M.E., Howe, K.M., Reich, P.B., Siemann, E., Groth, J., 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecol. Lett.* 2, 286–293.
- Koricheva, J., Mulder, C.P.H., Schmid, B., Joshi, J., Huss-Danell, K., 2000. Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. *Oecologia* 125, 271–282.
- Landis, D.A., Werling, B.P., 2010. Arthropods and biofuel production systems in North America. *Insect Sci.* 17, 220–236.
- Le Feon, V., Schermann-Legionnet, A., Delettre, Y., Aviron, S., Billeter, R., Bugter, R., Hendrickx, F., Burel, F., 2010. Intensification of agriculture, landscape composition and wild bee communities: a large scale study in four European countries. *Agric. Ecosyst. Environ.* 137, 143–150.
- Le Feon, V., Burel, F., Chifflet, R., Henry, M., Ricroch, A., Vaissiere, B.E., Baudry, J., 2013. Solitary bee abundance and species richness in dynamic agricultural landscapes. *Agric. Ecosyst. Environ.* 166, 94–101.
- LeBuhn, G., Droege, S., Connor, E.F., Gemmill-Herren, B., Potts, S.G., Minckley, R.L., Griswold, T., Jean, R., Kula, E., Roubik, D.W., Cane, J., Wright, K.W., Frankie, G., Parker, F., 2013. Detecting insect pollinator declines on regional and global scales. *Conserv. Biol.* 27, 113–120.
- Legendre, P., Legendre, L., 1998. *Numerical Ecology*. Elsevier, New York, NY.
- Losey, J.E., Vaughan, M., 2006. The economic value of ecological services provided by insects. *Bioscience* 56, 311–323.
- McGarigal, K., Cushman, M.C., Ene, E., 2012. Computer software program produced by the authors at the University of Massachusetts. In: FRAGSTATS v4: Spatial Pattern Analysis Program for Categorical and Continuous Maps. University of Massachusetts, Amherst, Available at the following web site: <http://www.umass.edu/landeco/research/fragstats/fragstats.html>.
- Mandelik, Y., Winfree, R., Neeson, T., Kremen, C., 2012. Complementary habitat use by wild bees in agro-natural landscapes. *Ecol. Appl.* 22, 1535–1546.
- Milfont, M.D., Rocha, E.E.M., Lima, A.O.N., Freitas, B.M., 2013. Higher soybean production using honeybee and wild pollinators, a sustainable alternative to pesticides and autopolination. *Environ. Chem. Lett.* 11, 335–341.
- Morandin, L.A., Kremen, C., 2013. Bee preference for native versus exotic plants in restored agricultural hedgerows. *Restor. Ecol.* 21, 26–32.
- Moure, J.S., Hurd Jr., P.D., 1987. *An Annotated Catalog of the Halictid Bees of the Western Hemisphere (Hymenoptera: Halictidae)*. Smithsonian Institution Press, Washington, DC.
- Nicholls, C.I., Altieri, M.A., 2013. Plant biodiversity enhances bees and other insect pollinators in agroecosystems: a review. *Agron. Sustainable Dev.* 33, 257–274.
- Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? *Oikos* 120, 321–326.
- Potts, S.G., Vulliamy, B., Dafni, A., Ne'eman, G., Willmer, P., 2003. Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology* 84, 2628–2642.
- Priess, J.A., Mimler, M., Klein, A.M., Schwarze, S., Tschamtkke, T., Steffan-Dewenter, I., 2007. Linking deforestation scenarios to pollination services and economic returns in coffee agroforestry systems. *Ecol. Appl.* 17, 407–417.
- R Development Core Team, 2013. R: A Language and Environment for Statistical Computing. R Development Core Team, Vienna, Austria.
- Rehan, S.M., Sheffield, C.S., 2011. Morphological and molecular delineation of a new species in the *Ceratina dupla* species-group (Hymenoptera: Apoidea: Xylocopinae) of eastern North America. *Zootaxa*, 35–50.
- Richards, M.H., Rutgers-Kelly, A., Gibbs, J., Vickruck, J.L., Rehan, S.M., Sheffield, C.S., 2011. Bee diversity in naturalizing patches of Carolinian grasslands in southern Ontario, Canada. *Can. Entomol.* 143, 279–299.
- Scherber, C., Eisenhauer, N., Weisser, W.W., Schmid, B., Voigt, W., Fischer, M., Schulze, E.D., Roscher, C., Weigelt, A., Allan, E., Bessler, H., Bonkowski, M., Buchmann, N., Buscot, F., Clement, L.W., Ebeling, A., Engels, C., Halle, S., Kertscher, I., Klein, A.M., Koller, R., Konig, S., Kowalski, E., Kummer, V., Kuu, A., Lange, M., Lauterbach, D., Middelhoff, C., Migunova, V.D., Milcu, A., Muller, R., Partsch, S., Petermann, J.S., Renker, C., Rottstock, T., Sabais, A., Scheu, S., Schumacher, J., Temperton, V.M., Tschamtkke, T., 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* 468, 553–556.
- Siemann, E., Tilman, D., Haarstad, J., Ritchie, M., 1998. Experimental tests of the dependence of arthropod diversity on plant diversity. *Am. Nat.* 152, 738–750.
- Stanley, D.A., Stout, J.C., 2013. Quantifying the impacts of bioenergy crops on pollinating insect abundance and diversity: a field-scale evaluation reveals taxon-specific responses. *J. Appl. Ecol.* 50, 335–344.
- Steffan-Dewenter, I., Kuhn, A., 2003. Honeybee foraging in differentially structured landscapes. *Proc. R. Soc. B—Biol. Sci.* 270, 569–575.
- Tyner, W.E., 2008. The US ethanol and biofuels boom: its origins, current status, and future prospects. *Bioscience* 58, 646–653.
- USDA, 2013. National agricultural statistic service cropland data layer. In: 2012 Cropland Data Layer. USDA-NASS, Washington, DC.
- Werling, B.P., Meehan, T.D., Gratton, C., Landis, D.A., 2011. Influence of habitat and landscape perennality on insect natural enemies in three candidate biofuel crops. *Biol. Control* 59, 304–312.
- Winfree, R., Griswold, T., Kremen, C., 2007. Effect of human disturbance on bee communities in a forested ecosystem. *Conserv. Biol.* 21, 213–223.
- Winfree, R., Aguilar, R., Vazquez, D.P., LeBuhn, G., Aizen, M.A., 2009. A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90, 2068–2076.
- Wolf, A.T., Ascher, J.S., 2008. Bees of Wisconsin (Hymenoptera: Apoidea: Anthophila). *Great Lakes Entomol.* 41, 129–168.
- Woodcock, B.A., Edwards, M., Redhead, J., Meek, W.R., Nuttall, P., Falk, S., Nowakowski, M., Pywell, R.F., 2013. Crop flower visitation by honeybees, bumblebees and solitary bees: behavioral differences and diversity responses to landscape. *Agric. Ecosyst. Environ.* 171, 1–8.
- Wright, C.K., Wimberly, M.C., 2013. Recent land use change in the Western Corn Belt threatens grassland and wetlands. *Proc. Nat. Acad. Sci. U.S.A.* 110, 4134–4139.
- Wu, M., Demissie, Y., Yan, E., 2012. Simulated impact of future biofuel production on water quality and water cycle dynamics in the Upper Mississippi river basin. *Biomass Bioenergy* 41, 44–56.