Ant biodiversity and ecosystem services in bioenergy landscapes

Jackson A. Helms IV[1*], Selassie E. Ijelu[2], Bill D. Wills[3,4], Douglas A. Landis[4], Nick M. Haddad[1]

[1] Kellogg Biological Station, Department of Integrative Biology, Michigan State University, Hickory Corners, MI 49060, USA

[2] University of Saint Francis, Fort Wayne, IN 46808, USA

[3] Department of Biological Sciences, Auburn University, Auburn AL 36849, USA

[4] Department of Entomology & DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824, USA

[*] Corresponding author: jacksonhelmsiv@gmail.com

Abstract

1. Most strategies for limiting global climate change invoke the use of bioenergy, but biofuel crops vary in climate mitigation potential and in the provision of other ecosystem services. The predominant biofuel in North America is ethanol produced from corn *Zea mays*. Corn is grown on ~360,000 km^2 of land in the U.S. and ~40% of the yield is used for ethanol production. Despite its prevalence, corn ethanol is a poor climate change mitigator and the spread of intensive corn agriculture also leads to the loss of biodiversity and an unknown complement of associated ecosystem services.

2. To test for effects of land use intensity on the provision of ecosystem services from biofuel crops, we worked in long-term experiments in which land use intensity varied from annual corn production to less intensive native perennial biofuel crops (switchgrass and restored prairie) and unmanaged native forests. Within our experiments we studied communities of ants (Formicidae: Hymenoptera), including their diversity, abundance, functional traits, and predation of biofuel crop pests.

3. Native perennial biofuel crops supported up to 185% more ant species than corn fields and provided up to 55% more natural pest suppression. They also contained higher functional diversity by supporting social parasites and seed dispersing ants that were absent in corn. Biofuel crops did not differ in ant activity or the prevalence of exotic ants.

4. *Synthesis and applications*. Our results highlight tradeoffs in bioenergy production and suggest ways to maximize benefits for wildlife and people. Converting some corn fields to prairie or other native vegetation could restore landscapes while mitigating climate change and meeting energy needs.

Keywords

Biofuels, Cellulosic Ethanol, Climate Change, Formicidae, Greenhouse Gas Mitigation, Pest Suppression, Seed Dispersal, Social Parasites

Introduction

Nearly all scenarios in which humans limit climate change to acceptable levels invoke the use of bioenergy (IPCC, 2018). Transitioning to biofuels made from autotrophic organisms can reduce net greenhouse gas emissions because the carbon produced during biofuel combustion is first removed from the atmosphere through photosynthesis. Biofuel crops are thus increasingly grown around the world to reduce reliance on fossil fuels and to slow or mitigate climate change (Nakada, Saygin, & Gielen, 2014). But the rapid rise of biofuels makes it difficult for energy and land use policies to accommodate the changes, resulting in major or controversial landscape transformations occurring before the full environmental impacts are known, including effects on climate change, biodiversity, and the provision of ecosystem services (Lark, Salmon, & Gibbs, 2015; Robertson et al., 2017; Lustgarten, 2018). To address this, we complement research on the climate impacts of bioenergy by evaluating the biodiversity and ecosystem services supported by landscapes managed for bioenergy crop production.

The potential for biofuel crops to mitigate climate change or maintain ecological processes varies with species, management, and land use history. Converting natural habitats like forests or grasslands to biofuel crops, for example, leads to net carbon emissions regardless of biofuel efficiency (Searchinger et al., 2008; Ruan & Robertson, 2013). Most biofuel production in the U.S. consists of corn grain ethanol (USEPA, 2018). Corn *Zea mays* is an annual crop requiring repeated planting, soil disturbance, and inputs of chemical fertilizers and pesticides, all of which

56 are associated with greenhouse gas emissions that reduce its efficiency as a climate mitigator
57 (Robertson et al., 2017). Heavy reliance on corn for bioenergy also leads to the conversion of
58 wildlife habitat to farmland (Fargione et al., 2009; Lark et al., 2015), reduced ecosystem services
59 like natural pest suppression in nearby non-corn crops (Landis, Gardiner, van der Werf, &
60 Swinton, 2008), and other negative environmental impacts from agricultural intensification
61 (Foley et al., 2005; USEPA, 2018).

62 Producing cellulosic ethanol from native perennial vegetation, on the other hand, may alleviate
63 many of the environmental impacts associated with corn agriculture (Tilman, Hill, & Lehman,
64 2006; Werling et al., 2014; Robertson et al., 2017). Seminatural vegetation like fields of
65 switchgrass *Panicum virgatum* or restored prairie can be harvested for ethanol production at
66 yields approaching those of corn (Sanford et al., 2016) but require no annual planting or soil
67 disturbance and few if any chemical inputs (Robertson et al., 2017) and have little impact on
68 landscape water balances (Hamilton, Hussain, Bhardwaj, Basso, & Robertson, 2015). For these
69 reasons native perennial biofuels are superior climate mitigators (Robertson et al., 2017). From a
70 biodiversity perspective, they also serve as wildlife habitat and support multiple ecosystem
71 services such as pollination and suppression of pest insects (Werling et al., 2014; Stahlheber,
72 Watson, Dickson, Disney, & Gross, 2016; Landis et al., 2018). Converting some corn fields to
73 native perennial biofuel crops may thus restore natural landscapes and slow climate change while
74 meeting energy needs.

75 We compare the conservation value of biofuel crops by studying their impacts on ants
76 (Hymenoptera: Formicidae), which are among the most abundant and functionally diverse
77 components of the soil communities that make up most of the biodiversity of agroecosystems
78 and mediate the bulk of their ecosystem services (Hölldobler & Wilson, 1990; Brussaard et al.,

1997; Giller, Beare, Lavelle, Izac, & Swift, 1997). Ants are ideal organisms for testing hypotheses about biodiversity and land use change because they contribute to many ecosystem processes (Folgarait, 1998) and interact with other organisms as predators, prey, and symbionts, making them useful ecological indicators (Agosti, Majer, Alonso, & Schultz, 2000; Andersen & Majer, 2004; Underwood & Fisher, 2006). In North American grasslands ants play key roles as soil movers, consumers, and seed dispersers (Beattie, 1989; Nemec, 2014; Wills & Landis, 2018), respond predictably to prairie restoration efforts (Trager, 1990; Menke, Gaulke, Hamel, & Vachter, 2015; Trager, Winkler, & Helzer, 2017), and are useful indicators of restoration progress (Debinski et al., 2011; Moranz et al., 2013; Wodika, Klopf, & Baer, 2014). As dominant predators of soil arthropod communities in agricultural landscapes (Way & Khoo, 1992; Grieshop et al., 2012), they likely also mediate ecosystem services associated with pest insect suppression. Ants should thus help maintain the processes that enable biofuel production, especially in native perennial systems, and the ecosystem services they provide to people.

We use long-term experiments to evaluate ant contributions to ecosystem services in bioenergy landscapes, and to assess the effects on ant communities of producing annual corn versus native perennial biofuel crops. We frame our investigation in the context of the *perenniality hypothesis*, which states that permanent plant cover is the most desirable trait for biofuel cropping systems when considering climate and biodiversity impacts (Robertson et al., 2017). In doing so we test predictions that native perennial biofuels support 1) higher species richness, 2) higher diversity, 3) more pest suppression, 4) higher ant activity, and 5) fewer invasive species than lands devoted to corn production. Our results illustrate tradeoffs involved in biofuel production, emphasize the importance of considering factors beyond yield alone, and suggest ways to maximize the conservation value of working landscapes.

Materials and methods

Study sites

To compare biodiversity and ecosystem services among biofuel crops, we studied three long-term experiments managed by the Great Lakes Bioenergy Research Center (*GLBRC*, Slater et al., 2015; USDOE, 2018) at the Kellogg Biological Station in southwest Michigan, USA (42˚24′18″ N, 85˚24′02″ W, 275 m a.s.l.). The first experiment, the Biofuel Cropping System Experiment (*BCSE*, 42˚23′42″ N, 85˚22′24″ W, 286 m a.s.l., Fig. 1a), was established in 2008 and consists of biofuel crops maintained in replicate 30 x 40-meter plots. Prior data from this experiment suggested that annual and perennial biofuel crops supported distinct ant communities and associated ecosystem services (Table S1, Fig. S1). For the current study, we studied five plots each of continuous corn, switchgrass, and restored prairie treatments. The second and third experiments are both managed as part of the broader GLBRC Scale-up Experiment (GLBRC, 2018). The second, at Lux Arbor Reserve (42˚28′38″ N, 85˚26′42″ W, 296 m a.s.l., Fig. 1b), was established in 2010 on fields that had been farmed as tilled corn-soybean rotation since at least 1987. This experiment contains three large fields maintained as a single treatment each (corn 11 ha, switchgrass 14 ha, restored prairie 13 ha). The third experiment, Marshall Farms (42˚26′37″ N, 85˚18′34″ W, 291 m a.s.l., Fig. 1c), was also established in 2010 with each treatment assigned to a single large field (corn 17 ha, switchgrass 13 ha, restored prairie 11 ha). Prior to being planted in biofuels, the fields at Marshall Farms had been maintained since 1987 as semi-natural grassland under the Conservation Reserve Program. All three experiments were managed in the same way (detailed agronomic logs are available online, GLBRC, 2018). Prairie treatments were planted with a representative mix of native prairie vegetation (consisting of six grass species, three legumes, and nine non-leguminous forbs in the Biofuel Cropping System Experiment,

Sanford et al. 2016; and five grasses, one legume, and 13 non-leguminous forbs at Lux Arbor Reserve and Marshall Farms, Stahlheber et al., 2016), followed by opportunistic recruitment of additional species. Switchgrass treatments were planted as monocultures but around 20% of their plant biomass consisted of naturally recruiting forbs and grasses (Werling et al., 2014). The three biofuel crops—corn, switchgrass, and prairie—thus represent a gradient in plant diversity, habitat complexity and land use intensity. To further extend this gradient to include unmanaged non-agricultural systems, we also compared our results to native forests by studying three separate deciduous forest fragments that are part of the Kellogg Biological Station Long-Term Experimental Research study (DF1 42˚24′13″ N, 85˚23′31″ W 304 m a.s.l, DF2 42˚24′30″ N, 85˚23′48″ W 281 m a.s.l, and DF3 42˚24′45″ N, 85˚23′08″ W 295 m a.s.l.).

Ant communities

We sampled ant communities at each site during the growing season from 29 May to 24 July 2018 using pitfall traps. Traps consisted of clear plastic cups (9.8 cm diameter) filled with 95% ethanol and a few drops of unscented detergent to break surface tension. We buried the traps flush with the soil surface, mounted clear plastic rain covers ~10 cm overhead, and collected them after 48 hours. All worker ants collected in the traps were counted and identified to species using keys in regional guides (Coovert, 2005; Ellison, Gotelli, Farnsworth, & Alpert, 2012), and pinned voucher specimens from each trap were stored in the senior author's reference collection.

Sampling for each site was spread out over the growing season (median of four distinct calendar weeks of trapping for each treatment, range of two to six), with every treatment sampled both early (before 28 June) and late (after 8 July) in the season. Within the Biofuel Cropping System Experiment, we collected six pitfall traps from each of five plots of corn, switchgrass, and prairie, for a total target of 90 samples (30 from each treatment). Traps within each plot were

placed at three designated sampling stations located at least 5 meters from the nearest edge and 14 to 21 meters from each other. We first collected one trap from each sampling station, and several weeks later collected a second set of traps shifted 2 meters from the first. At the Lux Arbor Reserve and Marshall Farms experiments we collected 15 pitfall traps from each field, for a total target of 90 samples (15 from each of 3 treatments in both experiments). We first placed traps at 10 evenly spaced sampling stations within each field, and several weeks later collected five more traps from each field from locations at least 2 meters from the first set. Finally, we collected 10 pitfall traps from each of the three deciduous forests, for a total target of 30 forest samples. At each forest we first collected pitfall traps from each of five sampling stations located 19 to 61 meters apart within the forest interior, and several weeks later collected a second set of five traps shifted 2 meters from the first. Out of the total 210 pitfall traps, 11 were lost or damaged by wildlife or weather, leaving 199 samples for analysis.

Pest suppression

We compared prey removal rates among biofuel crops using a sentinel prey experiment. We placed frozen eggs of the fall armyworm *Spodoptera frugiperda*—a generalist predator of biofuel crops including corn and switchgrass (Prasifka et al., 2009)—in vertebrate exclosures and tracked their disappearance. Vertebrate exclosures consisted of plastic containers (22.5 x 16.5 x 10 cm) with circular openings (5.5 cm diameter) on two sides covered by wire mesh (grain 1.5 x 1.5 cm) that allowed insects to enter but not birds or mammals. In the center of each exclosure we placed a tin tray (6.2 cm diameter) containing 50 armyworm eggs. To sample potential predators, we placed a strip of sticky trap (2 x 7 cm) along the bottom of a far wall of the exclosure. We collected the exclosures after 48 hours, counted the remaining eggs, and calculated mortality rates.

171 Prey removal sampling was done on the same schedule and layout as the pitfall traps, with each
172 exclosure being placed on the ground at least 1 meter away from each pitfall trap. Out of the
173 target 210 vertebrate exclosures, 8 were lost or damaged by wildlife or weather, leaving 202 for
174 analysis.

175 *Data analysis*

176 To calculate ant species richness and diversity for each treatment we used the program EstimateS
177 9.1.0 (Colwell, 2013) with Chao2 sample-based rarefaction and the inverse Simpson's diversity
178 index. Because multiple workers may be captured from the same colony, we conservatively
179 treated the occurrence of a species in a pitfall trap as one observed colony, regardless of how
180 many workers were captured (Ellison, Record, Arguello, & Gotelli, 2007). All further analysis
181 was performed in the program R (R Core Team, 2018). We compared ant community
182 composition using non-metric multidimensional scaling based on Bray-Curtis dissimilarity in the
183 package *vegan* (Oksanen et al., 2018). To explore functional variation among ant communities,
184 we assigned each species to one of six guilds—honeydew gatherer, omnivore, predator, thief ant,
185 seed disperser, or social parasite—using information from regional guides (Coovert, 2005;
186 Ellison et al., 2012). For each treatment we also calculated average ant activity as the total
187 number of workers of any species captured in traps. Finally, because the presence of exotic ants
188 can be an indicator of habitat disturbance (Holway, Lach, Suarez, Tsutsui, & Case, 2002), for
189 each treatment we calculated the proportion of colonies that were invasive species (the number
190 of detections of Eurasian pavement ants *Tetramorium immigrans* divided by total species
191 detections).

192 We controlled for effects of variable weather in two ways. First, we calculated mean hourly
193 temperature over the first and second calendar days for each pitfall trap or vertebrate exclosure,

194 recorded by a weather station at the Kellogg Biological Station, and found that treatments did not
195 differ in the weather conditions at which they were sampled (Kruskal-Wallis $P = 0.18$). Second,
196 we tested whether mean hourly temperature accounted for variation in ant activity, which is the
197 variable most likely to be impacted by temperature (Bujan, Wright, & Kaspari, 2016), and found
198 no relationship ($P = 0.23$, $r^2 = 0.11$). Because pitfall traps varied in distance from each other,
199 with some as close as 2 meters, we also tested for spatial autocorrelation using a subset of the
200 data in which it was most likely to occur, the Biofuel Cropping System Experiment. In this case,
201 15 pitfall traps per treatment were placed 2 meters away from a set of 15 collected earlier in the
202 season. But we found no relationship between ant activity among paired sets of 2-meter distant
203 traps (corn, $P = 0.58$, $r^2 = 0.03$; switchgrass with 1 outlier removed, $P = 0.30$, $r^2 = 0.09$; prairie,
204 $P = 0.29$, $r^2 = 0.09$).

205 To allow for comparison among variables, we converted community traits and ecosystem
206 services (species richness, diversity, pest mortality, ant activity, and proportion of invasive
207 species) to effect sizes using the package *compute.es* (Del Re, 2013). For effect size we used the
208 standardized mean difference expressed as Hedge's *g* (Hedges & Olkin, 1985), which is the
209 number of standard deviations a value is from a baseline, including a correction factor for sample
210 size. For the baseline we used values for the corresponding corn treatment from each
211 experiment. Significant effect sizes (different from 0 at $P < 0.05$) indicate a difference from corn
212 fields, and we considered effect sizes of at least 0.8 to be large or obvious in accordance with
213 accepted usage (Cohen, 1988). Finally, to test predictions of the perenniality hypothesis we used
214 a meta-analysis approach that evaluated overall effects of native perennial biofuels versus corn
215 across all three experiments. We did this by calculating the grand mean effect size for each
216 community trait or ecosystem service, which weights the effect sizes from separate experiments

by their variance (Koricheva, Gurevitch, & Mengersen, 2013). Using the grand mean effect sizes, we tested whether native perennial biofuels support 1) higher species richness, 2) higher diversity, 3) more pest suppression, 4) higher ant activity, and 5) a lower proportion of invasive species.

Results

Native perennial biofuel crops supported ant communities that were richer and more functionally diverse than those in corn. Our 199 pitfall traps captured 5,310 ants from 539 colonies belonging to 31 species (Table 1). Species accumulation curves began to level off for all sites except two of the deciduous forests (Fig. S2), suggesting that sampling had approached completion. Estimated species richness per treatment ranged from four species in the Lux Arbor Reserve corn field to over 18 species in the Marshall Farms prairie (Table 2). Corn fields contained the lowest species richness in every experiment, with native perennial biofuels supporting 58 to 185% more species. Native perennials also contained ant species representing all six functional guilds, and forests supported all guilds except thief ants (Fig. 2). Corn fields, in contrast, lacked social parasites or specialized seed dispersers, resulting in reduced functional diversity and a presumed reduction of ant-mediated seed dispersal. These differences were reflected in the ordination space, in which ant communities clustered by habitat (Fig. S3). One ordination axis separated undisturbed forests from the three biofuel crops, while variation along the second axis appeared to mirror the gradient in land use intensity, with ant communities from corn being the simplest and those from the two native perennial treatments overlapping.

Different habitats also experienced variable predation pressure on pest insects. We measured the persistence of 10,100 armyworm eggs among 202 mammal exclosures. The more complex

communities associated with native perennial biofuels supported up to 55% more prey removal than corresponding corn fields (Table 2).

When comparing effect sizes, native perennial biofuels always contained many more species than corn fields (effect sizes all > 2), and usually supported much higher diversity (effect sizes > 4, except one switchgrass treatment less diverse at -1.0) and more pest suppression (effect sizes ≥ 0.8 except one non-significant prairie treatment) (Fig. S4, Table 3a). Ant activity and the proportion of invasive species, however, were more variable and inconsistent.

Synthesizing our results using grand means (Fig. 3, Table 3b), we confirm several predictions of the perenniality hypothesis. Native perennial biofuels supported much greater species richness (grand mean effect size 3.4 ± 0.09 for switchgrass, 4.4 ± 0.12 for prairie) and pest suppression (switchgrass 1.2 ± 0.04, prairie 0.9 ± 0.04) than corn, and prairies also supported much greater diversity (6.6 ± 0.07). Native perennials did not differ from corn in ant activity or the proportion of invasive species, and switchgrass did not support higher ant diversity.

Discussion

We demonstrate that native perennial biofuel crops harbor more complex ground dwelling insect communities and provide more associated ecosystem services than lands devoted to corn production. Consistent with the perenniality hypothesis, native perennial biofuel crops support more ant species, higher rates of natural pest suppression, and potentially higher ant diversity than corn fields. The more complex ant communities of native perennial biofuels also support higher functional diversity by including social parasites and specialized seed dispersers that are absent in corn. By focusing on ground dwelling arthropods, our results provide a more complete understanding of the biodiversity and ecosystem services supported by diverse and lightly

managed bioenergy landscapes. These biodiversity-related services of perennial biofuels are in addition to their superior climate mitigating potential and other positive environmental impacts (Robertson et al., 2017). Strips of restored prairie, for example, also reduce runoff of sediment and nutrients from corn fields into waterways (Meehan et al., 2013; Schulte et al., 2017). Restoring corn fields to native perennial vegetation can thus provide diverse conservation benefits for wildlife and people while also meeting energy needs.

Scaling up our results, a switch from corn to native perennial vegetation would have conservation implications at a national scale. The production of corn ethanol is a major driver of land use in the U.S., commandeering land that could otherwise serve as wildlife habitat or be used for food production. An average of 90 million acres (~360,000 km^2) of U.S. farmland is planted in corn each year, from which ~40% of the grain yield is used for ethanol production (USEPA, 2018). Converting just 1% of land currently devoted to growing corn to the production of cellulosic ethanol from native perennial biofuels would restore several thousand square kilometers of semi-natural landscapes and the ecosystem services they provide. Restoration could perhaps be done most easily on lands that are the least productive for corn. Native perennial biofuels can also be grown on agricultural land that is not currently in production or is only lightly used. If planted in perennial biofuels, these marginal lands could meet up to 25% of U.S. ethanol demands without converting any current crop lands (Gelfand et al., 2013).

Although we focus on perenniality as the primary factor mediating biodiversity and ecosystem services in biofuel crops (Robertson et al., 2017), other crop traits almost certainly contribute. In our experiments, for example, perenniality alone did not determine diversity. Restored prairies supported a consistently higher index of ant diversity but switchgrass fields did not. Similarly, three native perennial treatments supported fewer colonies of invasive species, but three others

did not. These results suggest that additional factors such as land use history, plant diversity, or productivity (Haddad et al., 2009) may impact insect communities and associated ecosystem services provided by biofuel crops.

Bioenergy landscapes devoted to native perennial biofuels, or those containing a mix of crops varying in management intensity, likely provide a more complete set of ecosystem services for surrounding non-fuel crops than do landscapes dominated by corn monoculture (Werling et al., 2014). The absence of seed dispersing ants in corn fields is especially noteworthy, as up to 5% of the world's plant species rely on ants for dispersal (Lengyel, Gove, Latimer, Majer, & Dunn, 2010), rising to over 30% of herbaceous species in some North American habitats (Beattie & Culver, 1981) and including some grassland plants (Wills & Landis, 2018). It is unclear whether the absence of seed dispersing ants in corn fields impacts the dispersal of native plants in surrounding habitats. Landscapes devoted to intensive corn production may experience limited seed dispersal in remaining areas of natural vegetation or in other crop types, due to smaller or more fragmented populations of seed dispersing ants. Other ecosystem services mediated by ants, including potential impacts on soil quality, pest suppression, and yield, may likewise be reduced by the conversion of large portions of landscapes to corn production. Further research may reveal feedbacks in which restoring native perennial vegetation provides additional benefits to farmers that help recoup the costs of reduced yield.

As in other agricultural systems, maximizing yield alone in the production of biofuels comes at the expense of reduced biodiversity, climate mitigation potential, and other ecosystem services that could otherwise be provided by working landscapes (Foley et al., 2005; Landis, 2017). Recognizing tradeoffs and seeking to optimize overall returns from all services combined, in contrast, can lead to better informed management practices that provide more benefits for

wildlife and people. Switching from annual corn to native perennial biofuel crops, which have a lower ethanol yield but outperform corn in the provision of other services (Sanford et al., 2016; Robertson et al., 2017; Landis et al., 2018), is a prime example of this ecosystem services-based focus. Incorporating ecosystem services into agricultural decision-making ultimately depends on the value stakeholders place on potential services, which may vary across landscapes (Castro et al., 2014), and conversion of agricultural lands to native perennial biofuels will likely depend on policies or incentives designed to reduce economic risk and opportunity costs for farmers and land managers (Robertson et al., 2017; Landis et al., 2018). Large scale investment in the ecosystem services provided by working landscapes can nevertheless lead to dramatic reductions in environmental degradation and supplement the creation of protected areas (Ouyang et al., 2016; Xu et al., 2017). Valuing or incentivizing additional ecosystem services in corn agriculture and ethanol production could likewise have major conservation implications (Foley, 2013) by deciding the fate of hundreds of thousands of square kilometers of land, and potentially leading to the ecological restoration of entire landscapes.

Authors' contributions

All authors conceived the ideas and designed the methods; JAH, SEI, and BDW collected the data; JAH led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data used in this manuscript will be archived upon publication and publicly available on the KBS LTER website (https://lter.kbs.msu.edu/data/) in accordance with National Science Foundation Long-Term Ecological Research policy.

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Figures

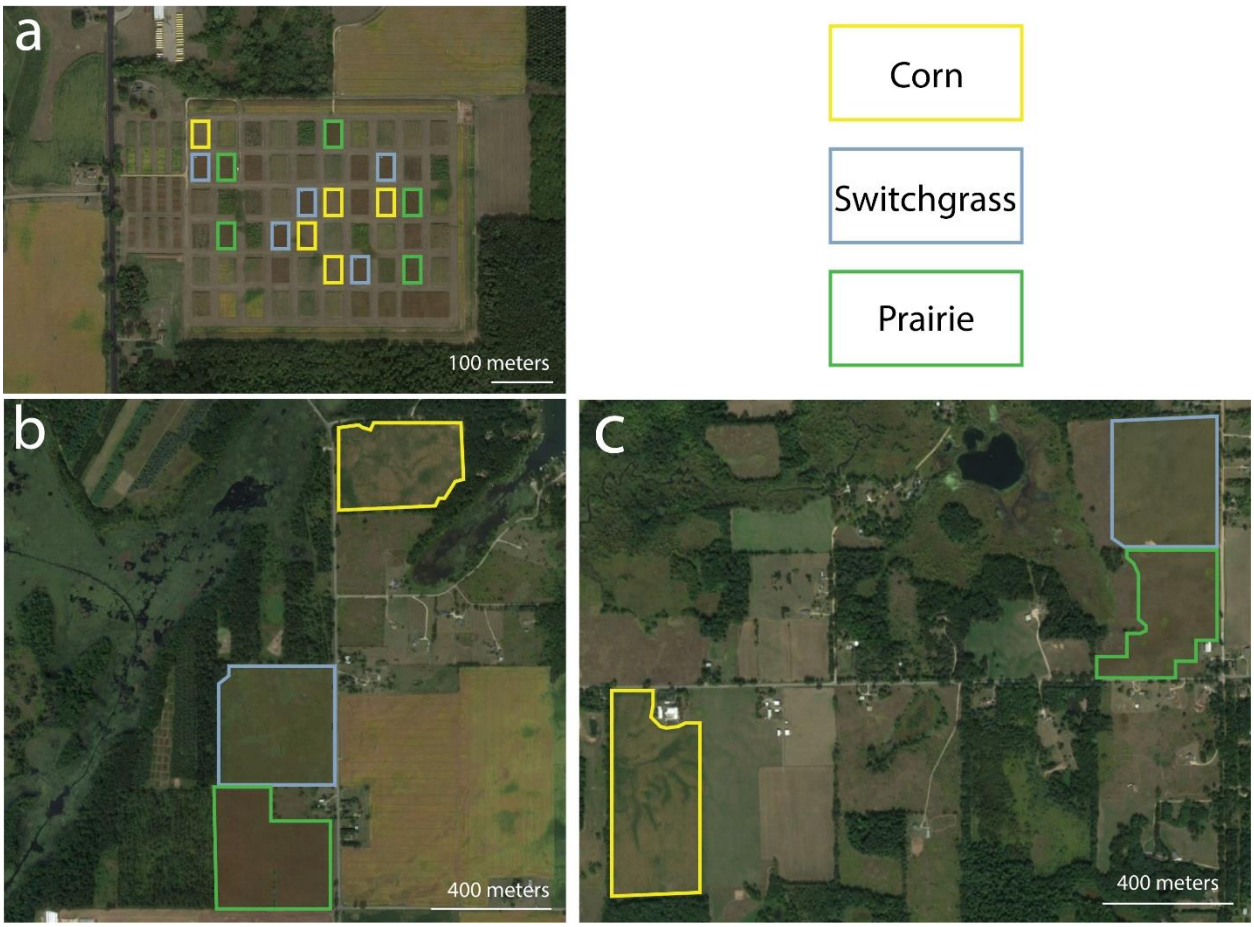
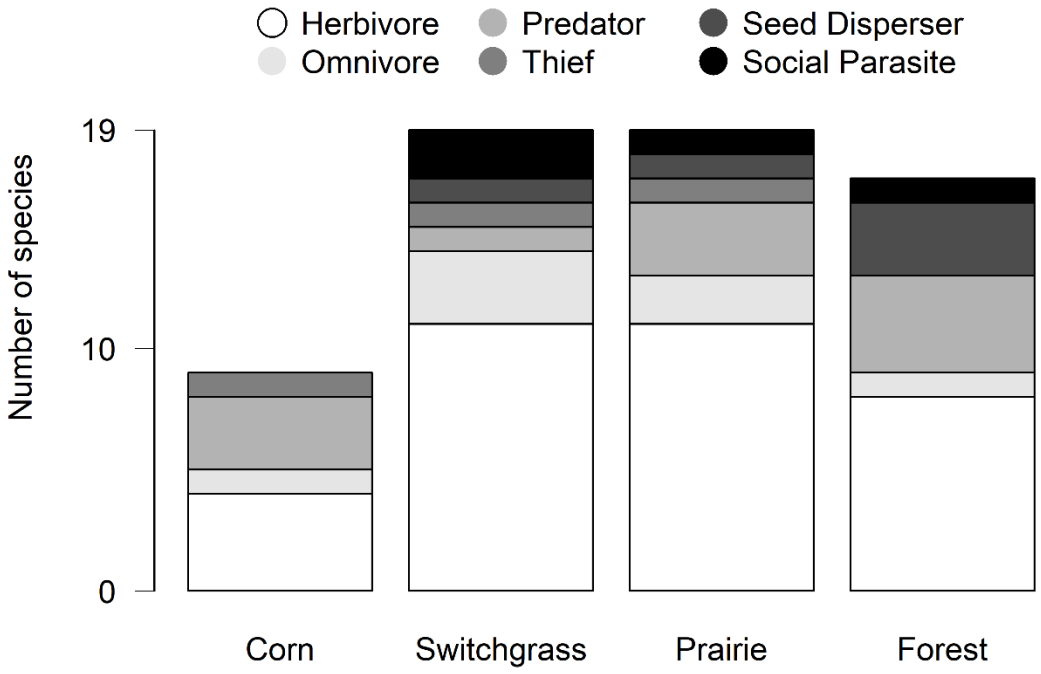
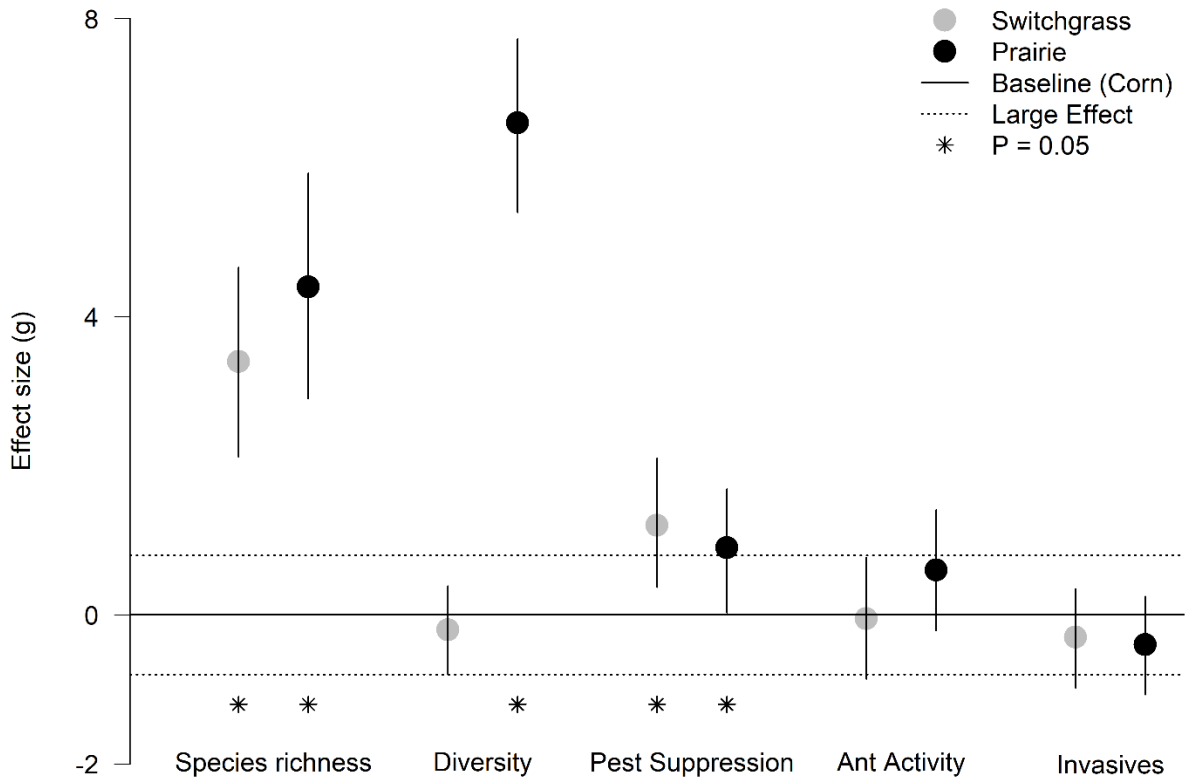


Figure 1. We studied three long-term experiments—one with multiple small plots of each biofuel treatment (a, Biofuel Cropping System Experiment), one with large fields for each treatment (b, Lux Arbor Reserve), and one with large fields on former Conservation Reserve Program land (c, Marshall Farms)



499

500 Figure 2. Corn fields lack seed dispersers and social parasites, whereas perennial biofuels and
501 native forests support a broad mix of ant guilds. Bars show number of species grouped by
502 treatment over all three experiments.



503

504 Figure 3. Overall effects (Hedge's *g*) of native perennial biofuels versus corn on ant communities
505 and ecosystem services. Dots show grand means and 95% confidence intervals for each
506 treatment across all three experiments. Asterisks show effects that differ from zero, and dotted
507 lines show an effect size of ±0.8, a standard cutoff for large or obvious effects.

Tables

Table 1. Ants collected in three long-term experiments and in native forests at Kellogg Biological Station, Michigan, USA. Values are percentages of pitfall traps that captured a given species from each experiment and habitat.

Species	Guild	Corn			Switchgrass			Prairie			Forest		
		B	L	M	B	L	M	B	L	M	1	2	3
Aphaenogaster picea	Seed Disperser	-	-	-	-	-	-	-	-	-	22.2	40.0	100
Aphaenogaster rudis	Seed Disperser	-	-	-	-	-	92.9	-	-	93.3	22.2	10.0	-
Brachymyrmex depilis	Honeydew	20.7	18.2	7.1	3.3	6.7	14.3	16.7	-	13.3	-	-	14.3
Camponotus pennsylvanicus	Honeydew	-	-	-	-	-	-	-	-	-	88.9	70.0	28.6
Formica glacialis	Honeydew	-	-	7.1	6.7	-	35.7	3.3	-	-	-	-	-
Formica incerta	Honeydew	-	-	-	3.3	26.7	7.1	10.0	-	60.0	-	-	-
Formica neogagates	Honeydew	-	-	-	-	-	-	-	-	6.7	-	-	-
Formica pallidefulva	Honeydew	-	-	-	10.0	13.3	-	20.0	-	20.0	-	-	-
Formica rubicunda	Social Parasite	-	-	-	-	-	-	-	-	13.3	-	-	-
Formica subsericea	Honeydew	-	-	-	-	20.0	-	-	13.3	53.3	-	-	-
Lasius alienus	Honeydew	-	-	-	-	-	7.1	-	-	6.7	22.2	-	71.4
Lasius claviger	Social Parasite	-	-	-	3.3	-	-	-	-	-	-	-	-
Lasius nearcticus	Honeydew	-	-	-	-	-	-	-	-	-	-	-	14.3
Lasius neoniger	Honeydew	72.4	-	7.1	66.7	46.7	14.3	80.0	33.3	26.7	-	-	-
Lasius umbratus	Social Parasite	-	-	-	-	-	-	-	-	-	-	20.0	-
Myrmecina americana	Predator	-	-	14.3	-	-	-	-	-	6.7	11.1	20.0	42.9
Myrmica americana	Omnivore	-	-	-	3.3	-	-	-	-	-	-	-	-
Myrmica detritinodis	Honeydew	-	-	-	-	-	28.6	-	-	20.0	33.3	-	-
Myrmica incompleta	Honeydew	-	-	-	3.3	-	-	-	-	-	-	-	-
Myrmica punctiventris	Seed Disperser	-	-	-	-	-	-	-	-	-	33.3	90.0	85.7
Ponera pennsylvanica	Predator	3.4	-	-	-	-	-	3.3	-	-	-	-	14.3
Prenolepis imparis	Honeydew	37.9	36.4	78.6	-	6.7	14.3	20.0	6.7	53.3	11.1	20.0	-
Solenopsis molesta	Thief	27.6	9.1	-	13.3	-	-	53.3	13.3	26.7	-	-	-
Stenamma brevicorne	Predator	-	-	28.6	-	6.7	42.9	6.7	6.7	6.7	11.1	-	14.3
Stenamma impar	Predator	-	-	-	-	-	-	-	-	-	11.1	-	28.6
Tapinoma sessile	Omnivore	-	-	-	6.7	20.0	14.3	30.0	-	13.3	11.1	-	-
Temnothorax ambiguus	Honeydew	-	-	-	6.7	26.7	50.0	-	-	40.0	-	-	-
Temnothorax americanus	Social Parasite	-	-	-	-	-	7.1	-	-	-	-	-	-
Temnothorax curvispinosus	Honeydew	-	-	-	-	-	-	-	-	-	11.1	30.0	28.6
Temnothorax longispinosus	Honeydew	-	-	-	-	-	-	-	-	-	-	-	14.3
Tetramorium immigrans	Omnivore	82.8	90.9	64.3	70.0	40.0	35.7	76.7	60.0	46.7	-	-	-
Total		**6**	**4**	**7**	**12**	**10**	**13**	**11**	**6**	**17**	**12**	**8**	**12**

Site abbreviations: B = Biofuel Cropping System Experiment, L = Lux Arbor Reserve, M = Marshall Farms

512 Table 2. Ant communities and ecosystem services for each experiment and habitat

Experiment	Treatment	Pitfall Traps (*n*)	Sentinel prey (*n*)	Species Richness (Chao2)	Diversity (Inverse Simpson's)	Pest Mortality (%)	Ant Activity (# workers)	Invasives (Proportion of colonies)
Biofuel Cropping System Experiment	Corn	29	29	6.00 (0.48)	4.07 (0.06)	51.1 (27.13)	26.9 (41.04)	0.34 (24/71)
	Switchgrass	30	28	14.42 (3.07)	3.99 (0.09)	79.3 (19.78)	22.9 (18.40)	0.36 (21/59)
	Prairie	30	30	11.48 (1.26)	5.97 (0.08)	79.1 (19.10)	68.8 (97.64)	0.24 (23/96)
Lux Arbor Reserve	Corn	11	12	4.00 (0.23)	2.51 (0.16)	60.3 (19.23)	10.0 (7.39)	0.59 (10/17)
	Switchgrass	15	15	11.40 (2.44)	7.27 (0.27)	86.4 (14.90)	14.1 (16.74)	0.19 (6/32)
	Prairie	15	15	6.31 (0.88)	3.43 (0.22)	78.3 (23.21)	16.9 (14.23)	0.45 (9/20)
Marshall Farms	Corn	14	15	8.39 (2.43)	3.75 (0.23)	69.1 (15.91)	15.5 (14.86)	0.31 (9/29)
	Switchgrass	14	15	13.56 (1.12)	7.6 (0.22)	85.7 (12.60)	12.9 (7.52)	0.10 (5/51)
	Prairie	15	15	18.40 (2.11)	10.3 (0.27)	75.2 (15.44)	31.3 (24.85)	0.09 (7/76)
Native Forests	Forest 1	9	8	15.33 (3.79)	6.7 (0.51)	74.5 (21.60)	7.8 (5.69)	0 (0/26)
	Forest 2	10	10	8.00 (0.11)	5.4 (0.27)	55.4 (34.84)	9.6 (7.45)	0 (0/30)
	Forest 3	7	10	14.14 (2.77)	7.4 (0.36)	78.0 (23.38)	24.4 (18.05)	0 (0/32)

Values for *Species Richness*, *Diversity*, *Pest Mortality*, and *Ant Activity* are means with standard deviations in parentheses. Those for *Invasives* show proportion for entire treatment with raw totals in parentheses.

513

514 Table 3. Effect sizes for ecosystem services in native perennial biofuels versus corn

a. Effect Sizes

Experiment	Treatment	Species Richness	Diversity	Pest Suppression	Ant Activity	Invasives
BCSE	Switchgrass	**3.75 (0.19)**	**-1.04 (0.02)**	**1.17 (0.08)**	-0.12 (0.07)	0.05 (0.04)
	Prairie	**5.64 (0.34)**	**26.77 (1.81)**	**1.18 (0.08)**	**0.55 (0.07)**	-0.27 (0.04)
Lux Arbor	Switchgrass	**3.83 (0.43)**	**21.37 (1.16)**	**1.49 (0.18)**	0.29 (0.15)	**-0.98 (0.13)**
	Prairie	**3.25 (0.35)**	**4.76 (0.08)**	**0.81 (0.15)**	0.56 (0.15)	-0.3 (0.13)
Marshall Farms	Switchgrass	**2.65 (0.26)**	**17.04 (0.75)**	**1.13 (0.15)**	-0.21 (0.14)	**-0.76 (0.11)**
	Prairie	**4.29 (0.45)**	**26.02 (1.71)**	0.38 (0.13)	0.74 (0.14)	**-0.83 (0.10)**

Values are effect sizes relative to the corn treatment in the same experiment (Hedge's *g*) with variances in parentheses. Bold numbers differ from corn at $P = 0.05$. *BCSE* is the Biofuel Cropping System Experiment.

b. Grand Means

Treatment	Species Richness	Diversity	Pest Suppression	Ant Activity	Invasives
Switchgrass	**3.40 (0.09)**	-0.21 (0.02)	**1.23 (0.04)**	-0.05 (0.04)	-0.32 (0.02)
Prairie	**4.41 (0.12)**	**6.56 (0.07)**	**0.86 (0.04)**	0.60 (0.04)	-0.41 (0.02)

Values are grand means of effect sizes relative to corn across all experiments (Hedge's *g*) with variances in parentheses. Bold numbers differ from corn at $P = 0.05$.

515

Supporting Information for

Ant biodiversity and ecosystem services in bioenergy landscapes

Jackson A. Helms IV, Selassie E. Ijelu, Bill D. Wills, Douglas A. Landis, Nick M. Haddad

Supporting Tables & Figures

S1. Preliminary sampling methods

To initially explore how biofuel plantings impact ground predators and pest suppression services in potential bioenergy crops we sampled the ground foraging arthropod community and recorded sentinel egg removal within selected treatments in the Great Lakes Bioenergy Research Center's Biofuel Cropping System Experiment (https://lter.kbs.msu.edu/research/long-term-experiments/glbrc-intensive-experiment/). The experiment was initially started in 2008 and we sampled three times during the 2016 growing season (June, July, and August). Sampled treatments included corn (treatment name G3), soybean (G4) switchgrass (G5), early successional fields (G9), and restored prairie treatments (G10). Sampling began two weeks after the last annual crop was planted (e.g., soybean; circa June 13, 2016) and was repeated two additional times near the middle of each following month. During each sampling period we set and retrieved 75 pitfall samples, for a total of 225 pitfalls.

In each of $n = 5$ treatment replicates (approximately 30 m x 40 m), we placed three pitfalls (110 mm diameter) 0.5 m from the sampling path, near existing sampling locations ($n = 3$) and avoiding any permanent sampling structures. The pitfalls were left running (e.g., open) for two

weeks. From pitfalls we recorded the number of ants, spiders, predatory beetles, crickets, and slugs. Crickets and slugs were included as previous observations had shown they contribute to sentinel egg removal.

We estimated pest suppression services using sentinel egg cards. An egg card consisted of ~100 corn earworm (*Helicoverpa zea*) eggs (frozen to prevent hatching) attached to cardstock and placed in a 100 mm petri dish. One dish was covered with a 0.5 m^2 wire mesh providing ants access to eggs and excluding predatory beetles, and a second covered with a fine mesh excluding all other insects from access to egg cards. Pitfalls and egg cards were separated by 0.5 m. During each 48-hour sampling period we set and retrieved 150 egg cards for a total of 450 egg cards in total.

The number of ant and non-ant predators was pooled across all pitfalls collected across an entire season (three pitfalls for each of the three sampling periods) and log transformed. As ants are social insects we estimated their activity as the number of workers captured in pitfalls, rather than counting the number of colonies. Percent egg removal was similarly estimated by pooling the number of eggs exposed and removed across the entire season within an individual field.

37 Table S1. Ants previously collected in the Biofuel Cropping System Experiment in 2016

Species	Guild	Soy	Corn	Switchgrass	Early Successional	Prairie
Brachymyrmex depilis	Honeydew	X	X	X	X	X
Crematogaster cerasi	Omnivore	-	-	-	X	X
Formica subsericea	Honeydew	X	X	X	X	X
Lasius alienus	Honeydew	X	X	-	-	-
Lasius flavus	Honeydew	-	-	-	-	X
Lasius interjectus	Honeydew	-	-	X	-	-
Lasius latipes	Social Parasite	-	-	X	X	X
Lasius neoniger	Honeydew	X	X	X	X	X
Lasius subglaber	Honeydew	X	-	-	X	-
Myrmica detritinodis	Honeydew	-	X	-	X	X
Myrmica incompleta	Honeydew	X	X	-	X	-
Ponera pennsylvanica	Predator	X	X	X	X	X
Prenolepis imparis	Honeydew	X	X	X	X	X
Solenopsis molesta	Thief	X	X	X	X	X
Stenamma brevicorne	Predator	X	X	-	X	-
Tapinoma sessile	Omnivore	-	-	X	-	X
Temnothorax ambiguus	Honeydew	-	X	X	-	X
Temnothorax curvispinosus	Honeydew	-	-	-	-	X
Tetramorium immigrans	Omnivore	X	X	X	X	X
Species Richness		**11**	**12**	**11**	**13**	**14**
Ant Activity		664	804	848	2626	1950
Simpson's 1 - D Diversity Index		0.71	0.67	0.53	0.73	0.66

Ant Activity shows the total number of foraging worker ants captured from each treatment in pitfall traps from June to August (n = 45 traps per treatment). *Simpson's 1 – D Diversity Index* is based on the same pitfall traps.

38

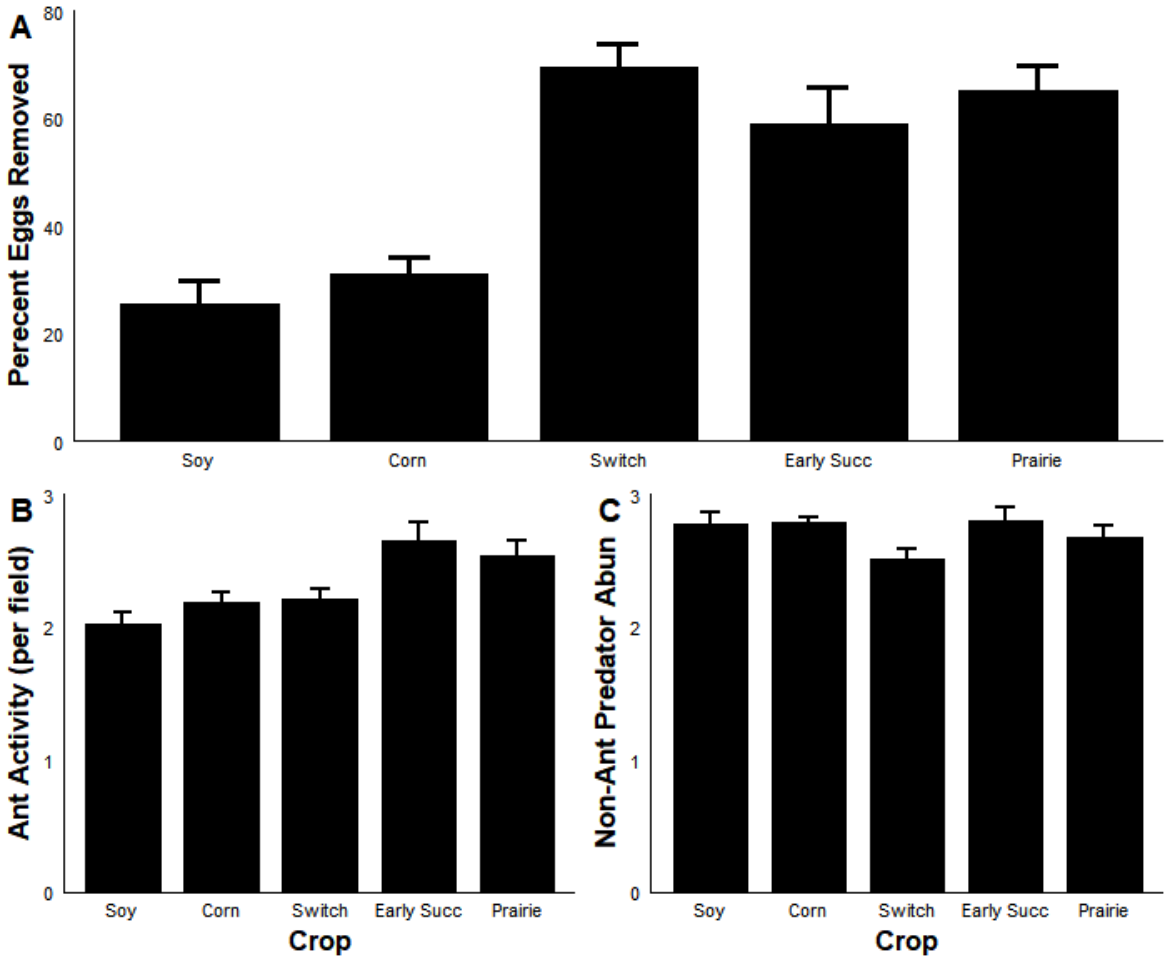


Figure S1. Preliminary data from the Biofuel Cropping System Experiment in 2016 showed variation among biofuel crops (soybean, corn, switchgrass, early successional vegetation, restored prairie) in (A) pest egg mortality, (B) ant activity, and (C) the abundance of other invertebrate predators. Data are from June to August, bars show means with standard errors, y-axes in (B) and (C) are log-scale. Egg removal rates were measured in non-ant predator exclosures containing ~100 frozen eggs of the corn earworm (*Helicoverpa zea*) and placed in each treatment for 48 hours (n = 45 per treatment). Ant activity is the number of workers of any species captured in pitfall traps (110 mm diameter) placed in each treatment (n = 42 to 45 per treatment) for two weeks. Non-ant predator abundance was counted from the same pitfall traps as the ants.

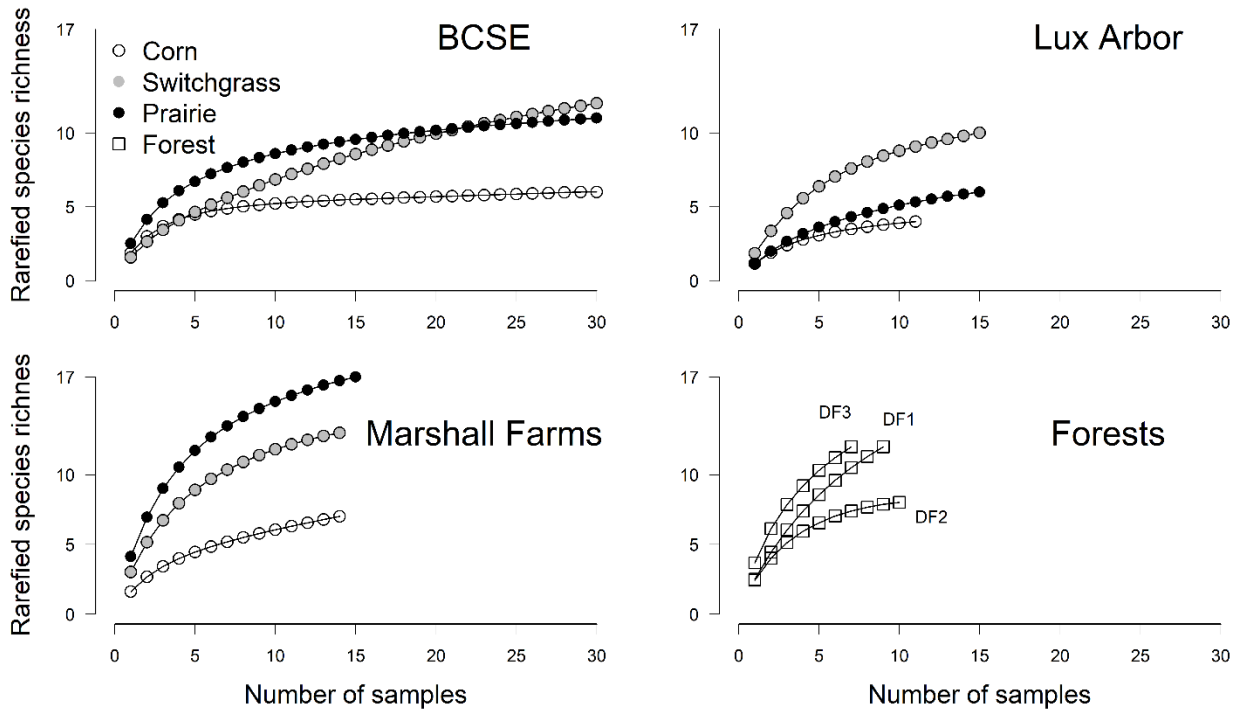
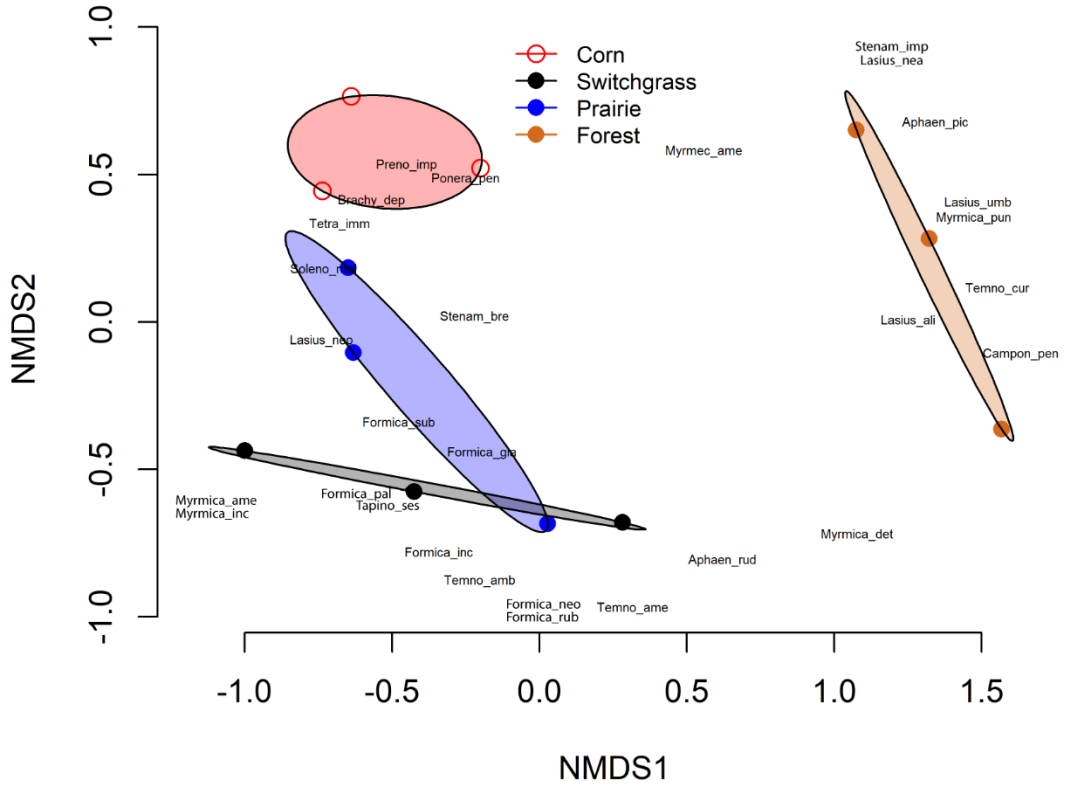


Figure S2. Species accumulation curves of ants in biofuel crops and in native forests. *BCSE* is the Biofuel Cropping System Experiment, and *DF1*, *DF2*, and *DF3* are the three deciduous forest fragments.



54

55 Figure S3. Ant communities differ by treatment. Forest communities are the most distinct
56 (separated along NMDS1), with remaining communities located along a gradient in plant
57 diversity and land use intensity (NMDS2) from annual corn to perennial prairie and switchgrass.
58 Ordination is non-metric multidimensional scaling using Bray-Curtis dissimilarity based on the
59 number of colonies of each species. Treatments are grouped by ellipsoid hulls.

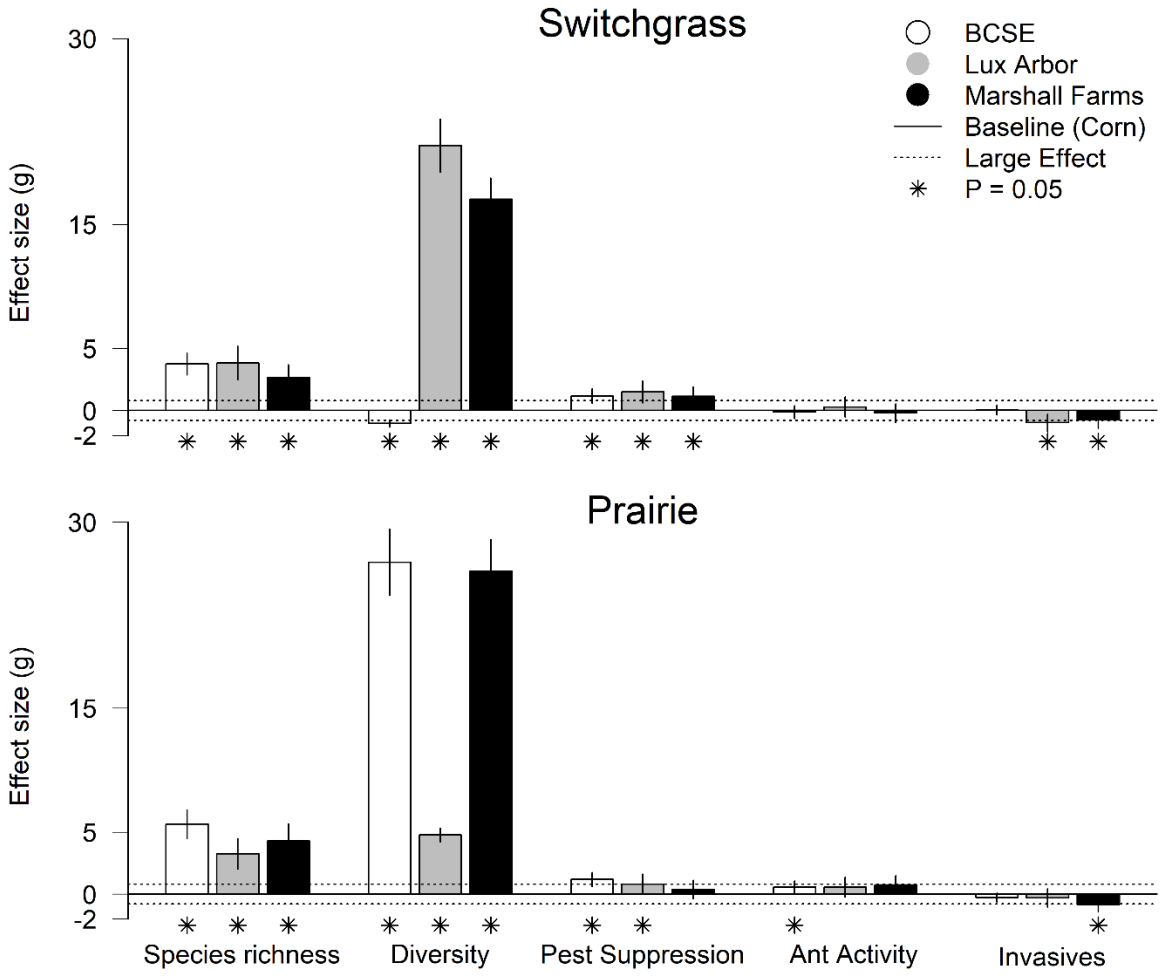


Figure S4. Ant communities and ecosystem services in native perennial biofuels compared to corn. Bars show effect sizes (Hedge's *g*) with 95% confidence intervals for each treatment relative to corn in each of three experiments. Asterisks show effects that differ from zero, and dotted lines show an effect size of ±0.8, a standard cutoff for large or obvious effects. *BCSE* is the Biofuel Cropping System Experiment.