APPLIED ECOLOGY

Contrasting effects of bioenergy crops on biodiversity

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Agriculture is driving biodiversity loss, and future bioenergy cropping systems have the potential to ameliorate or exacerbate these effects. Using a long-term experimental array of 10 bioenergy cropping systems, we quantified diversity of plants, invertebrates, vertebrates, and microbes in each crop. For many taxonomic groups, alternative annual cropping systems provided no biodiversity benefits when compared to corn (the business-as-usual bioenergy crop in the United States), and simple perennial grass–based systems provided only modest gains. In contrast, for most animal groups, richness in plant-diverse perennial systems was much higher than in annual crops or simple perennial systems. Microbial richness patterns were more eclectic, although some groups responded positively to plant diversity. Future agricultural landscapes incorporating plant-diverse perennial bioenergy cropping systems could be of high conservation value. However, increased use of annual crops will continue to have negative effects, and simple perennial grass systems may provide little improvement over annual crops.

INTRODUCTION

Most pathways to addressing climate change require expanded use of bioenergy (*1*). However, widespread adoption of bioenergy crop feedstocks can transform landscapes, strongly affecting ecosystems and biodiversity (*2*–*5*). The direction and magnitude of these changes will depend on which cropping systems are adopted, which types of ecosystems they replace on the landscape, and how these crops are managed. There are currently competing visions for which crops could be grown and how they should be incorporated into managed landscapes. In the United States, corn is a dominant crop and is the business-as-usual option for producing ethanol from grain, with ~40% of harvest currently allocated to biofuel production (*6*). However, expanding the footprint of corn or other intensive cropping systems dismantles natural ecosystems, reduces biodiversity, and undermines key ecosystem services that agriculture ultimately depends on (*4*, *7*–*9*). Corn ethanol may also be more carbon intensive than gasoline after emissions from land use change are taken into account (*5*). Given these sustainability concerns, low-input perennial crops are a compelling alternative to annual systems as a bioenergy source. Biodiversity in perennial biofuel crops is usually higher than in arable crops (*8*, *10*, *11*). In addition, in general, adding perennial features to agricultural landscapes can promote valuable ecosystem services locally and at landscape scales by increasing structural complexity (*8*, *12*–*16*). Still, perennial biofuel systems tend to be less biodiverse than natural and seminatural reference systems (*2*, *11*, *17*), so their net effects on ecosystems and biodiversity (in addition to their net carbon balance) will depend both on characteristics of the crops in question and on which ecosystems they replace. In some contexts, biofuel crops could replace natural or seminatural systems, whereas in others, they may replace arable crops, particularly on land where traditional crops are unprofitable (*18*–*21*).

We address two important knowledge gaps concerning biodiversity in bioenergy crops. First, there are many potential cropping systems that could become widely adopted, but there have been virtually no empirical experiments carried out to compare candidate cropping systems simultaneously and measure how they affect local biodiversity. Instead, most of our ability to forecast comes from literature reviews and meta-analyses composed of narrower studies (*2*, *10*, *17*), databases (*11*), or simulations (*22*). These syntheses have shown us that perennial and/or second-generation bioenergy crops tend to be more biodiverse than annuals and that both types of systems are less biodiverse than natural systems. However, they are not well suited to make detailed comparisons between perennial crop types. Second, the taxonomic scope of past work on this topic has been limited, often focusing on one to two animal groups per study (*23*–*28*), but available evidence suggests that different lineages of organisms can respond inconsistently to cropping systems (*2*, *10*, *11*, *17*). We also have only a nascent understanding of soil microbial communities across different bioenergy crops despite their roles governing key ecological processes (*29*–*36*). As a result, our ability to make broad statements about the impacts of candidate bioenergy crops on biodiversity and ecosystem functioning, particularly within the wide range of low-input perennial systems that could be adopted, is limited.

To address these knowledge gaps, we conducted broad biodiversity censuses in 10 bioenergy cropping systems grown in a well-established long-term experimental array (28 m–by–40 m plots, five replicates for each crop). We surveyed a spectrum of crop and grassland types that could be widely adopted or expanded in North America, including three intensive annual systems (corn, sorghum, and sorghum with winter cover crop), four simple grass-based perennial systems (*Miscanthus*, mature and newly establishing switchgrass stands, and a native prairie grass mix), and three complex perennial polycultures (reconstructed prairie, successional vegetation, and short-rotation poplar). Within the annual systems, corn is currently the dominant North American bioenergy feedstock; energy sorghum has been developed as an alternative,

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having yields that approach corn but with greater resource use efficiency (*37*, *38*). Among the simple perennial systems that we censused, *Miscanthus* × *giganteus* is a perennial and fast-growing sterile hybrid grass that produces dense, high-yielding bamboo-like thickets (*37*, *38*), and switchgrass (*Panicum virgatum*) is a perennial prairie grass native to North America from which several high-yielding varieties have been developed for bioenergy (*39*, *40*). We censused communities in two switchgrass systems, including mature stands originally seeded in 2008 and newly establishing stands that contained dead cover crop residue (*Trifolium pratense*) in the spring and increasing switchgrass cover as the season progressed. We also censused a native grass mix containing switchgrass plus four native perennial grasses that are characteristic of prairies in the U.S. Midwest. Among the complex perennial polycultures that we sampled, reconstructed prairie plots contained a seeded mix of 18 native prairie species (6 grasses, 9 forbs, and 3 legumes) plus limited volunteers. Successional vegetation plots contained unmanaged volunteer vegetation, and short-rotation coppice poplar plots contained *Populus* NM-6 (*Populus nigra* × *Populus maximowiczii*), with a diverse and mostly unmanaged understory. Poplars were in their third year of growth during data collection.

Within each of the 10 cropping systems, we profiled macro- and microorganismal groups including plants, bees, butterflies, ground beetles, ants, birds, small mammals, prokaryotes, fungi, and microeukaryotes (i.e., eukaryotes that are not plants, animals, or fungi; fungi were censused separately). We used a mix of in field identification, trapping, and metagenomics based on environmental DNA. We had three objectives. First, we compared taxonomic richness of all organismal groups, as well as abundance of each animal group, across the 10 cropping systems. We expected these community attributes to vary across systems, and beyond the general pattern of higher diversity in perennial crops than in annuals, we expected to observe strong variation across the different perennial systems. Second, we measured community dissimilarity for each taxonomic group, partitioned into nestedness and turnover. These are independent sources of dissimilarity and are relevant to biodiversity conservation because they indicate whether communities differ because they contain unique species assemblages or because one is a species-poor subset of the other. Last, we examined the gradient of plant diversity and composition that these cropping systems comprise and demonstrate its role in shaping diversity of other groups of organisms. We expected this relationship to take the form of positive correlations between plant richness and that of other groups of organisms.

RESULTS

Richness and abundance depend strongly on bioenergy crop type

Richness and abundance of nearly all groups differed strongly among cropping systems (Figs. 1 to 3; see fig. S1 for results based on evenness-adjusted diversity measures, which corroborate richness-based figures in the main text). Because corn is the business-as-usual bioenergy crop grown in the United States, we use it as a baseline to compare other cropping systems against using effect sizes (Hedges' *g*; Fig. 1). We consider effect sizes with 95% confidence intervals that do not encompass zero to be significant. See table S1 and figs S2 to S3 for results of additional statistical tests assessing whether mean species richness differed by cropping system for each taxonomic group. To summarize succinctly across many cropping systems, at times, we refer to them collectively as annual systems (corn and sorghum), simple perennial systems (*Miscanthus*, switchgrass, and native grasses), and complex perennial systems (prairie, successional vegetation, and poplar).

For plants and most animal groups, biodiversity gains over corn were distinctly larger in complex perennial polyculture systems than in simple perennial systems (Fig. 1). Within the complex habitats, effects relative to corn were positive in all cases for all taxonomic groups except small mammals and ground beetles. In addition, positive differences were always significant except for birds in prairie and successional vegetation. In contrast, biodiversity gains for plants and animals in the simple perennial systems were weaker. *Miscanthus* was particularly species poor, and richness within this crop did not differ from corn for any taxonomic group. In both switchgrass systems, bee group richness was lower than corn, but ant richness was higher. In contrast to other simple perennial treatments, diversity in the mix of native grasses was usually higher than corn, with significant and positive effects for plants, butterflies, bumblebees, and ants. Last, species richness in the two sorghum-based systems tended to be similar to or lower than corn. In the continuous sorghum system, plant species richness was lower than corn, whereas small mammal richness was higher. This same pattern was present, but weaker, in the sorghum + winter cover crop system; in this treatment, bee functional group diversity was also lower.

We highlight some of the more notable differences by averaging species richness across all plots in each of the three treatment categories. Plant richness in complex perennial systems was 3.6× that of corn and 2.8× that in simple perennial systems. Butterflies and bumblebees were, on average, >9× more species rich in complex perennial systems than in corn and contained 3.5× and 6.3× more species, respectively, than in simple perennial systems. Similarly, richness of both bee groups and birds in complex perennial systems was more than double that in corn or simple perennial systems, whereas simple perennial systems contained fewer taxa, on average, than corn. Last, ant richness in complex perennial systems was 2.2× that of corn and 1.5× that of simple perennial systems (fig. S2).

Abundance of animals also varied strongly among cropping systems (Fig. 2). As with richness, it differed most strongly from corn in the complex perennial habitats. Bee and butterfly abundance were consistently highest in these treatments, whereas effects on ant abundance (i.e., activity density) tended to be positive but were weaker. Differences in bird and mammal abundance were weak and/or somewhat negative except in poplar, where birds were most abundant. Patterns of animal abundance in switchgrass and the native grass mix were eclectic, whereas in *Miscanthus* and sorghum, abundances tended to be similar to or lower than in corn.

Within the microbial groups, richness tended to be greater in perennial systems than in corn but was eclectic depending on the habitat compartment that we sampled (soil matrix, roots, or leaves) and the group in question (fungi, prokaryotes, and microeukaryotes; Fig. 3 and fig. S3). Effects within the root zone were most consistent; richness of all three microbial groups here was markedly higher than in corn across all seven perennial treatments. Patterns within the phyllosphere were more complex. Effects on microeukaryotes were inconsistent, but richness tended to be higher in the complex polyculture treatments; in contrast, effects on fungal

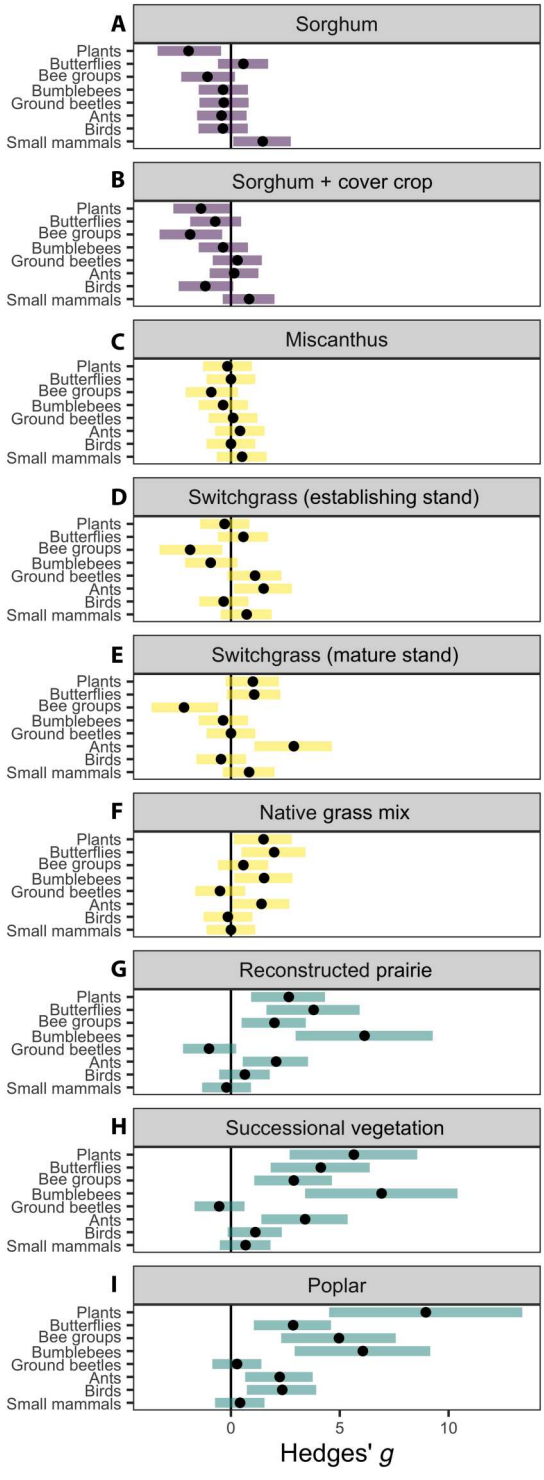

Fig. 1. Effects of each crop type on plant and animal species richness relative to corn. Species richness of plants and several animal groups varied strongly among bioenergy cropping systems and was often markedly higher in complex perennial polycultures than in simple perennial treatments or annual treatments (**A** to **I**). Here, we show species richness differences expressed as effect size (Hedges' *g*) relative to corn, the business-as-usual bioenergy crop in the United States. Error bars are 95% confidence intervals, and cropping systems are color-coded to denote annual systems (purple), simple perennial monocultures or near-monocultures (yellow), and complex perennial polycultures (blue). Data are from five replicate plots per cropping system.

richness were consistently weak. In contrast, prokaryote richness in the phyllosphere was significantly lower than corn in all perennial treatments except the newly establishing switchgrass system. Within the soil matrix, microeukaryote richness did not differ strongly among cropping systems. In contrast, soil fungal richness was higher than corn across all perennial systems. Prokaryote richness in the soil matrix tended to be lower than in corn and was significantly so in the *Miscanthus*, native grass, and successional vegetation systems. Last, in sorghum-based systems, patterns of microbial richness were similar to corn. In the continuous sorghum treatment, richness never differed strongly from corn for any group of microorganisms. In the sorghum and winter cover crop system, most groups of microorganisms assessed had lower richness than in corn, except for microeukaryotes within the root zone,

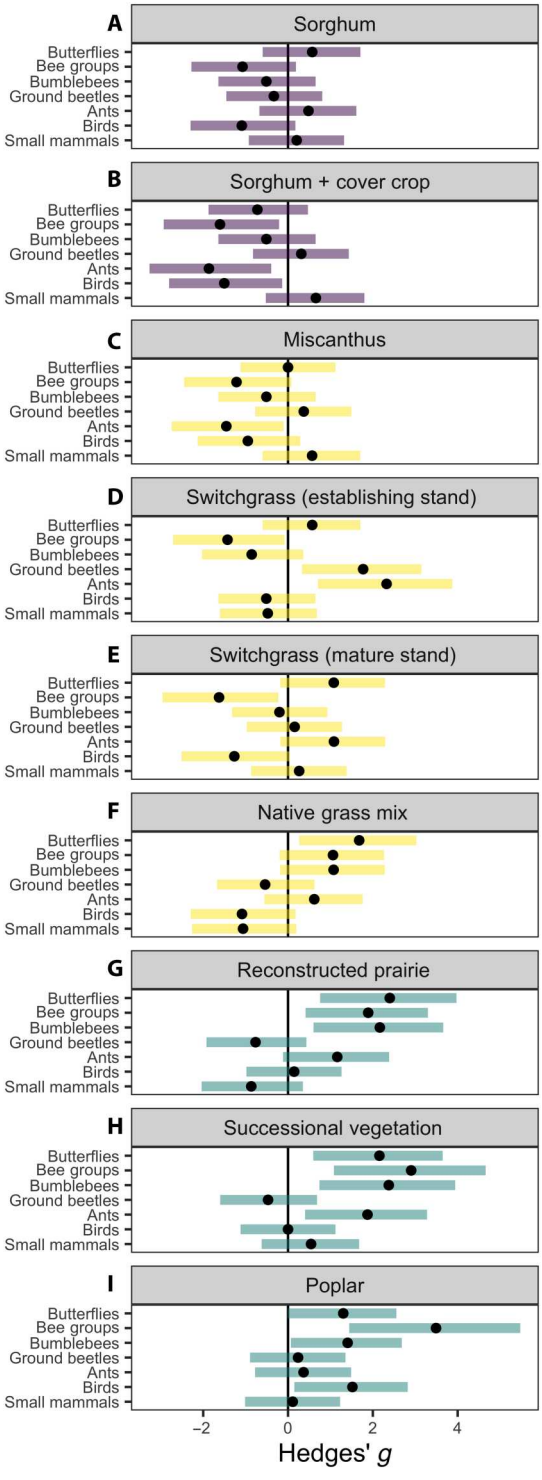

Fig. 2. Effects of each crop type on animal abundance relative to corn. Abundance of most animal groups varied strongly among bioenergy cropping systems and was highest in complex perennial polycultures (**A** to **I**). Differences in abundance are expressed as effect size (Hedges' *g*) relative to corn, and error bars are 95% confidence intervals. Cropping systems are color-coded to denote annual systems (purple), simple perennial monocultures or near-monocultures (yellow), and complex perennial polycultures (blue). Data are from five replicate plots per cropping system.

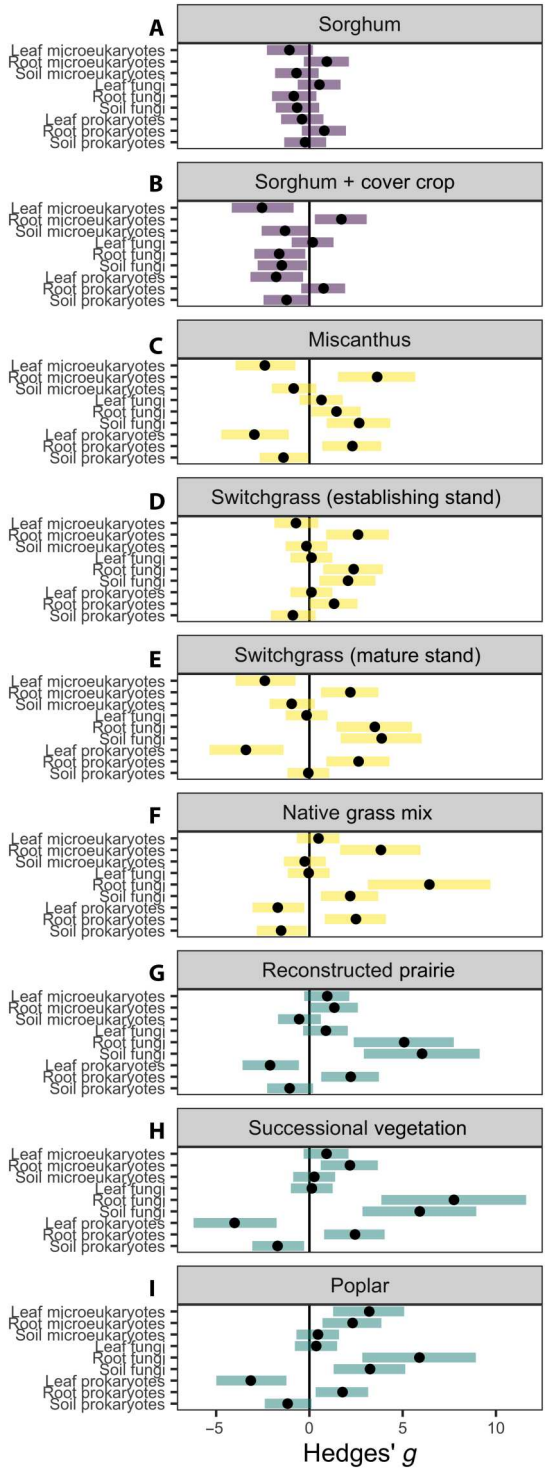

Fig. 3. Effects of each crop type on taxonomic richness of microbes. Taxonomic richness of fungi, prokaryotes, and microeukaryotes varied strongly among bioenergy cropping systems with effects depending on the habitat compartment that we sampled (**A** to **I**). Richness differences are expressed as effect size (Hedges' *g*) relative to corn, and error bars are 95% confidence intervals. Cropping systems are color-coded to denote annual systems (purple), simple perennial monocultures or near-monocultures (yellow), and complex perennial polycultures (blue). Data are from five replicate plots per cropping system.

which were significantly more diverse, and leaf fungi and root prokaryotes, which were not strongly affected.

Community dissimilarity

We examined dissimilarity in species pools within each taxonomic group, pooling the communities across crop types within the annual crop, simple perennial, and complex perennial groupings to compile three overall communities and making pairwise comparisons for each taxonomic group (Figs. 4 and 5).

Differences in species composition, quantified on the basis of Sorenson dissimilarity, were almost always strongest between complex perennial and annual systems, slightly weaker between simple perennial and annual systems, and weakest between the two perennial groupings (Figs. 4 and 5). For macroorganisms, many groups were highly nested; that is, communities in the species-poor cropping systems were exclusive or near-exclusive subsets of the species pool found in the more diverse treatments. This was strongly the case for ants, bees, and butterflies and, to a lesser degree, for plants. In contrast, differences in ground beetle and bird communities were mostly due to turnover, i.e., the different crop categories contained unique communities with few species in common. For microbial communities, community dissimilarity

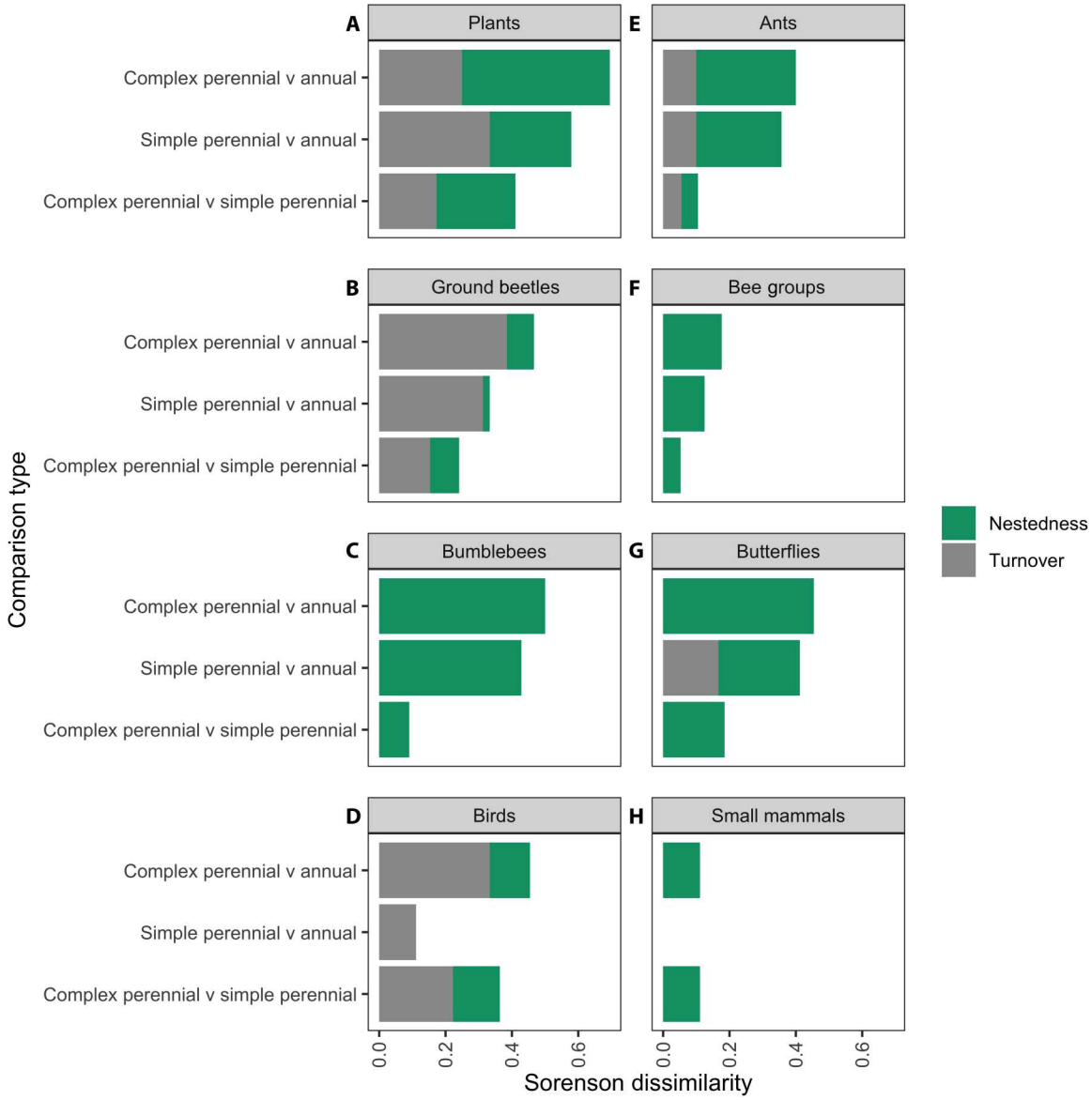

Fig. 4. Plant and animal community dissimilarity, nestedness, and turnover between annual, simple perennial, and complex perennial systems. Pairwise measurements of community dissimilarity for plants and animals between annual, simple perennial, and complex perennial cropping systems, partitioned into turnover and nestedness (**A** to **H**). We found strong differences in community composition among cropping system types, which, for some taxonomic groups, were largely due to nestedness (i.e., species-poor communities were subsets of richer communities found elsewhere). For this analysis, we compiled the overall community across all plots within these three categories and calculated Sorensen dissimilarity for each habitat pair. Sorenson dissimilarity is bounded between 0 (identical communities) and 1 (communities with no overlap).

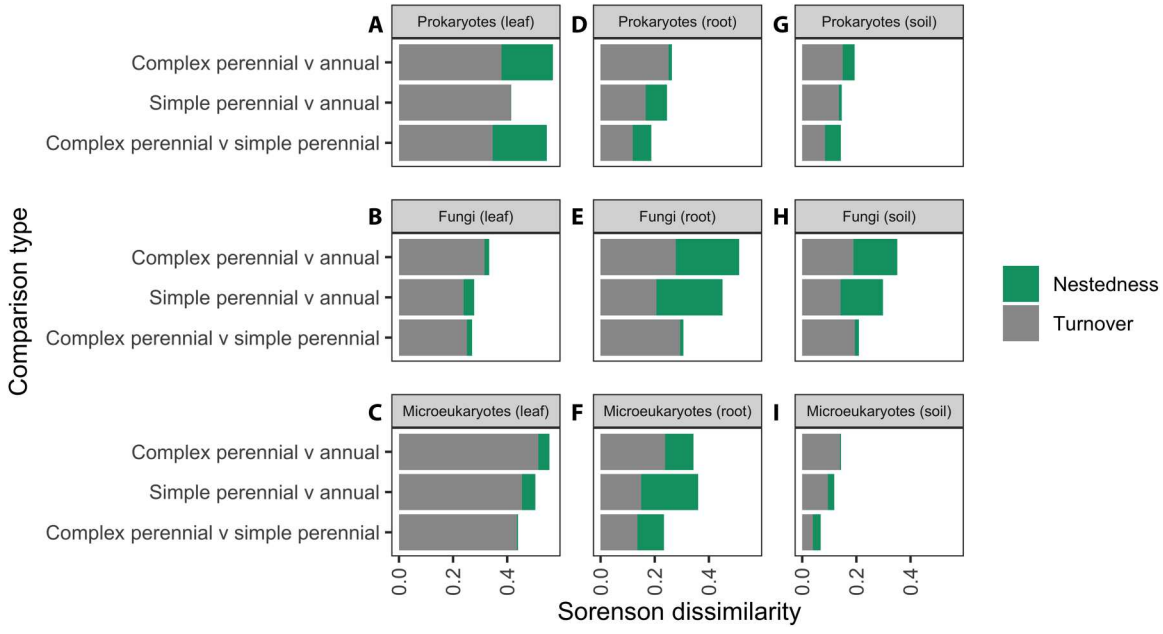

Fig. 5. Microbial community dissimilarity, nestedness, and turnover between annual, simple perennial, and complex perennial systems. Pairwise measurements of community dissimilarity for microbes between annual, simple perennial, and complex perennial cropping systems, partitioned into turnover and nestedness (**A** to **I**). Sorenson dissimilarity is bounded between 0 (identical communities) and 1 (communities with no overlap).

was more strongly due to turnover; this was especially pronounced for phyllosphere fungal and microeukaryote communities, which were highly unique in each habitat type with almost no nestedness.

Responses to plant diversity and functional group composition

The perennial treatments in this study comprised a gradient that blended plant species richness and functional group composition, which strongly predicted richness of many other taxonomic groups (Fig. 6 and table S2; we exclude the annual crops corn and sorghum from this analysis). Plant species richness was very strongly and negatively correlated with percent grass cover ($r_s = -0.92$), was very strongly and positively correlated with forb ($r_s = 0.90$) and woody cover ($r_s = 0.87$), and somewhat strongly and positively correlated with legume cover ($r_s = 0.62$; fig. S4). When we modeled richness of other taxonomic groups as a function of plant species richness using generalized additive models (GAMs), there was a strong positive, significant, and linear or near-linear response for bee groups, butterflies, ants, and leaf microeukaryotes. Microeukaryotes in soil followed a similar pattern but more weakly ($P = 0.07$). For other target groups, the overall relationship was positive and significant but saturated or decreased slightly at the highest values of plant richness; this occurred for bumblebees, fungi in the root zone, and fungi in the soil matrix. Bird richness increased modestly when plant species richness was low, and much more steeply as plant richness increased. Ground beetle richness was high in very species-poor and species-rich plant communities but low in intermediately diverse plots. The only decreasing trends were for root microeukaryotes and phyllosphere prokaryotes ($P = 0.07$ and $P = 0.05$, respectively). Last, richness of small mammals, root prokaryotes, and soil prokaryotes did not respond strongly to changes in plant species richness.

DISCUSSION

Our findings illustrate the widely contrasting effects that bioenergy crop expansion could have for biodiversity. For many animal groups, the strongest differences in species richness were not between annual and perennial crops but between the different types of perennial crops. Specifically, biodiversity gains in simple perennial systems relative to corn were often minimal and were dwarfed by those in complex perennial systems with more plant species (Fig. 1). Therefore, dichotomizing between annual and perennial bioenergy systems can overlook the glaring biodiversity differences that occur between different types of perennial bioenergy habitats. We also found that for several groups, particularly bees, butterflies, and ants, communities were highly nested (Fig. 4). That is, species-poor communities in low-quality habitats were merely subsets of communities found in more diverse crops rather than containing unique species. We interpret this as meaning species-poor communities in simpler cropping systems contain extreme habitat generalists and/or species that arrive through local and landscape spillover but would be unlikely to persist using resources in those habitats alone (*41*). We conclude that while simple perennial cropping systems offer many important benefits over annual systems, such as reduced input requirements and increased carbon storage, nutrient, and soil retention, the best outcomes for conservation of macroorganisms lie in complex perennial bioenergy habitats with higher plant diversity.

Patterns of richness in microbial communities differed from those of plants and animals, indicating that different factors shape the dispersal and persistence of microorganisms. In most (but not all) instances, richness was higher in perennial systems than in corn, but unlike for plants and animals, it did not increase sharply in complex perennial systems compared to simple ones. Prokaryote responses in the soil matrix and phyllosphere were opposite most

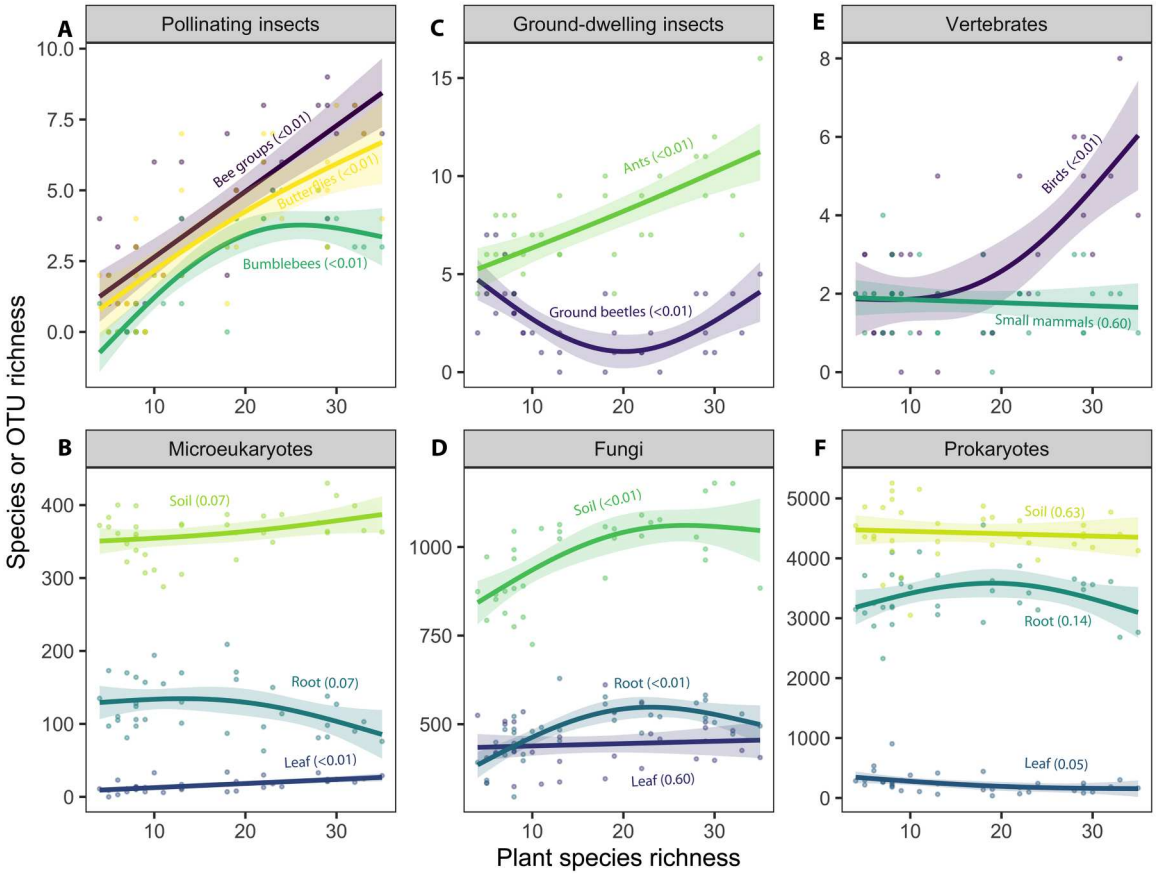

Fig. 6. Plant species richness predicts richness of other groups. Perennial cropping systems in this study form a gradient of plant species richness and community composition that predicts diversity of many of the other taxonomic groups that we censused (**A** to **F**). Richness of many groups increased strongly with plant richness and showed little to no sign of saturating (bee groups, butterflies, ants, birds, and leaf microeukaryotes). Richness of other groups increased but saturated or declined slightly at the highest levels of plant richness (bumblebees and soil/root fungi), whereas others decreased before increasing (ground beetles) or responded negatively (leaf prokaryotes). Other groups exhibited no significant trend (small mammals, soil/root microeukaryotes, leaf fungi, and soil/root prokaryotes). Points are raw species richness; fit lines are based on generalized additive models ($k = 3$, $N = 35$) fit individually to each taxonomic group. Numbers in parentheses are model P values; see table S2 for detailed model outputs.

other groups and decreased relative to corn in many of the treatments, suggesting that drivers of bacterial diversity differ from those of other groups. Sources of community dissimilarity also contrasted between micro- and macroorganisms, as microbial community differences were much more strongly driven by turnover than nestedness in most cases (Fig. 5).

This study used an experimental field array instead of field-scale cropping systems. This allowed for intensive, standardized, simultaneous side-by-side comparisons that eliminated variation in field size, landscape species pool, and physical geography. It also allowed us to evaluate crops that have not yet been widely deployed on the landscape and to include realistic harvest practices. Because plots in the array were at a smaller spatial scale than what is agronomically realistic, the differences that we measured between crops are likely to be conservative. We base this on three lines of reasoning. First, communities in small patches and field edges are partly made up of organisms donated from nearby habitats (*41*). If we had censused larger fields, then the influence of cross-habitat spillover would likely be reduced and differences in richness and community composition would be more pronounced. Second, large fields in aggregate produce coarse-grained landscapes, which offer limited resource complementarity for organisms that forage across multiple habitat types (*42*). In contrast to this, in our study, we observed (for example) bees nesting in open soil or crop residue in corn and sorghum but nectaring on flowers that occurred only in other habitats. At realistic scales, resource complementarity between habitats would be reduced. Third, when widely deployed in a landscape, crops can strongly shape the species pool of that landscape (*43*), and in simple landscapes dominated by species-poor crops, many species would be absent altogether. This type of effect was outside the scope of our experiment, which tested whether various crops were being used or colonized by different subsets of a single landscape-scale species pool. Last, we note that for some taxonomic groups with limited dispersal ability, the crop types in this experiment were relatively spatially independent from one another, whereas for others (e.g., bees, butterflies, and birds), the array functioned as a choice experiment in which all crop types were available, but they were more likely to visit and be detected in cropping systems that contain resources that they use.

Within a crop type, management intensity and intracrop variation can strongly shape biodiversity outcomes. Richness of many groups in our experiment was highest in poplar; short-rotation coppicing systems can be quite biodiverse, but in practice, this depends on management, understory vegetation, and growth stage of the planting (*44*–*48*). In our experiment, poplar plots contained a diverse understory of volunteer plants, which may or may not occur depending on management and landscape context. We expect that coppicing systems with fewer plant species in the understory would not support the diverse animal communities observed in our study. Communities in coppicing systems also change strongly over time, particularly as the canopy closes and after harvest, and at landscape scales, diversity can be optimized by staggering stand age and timing of coppicing (*47*). Along the same lines, the switchgrass stands in our study were treated with broadleaf-specific herbicides and contained almost no forbs. If management had allowed more weeds to persist, then richness of some groups probably would have been higher. Past studies of switchgrass found that animal diversity levels were closer to prairie than what we measured here, but often, a large amount of plant biomass in those switchgrass treatments was made up of other species (*49*, *50*). Last, in annual systems, cover crops can provide a range of ecosystem services and generally enhance biodiversity (*51*). However, we found no evidence that cover crops enhanced biodiversity in the sorghum treatments in this study, and for a few groups (e.g. ants), abundance decreased where cover crops were used. We suspect that the increased disturbance associated with planting and terminating the crop outweighed any potential benefits. In general, much more research is needed to design grass- and woody-based management systems that optimize both bioenergy yield and biodiversity gains.

Microbial richness responses were quite eclectic, differing strongly depending on the kingdoms and habitat compartments that we sampled. Recent meta-analyses show that richness and abundance of soil fungi and prokaryotes are generally positively correlated with plant species richness (*52*, *53*). In our study, fungal richness conformed to this trend in both soils and roots, but prokaryotes did not. We are unsure of why this difference occurred, but there is considerable variability in the plant-microbial diversity correlation that can occur because of differences in habitat type and sampling scale (*53*), and some evidence suggests that fungi may respond more strongly to plant diversity than prokaryotes do (*52*). The lack of effect on prokaryote richness could also have to do with the relatively small spatial scale of our experimental array or because turnover in species and functional groups occurred without strong changes in total richness. Breaking each microbial group down into the many functional guilds that they include will yield important additional information about how microbial diversity and function vary across these plant communities.

Pathways to biodiverse bioenergy landscapes

Bioenergy adoption can cause adverse land use change in which croplands are expanded, natural habitats are destroyed, and landscapes are simplified (*54*–*56*). Most evidence indicates that converting any seminatural system to bioenergy crops will incur biodiversity costs even if the crops are perennial (*2*, *17*). While we did not census natural reference habitats such as hardwood forests in this study, the costs of converting this type of ecosystem to bioenergy crops are likely to be high both in terms of biodiversity impact and carbon debt. Our results show that the biodiversity cost of converting seminatural habitats to biofuels will depend strongly on crop type. For example, converting successional grasslands on abandoned farmland to miscanthus would result in strong biodiversity loss. However, converting them to higher-yielding prairie or woody coppicing systems could have neutral or positive effects for biodiversity.

In some contexts, demand for bioenergy could enhance biodiversity and ecosystem services in agricultural landscapes if diverse perennial systems are adopted. Farmers have increasing access to precision tools like spatially explicit yield monitoring and profitability mapping, allowing them to identify subfield areas that consistently lose money, contribute disproportionately to soil loss and nutrient pollution, and would be better suited to perennial crops (*18*, *19*). Converting unprofitable subfield areas minimizes indirect land use change by removing crops from the least productive areas; precision techniques could also compensate for yield losses incurred if they increase yield in other parts of fields due to enhanced management (i.e., variable rate fertilization). Adding perennial bioenergy habitat in this manner would increase local biodiversity and enhance the broad suite of ecosystem services that occur when perennial elements are incorporated into arable fields (*15*, *16*). It would also add both compositional and configurational complexity to simplified landscapes, bringing about improvements to biodiversity and ecosystem services at larger spatial scales (*8*, *13*, *14*, *43*, *57*, *58*).

MATERIALS AND METHODS

Experimental design

This study took place in the Bioenergy Cropping Systems Experiment (BCSE; 42°23′42″N, 85°22′24″W), a long-term array at Kellogg Biological Station, Michigan State University, USA. Biodiversity censuses occurred in 2021 during three periods: early summer (approximately the first 2 weeks of June), midsummer (the last 2 weeks of July), and late summer (the last week of August and first week of September). See table S3 for details of when each taxonomic group was sampled. All taxonomic groups were sampled during all three sampling periods except plants and microbes, which were sampled once. We censused the plant community during the August/September period, as this time most closely approximated peak biomass, and collected samples for the microbial communities in July. In this article, we report the season-long total of all censuses.

The BCSE array includes five replicates of each of 10 bioenergy cropping systems in 28 m–by–40 m plots, with replicates grouped spatially into blocks. The array was established in 2008. In 2021, the year of our study, it included three annual systems: continuous no-till corn (*Zea mays*), continuous energy sorghum (*Sorghum bicolor* photoperiod-sensitive hybrid ES5200), and energy sorghum (*S. bicolor* photoperiod-insensitive hybrid TAM 17900) with a winter cover crop. Simple perennial systems included switchgrass (*P. virgatum* var. Cave-in-rock), miscanthus (*Miscanthus* × *giganteus*), and a transitional treatment that was fallowed and planted in red clover (*T. pratense*) from 2019 to 2020 but sprayed with herbicide and newly seeded with switchgrass in 2021. Thus, over the course of the growing season, it transitioned from residue to ruderal annual grasses mixed with switchgrass and, eventually, newly established switchgrass mixed with these other species. The mature switchgrass plots were sprayed periodically in 2021 with broadleaf herbicides to

remove forbs. *Miscanthus* plots formed dense thickets that contained limited cover of agricultural weeds beneath the canopy. The native grass mix was composed of the prairie grasses *Andropogon gerardii*, *Sorghastrum nutans*, *Elymus canadensis*, *P. virgatum*, and *Schizachirium scoparium* plus limited volunteer species that had colonized the plots. The reconstructed prairie seed mix included 18 species including the same grass species as the previous treatment plus one additional grass, three legumes, and nine forb species plus volunteer species (for species lists associated with these treatments, see https://lter.kbs.msu.edu/wp-content/uploads/2012/05/GLBRC-Species.pdf). Plots with successional vegetation contained volunteer species that originated from the seed bank or immigrated from other treatments. They were representative of diverse old-field communities in the upper Midwest but with higher abundance of flowering forbs (e.g., *Monarda fistulosa* and *Ratibida pinnata*) that had colonized from reconstructed prairies. Last, the hybrid poplar plots contained "NM-6" *P. nigra* × *P. maximowiczii*. Poplar was grown continuously in these plots since 2008 and coppiced in 2014, and all trees were replaced in 2019; thus, they were ~2 to 3 m in height at the time of this study. The understory of these plots was mostly unmanaged and was a diverse mix of old-field vegetation, agricultural weeds, and native forbs, with alleys between rows mowed annually during the summer to minimize competition with trees. We note that while the perennial treatments were seeded with only perennial species, the volunteer community throughout the array included some annual and biennial species as well. All treatments are harvested annually after killing frost in the fall (except poplar). Additional details about the experimental array, changes in crop type over time, and agronomic practices can be found at https://lter.kbs.msu.edu/research/long-term-experiments/glbrc-intensive-experiment/. See figs. S5 to S14 for representative photos of each treatment.

Each plot in the BCSE contains a 60-m interior path along which there are three sampling stations (for details, see https://lter.kbs.msu.edu/maps/images/glbrc-station-flags.pdf; fig. S15). During censuses for some taxa, we used the path as a walking transect; for others, we set up traps at sampling stations or collected samples at set increments along the pathway.

Plants

We censused the plant community in each plot using 1-m^2 quadrats. Quadrats were placed in 10 locations along the path within each plot (fig. S15). Identification and taxonomy follow (*59*). All plants were identified to the species level except for a small number that were grouped by genus instead of species (see the Supplementary Materials). There were 11 unknown but clearly unique taxa that we included in the dataset; four were unknown but not known to be unique taxa. These were excluded from the dataset (these represent 0.06% of overall plant cover).

Bees and butterflies

Within each plot, we walked a 60-m transect and recorded all bees and butterflies that we encountered [modified from (*60*)]. We collected transect data for four consecutive weeks during each of the three sampling periods. Transect walks lasted approximately 5 min per plot, excluding time spent recording data or capturing insects. They occurred during calm conditions between the hours of 9:00 and 17:00, and we rotated starting points to minimize temporal effects. Butterflies (including skippers) were recorded if they passed within 5 m in front of, above, or beside the observer, but not behind. We identified them visually or by taking photographs, capturing specimens as needed. We recorded bees along the transect to within ~2 m, as they are harder to detect beyond this distance. We focused on bees that landed on vegetation in the plot or used resources there; individuals that flew through without stopping were not recorded. Bees (except bumblebees) were not identified to species but grouped into the following categories following monitoring protocols developed by the Xerces Society (*61*): honeybee, tiny dark bee, medium dark bee, green sweat bee, striped sweat bee, chap-legged bee, striped hairy belly bee, metallic hairy belly bee, cuckoo bee, and bumblebee. Most of these groups include one to two genera (Supplementary Materials). Bumblebees (*Bombus*) were also identified to species; some species were identified visually without capture, whereas others were netted or occasionally preserved in ethanol for laboratory identification. Identification followed (*62*). We omit observations of four bumblebees and four butterflies that we were unable to capture for identification. Voucher specimens of bee groups and each *Bombus* species were preserved and housed in the laboratory collection maintained by D. Landis and will be incorporated into the Albert J. Cook research collection at Michigan State University.

Ants and carabid beetles

We used pitfall traps to census the ant and carabid beetle community in each plot. Traps were placed approximately 1.4 m northwest of each sampling station within all 50 plots (total of 150 trap locations). To minimize the effects of repeatedly digging and installing traps, we placed a socket made of polyvinyl chloride (PVC; diameter = 5.08 cm) in the soil at the beginning of the season several days before sampling. These were plugged with plastic mailing tube caps or with capped pitfall containers to create an even surface that was flush with the soil surface until pitfalls were deployed or opened. Traps were also sheltered from rain with a clear 15 cm–by–15 cm piece of plexiglass held in place with 15-cm lawn staples (fig. S16). We placed 120-ml plastic cups within each PVC socket. Cups were filled with ~60 ml of 95% ethanol with a drop of unscented dish soap to break surface tension. Traps were placed to be continuous with soil and litter with no lip protruding. Pitfalls were left in place for 48 hours and picked up in the same order as they were deployed to minimize differences in exposure. On hot days, we refilled ethanol at 24 hours if needed because of evaporation. We set pitfalls twice during each of the three sampling periods; thus, in all, we deployed 900 traps (50 plots × 3 stations × 6 deployments). Data from 16 of the 900 traps are not presented because of labeling issues or because they were destroyed by agronomic activities in the plots. We identified ants following (*63*) [and (*64*); for *Formica* spp.] and carabid beetles using (*65*). Organisms that we collected are pinned or preserved in ethanol and housed in the laboratory of D. Landis.

Small mammals

We used Sherman traps to census the small mammal community in all plots. Within each plot, we placed six traps, two at each of the three sampling stations (fig. S15). We baited traps with oats and provided a cotton ball that could be used as bedding during cold nights. Traps were placed ~1.4 m northwest and southeast of each sampling station with the entrance facing south. We placed them late in the day and then checked them the following three mornings

before ~10:00. Because traps were sometimes in exposed locations, we placed 30 cm–by–30 cm corrugated plastic shades above each trap, held in place with lawn staples leaving 1 to 2 cm between the trap and shade. Each plot was censused once (i.e., six traps for three nights) during each of the three sampling periods. Because it was not feasible to sample all 50 plots simultaneously, we placed traps in 20 plots for 1 week and in the remaining 30 1 to 2 weeks later. In total, this resulted in 2700 trap-nights divided evenly among the three sampling periods. Captured small mammals were identified to species and photographed to verify their identity as necessary and then released. They were not marked to quantify recapture rates, as our main objective was to assess species richness rather than abundance. Small mammal trapping occurred in accordance with Institutional Animal Care and Use Committee protocol 202100084.

Birds

We surveyed birds in each plot weekly for 4 weeks during each of the three sampling periods (table S3). Two observers walked the alley between plots with binoculars, observing the pair of plots on either side of the path simultaneously (one plot per observer) and recording all birds seen or heard within each plot. Each pair of plots was observed for 3 min during each sampling bout, and each bout was completed on a single morning, rotating starting locations within the array. Observations took place between approximately 6:00 and 9:00 a.m. Birds were also recorded if they were flushed from plots that had not yet been approached (i.e., the plots immediately ahead of the surveyors). We omit 10 observations of birds that flew away before they could be identified.

Microbes

We censused fungi, prokaryotes, and microeukaryotes [using ITS (internal transcribed spacer), 18*S*, and 16*S* DNA markers, respectively] in soil, leaf, and roots. The microeukaryote grouping is polyphyletic, but we include them because they are functionally diverse and important organisms whose diversity has not been assessed in bioenergy systems. Soil samples were collected from 10 locations within 1 m of the sampling path within each plot. For each of the 10 locations, we dug a 10-cm hole with a sterilized trowel (approximately the width of the trowel), removed the plant residues immediately on the top layer (when present), homogenized soil within the hole by stirring it with the trowel, and filled a sterile 100-ml cup with soil. Soils from all 10 cups were pooled into a sterile plastic bag and further homogenized after pooling by shaking the bag several times. Composite soil samples were immediately transported to the laboratory where a subsample of about 100 g of soil was sieved and dried in sterile paper bags with silica gel beads. Leaf samples were collected from 10 plants in each plot, located at regular intervals along the path. In each case, the collector walked to the designated sampling location and pointed without looking, sampling the resulting plant by removing a lower leaf, middle-aged leaf, and a new one. All 30 leaves from each plot were pooled. Then, still in the field, we used a sanitized hole puncher to remove a 5-mm disc from near the center of each leaf; leaf discs were then placed directly into 1 ml of Omega Mag-Bind Plant DNA Plus kit (Omega Bio-Tek, USA) extraction buffer solution. Similarly, root tissues were collected from 10 locations within each plot along the sampling path using the same strategy as for leaves. We used sterilized trowels to remove a portion of the roots (up to 10-cm depth) from the focal plant. It is likely that in polyculture plots, we collected roots from multiple species per location. In the field, each root sample was washed in a 0.1% Tween-20 solution and then washed three times with ddH_2O. Clean roots were transferred in a sterile paper bag and dried in silica gel.

Genomic DNA was extracted from approximately 0.5 g of dried soil with the MagAttract PowerSoil DNA KF (QIAGEN, USA), and 0.5 g of fine (ø ≤0.5 mm) roots with Omega Mag-Bind Plant DNA Plus Kit (Omega Bio-Tek, USA) on a KingFisher Flex robot (Thermo Scientific, USA). Polymerase chain reactions (PCRs) were performed using DreamTaq Green DNA Polymerase (Thermo Scientific, USA) with the ITS1f-ITS4 primers for fungi (*66*, *67*) and F1391-REukBr primers for soil micro invertebrates (*68*) and using the Platinum Taq DNA polymerase (Thermo Scientific, USA) with the 515F-806R PCR primers for prokaryotes (*69*).

Successful amplifications were determined on a QIAxcel Advanced machine with a DNA Fast Analysis kit (QIAGEN, USA). PCR products were then normalized with the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, USA) and pooled. The obtained amplicon pool was concentrated to 20:1 with Amicon Ultra 0.5-ml 50-K filters (EMD Millipore, Germany), purified from primer dimers with the HighPrep PCR Clean-up System (MagBio, USA), and paired-end sequenced on an Illumina MiSeq analyzer with the v3 600 cycles kit (Illumina, USA).

Raw ITS, 18*S*, and 16*S* sequence reads were checked for errors with FastQC (*70*). Only 18*S* and 16*S* reads were merged with PEAR (*71*); for ITS, only forward read was used for the downstream analysis. Reads were demultiplexed by barcode sequences in QIIME (*72*). Illumina adapters and sequencing primers were removed with Cutadapt (*73*). Before sequence dereplication, sequences were quality-filtered on the basis of maximum expected errors and trimmed to equal length (*74*, *75*). Operational taxonomic units (OTUs) were clustered using the UPARSE algorithm at 97% sequence similarity (*76*). OTU taxonomy was performed in CONSTAX2 (*77*, *78*) against the UNITE eukaryote database ver. 8.2 of 04.02.2020 (*79*) for the 18*S* and ITS sequences and SILVA version 138 (*80*) for the 16*S* sequences. 18*S* OTU taxonomy was then polished and improved using local BLAST (Basic Local Alignment Search Tool) queries against the whole National Center for Biotechnology Information nucleotide reference database (www.ncbi.nlm.nih.gov/). Nontarget taxa, OTUs not assigned to a kingdom, and OTUs identified as either chloroplast or mitochondria in either dataset, as well as singleton sequences, were removed from subsequent analysis. For the 18*S* dataset, we removed fungi (more accurately characterized using the ITS region) and metazoa (as many groups were already censused using other methods), leaving behind all other identified microeukaryotes, i.e., the groups Telonemia, Stramenopila, Alveolata, Rhizaria, Choanoflagellozoa, Cryptista, Euglenozoa, Filasteriae, and Ichthyosporea.

Statistical analysis

All analyses were conducted in R 4.1.2 (*81*). For microbial data, ITS, 18*S*, and 16*S*, otu_tables and metadata files were imported in R. Contaminant OTUs were removed from the datasets using the decontam R package (*82*). Sequence read depth for each sample and removed contaminant OTUs are provided in fig. S17. To eliminate biases due to uneven sequencing effort obtained in each sample and reduce data waste (*83*), we normalized the data using the cumulative sum scaling algorithm implemented in the metagenomeSeq R package (*84*).

We calculated species or OTU richness for each taxonomic group then, within each group, calculated Hedges' *g* effect size for richness in each crop type, relative to corn, using the R package effectsize (*85*). We also used linear models to determine whether species richness within each group differed by treatment (see the Supplementary Materials). While results in the main text focus on raw species richness, for groups with abundance data, we also calculated Hill's number (the exponent of Shannon Diversity) (*86*), which adjusts species richness estimates downward when communities are less even (Supplementary Materials).

To examine dissimilarity in community composition within each taxonomic group, we first pooled the overall communities for each group detected across all treatments and replicates within the annual crop, simple perennial, and complex perennial groupings, so we could compile three overall communities and focus on pairwise dissimilarity between them. We used the R package betapart (*87*) to calculate pairwise Sorensen dissimilarity and partition it into nestedness and turnover.

Last, we examined relationships between plant species richness, composition, and richness of other groups. For this analysis, we excluded corn and sorghum, focusing only on the perennial treatments in the experiment. Cropping systems in this experiment run a gradient from low-diversity, grass-dominated plots to high-diversity plots with greater cover of other functional groups; we quantified this correlation structure using Spearman's rank correlation (fig. S4). We then used generalized additive models (GAMs) to explore relationships between plant species richness and richness within other taxonomic groups, using the R package mgcv (*88*). We used GAMs because some of the relationships appeared linear, whereas others were highly nonlinear, and this method allowed us to fit flexible models with a summary statistic for the degree of nonlinearity (*edf*; see table S2). Separate models were run for each taxonomic group; in all cases, we used three basis functions to minimize overfitting, i.e., approximating curves in the data but not allowing for repeated changes in trend direction (Fig. 6). We estimated the smoothing parameter using restricted maximum likelihood.

Supplementary Materials

This PDF file includes:

Supplementary Text
Figs. S1 to S17
Tables S1 to S3

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Supplementary Materials for

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Supplementary Text

Results based on evenness-adjusted diversity measures

In the main text we report results based on raw species richness (Fig 1). To assess whether taking community evenness into account would affect our results, we compared diversity in each bioenergy crop to corn using effect sizes based on the exponent of Shannon Diversity, or 'effective numbers of species' (*86*). The effective number of species is equal to species richness when communities are perfectly even but decreases as unevenness increases. When making this calculation, there were a small number of instances where a group's richness in a plot was zero; we held this number at zero rather than calculating effective number of species, which would misleadingly equal 1. Evenness within plant communities was calculated based on estimated percent cover; we estimated percent cover of all species to the nearest 1% (or 5% if it occupied > 15% of the quadrat). Species occupying less than 0.5% of a quadrat were rounded accordingly to 0.25% or 0.5%. For butterflies, ground beetles, bees, birds, and small mammals, abundance was based on the number of individuals we detected. For ants, abundance estimates were based on the number of traps per plot that a species was detected in (0-3), rather than the number of workers we caught. This is a conservative estimate of the number of colonies present since multiple workers in the same trap are likely to be from the same colony and worker density varies intrinsically by species. We did not calculate evenness-adjusted richness estimates for microbes.

Effect size patterns based on effective numbers of species were very similar in direction and magnitude to those calculated directly from species richness (Fig S1, compare to Fig 1 in main text). The most obvious instance in which an effect differed strongly and changed direction was for bee group richness in successional vegetation, which was distinctly higher than in corn when based on species richness, but somewhat lower than in corn after accounting for evenness. This likely occurred because the bee community in successional vegetation was extremely uneven, with bumblebees and honeybees accounting for most observations despite richness being high. In contrast, in corn, bee richness and abundance were quite low (Figs 1, 2, main text) but evenness was high. Similarly, in newly-establishing switchgrass stands, plant species richness was slightly lower than in corn, but diversity was significantly higher after taking evenness into account. In this case, while there were more weed species present in corn plots than occurred in switchgrass, the community was highly uneven. In contrast, in switchgrass the community was made up of switchgrass with large amounts of a few other annual grass species.

Differences between treatments, assessed with ANOVA

In the main text we expressed differences in species richness as effect size relative to corn, the business-as-usual bioenergy crop in the US. Here we test if species richness differs by cropping system treatment and/or replicate (replicates were blocked spatially within the array) using two-way ANOVAs (Table S1). Species richness differed between cropping systems for all taxonomic groups except small mammals, leaf fungi, and soil microeukaryotes. Replicate had significant or near-significant effects on ants, soil prokaryotes, soil microeukaryotes, and leaf microeukaryotes, illustrating there are also some positional differences in biodiversity within the experimental

array. See Figs S2 and S3 for visual summaries of species richness and effective numbers of species for each group in each cropping system.

Photos of each treatment type

See Figs S5-S14 for representative photos of each treatment in the experimental array. Photos are taken from the southwest corner of each plot.

Plant Surveys

Within each plot, quadrats were placed in locations indicated in Figure S15. Plants were identified to species except for a small number of taxa that were not identified beyond the genus level; these included *Anthemis, Crataegus, Erigeron, Galium, Hieracium, Poa, Ranunculus,* and *Vitis.* Additionally, we did not differentiate between *Trifolium campestre* and *T. aureum*, nor between *Solidago canadensis, S. altissima*, and *S. gigantea*, treating each group as a single taxon.

Bee functional groups

Bees were identified in the field to coarse functional groupings, rather than to species, using the group assignments developed in the *Xerces Society Upper Midwest Citizen Science Monitoring Guide for Native Bees*. Groups were as follows; for more information see (*61*):

Honeybee: *Apis mellifera*
Bumblebee: *Bombus* sp. Members of this group were identified to species but are included in our calculations of bee group richness as a single taxonomic unit.
Chap-legged bee: members of family Apidae that carry dry pollen on lower legs. This group is mostly made up of the genera *Melissodes, Peponapis,* and *Anthophora.*
Medium dark bee: ground-nesting bees belonging to families Andrenidae and Colletidae.
Green sweat bee: metallic green members of family Halictidae, including genera *Augochlora, Augochlorella, Augochloropsis,* and *Agapostemon.*
Striped sweat bee: members of family Halictidae with dark, striped bodies; includes genera *Halictus* and *Lasioglossum*.
Tiny dark bee: A catch-all group including members of Halictidae, Apidae, and Colletidae that are too small to reliably assign to other categories in the field ($\leq$8 mm approximate length).
Striped hairy belly bee: striped members of family Megachilidae (genera include *Megachile, Heriades, Anthidium, Hoplitis*)
Metallic hairy belly bee: members of genus *Osmia* within Megachilidae.
Cuckoo bee: Nest-parasitic bees; phylogenetically diverse.

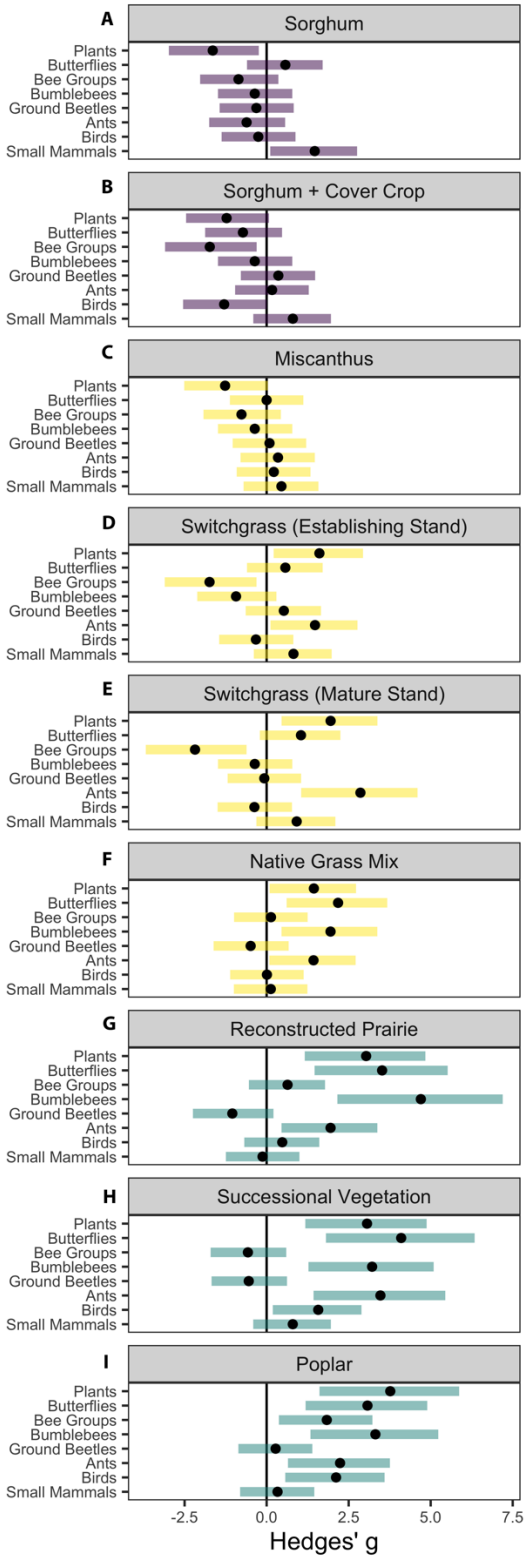

Figure S1. Effect sizes relative to corn calculated based on effective numbers of species (the exponent of Shannon diversity) instead of raw species richness as shown in Figure 1, main text.

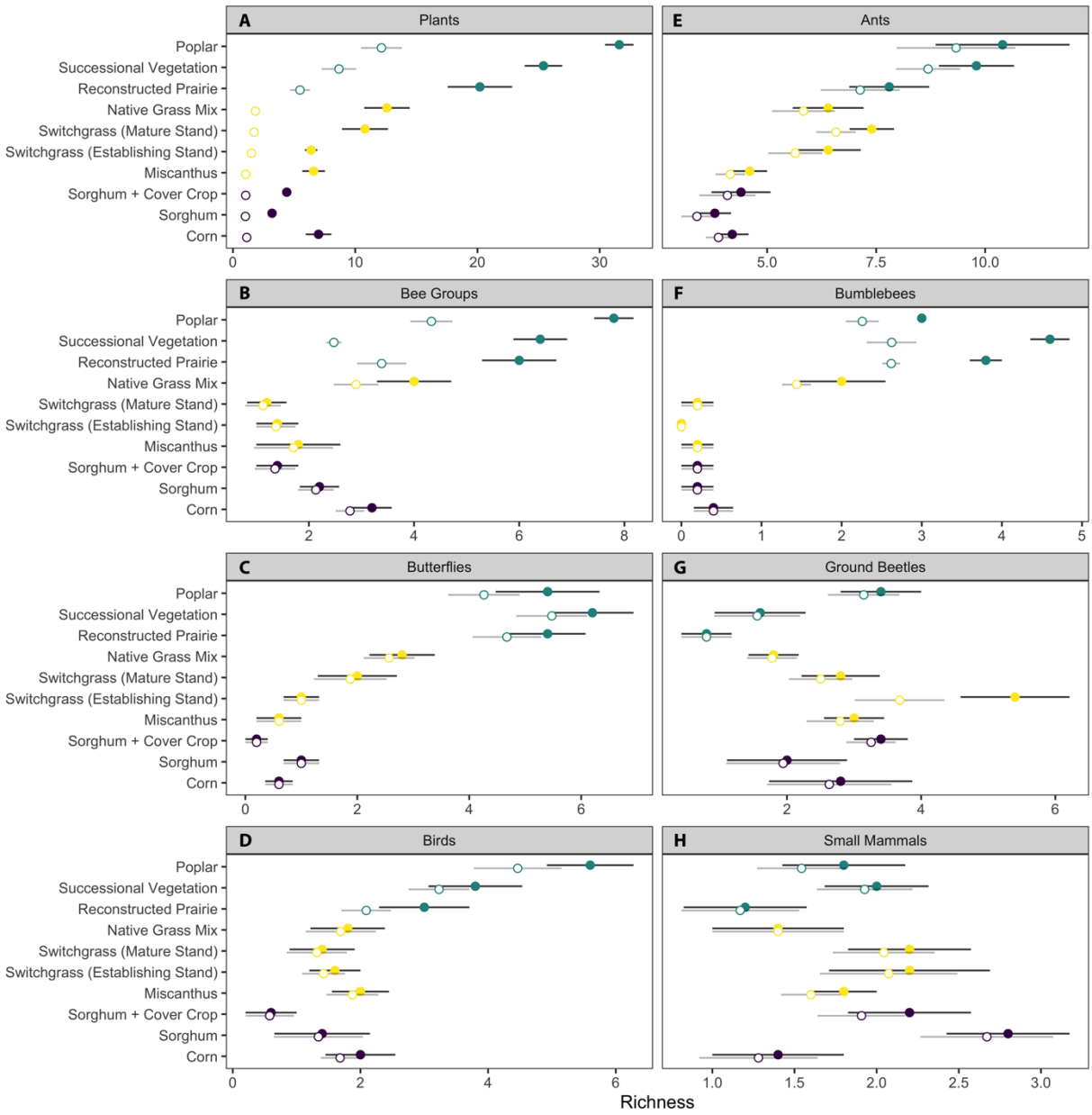

Fig S2. Diversity of macroorganisms by treatment. Points represent means; error bars are ±1 SE. Closed points are species richness; open points are effective numbers of species (i.e., the exponent of Shannon Diversity, taking evenness into account). Treatments are color coded as annual monocultures (purple), simple perennial systems (yellow), and complex perennial polycultures (blue). Note that axis scales differ between panels.

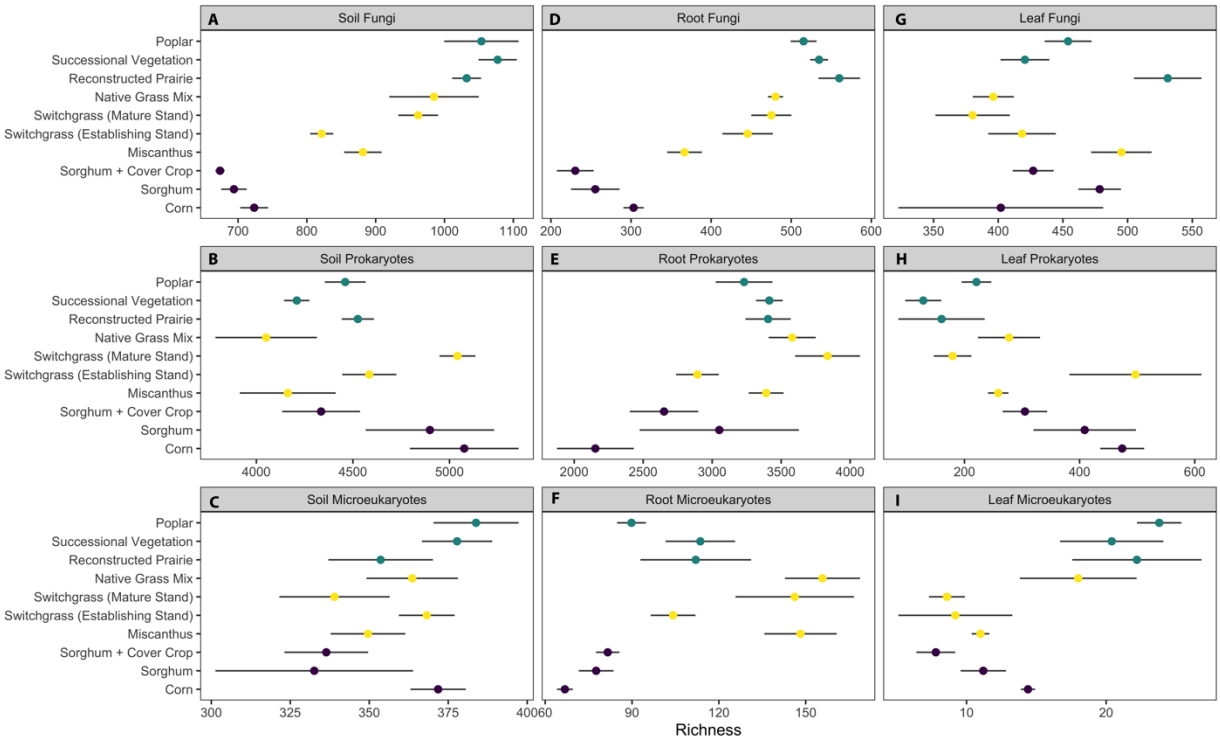

Figure S3. Microorganism OTU richness by treatment. Points represent means; error bars are ±1 SE. Treatments are color coded as annual monocultures (purple), simple perennial systems (yellow), and complex perennial polycultures (blue). Note that axis scales differ between panels.

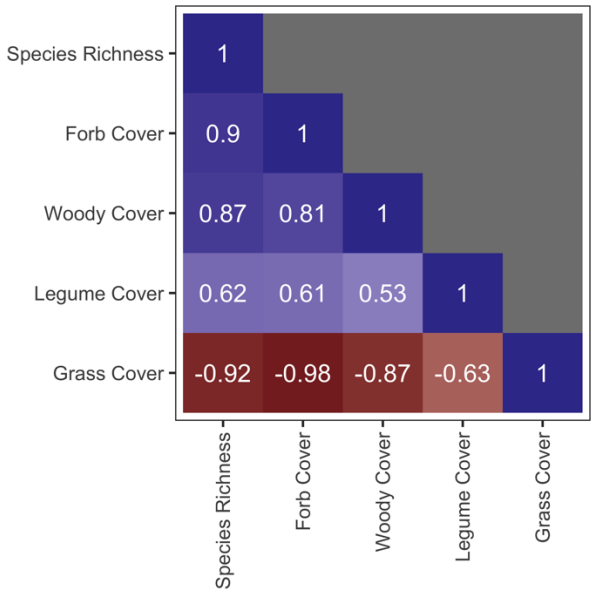

Figure S4 Pairwise Spearman rank correlations between plant species richness and functional group composition, expressed as percent cover of forbs, woody plants, legumes, and grasses. The plant species richness gradient found in our experimental array was accompanied by strong shifts from grass-dominated communities to those with more forbs, woody species, and (to a lesser extent), legumes.

Figure S5. Corn

Figure S6. Sorghum photoperiod sensitive hybrid ES5200

Figure S7. Sorghum photoperiod insensitive hybrid TAM 17900 (cover cropped in winter)

Figure S8. Newly establishing switchgrass

Figure S9. Mature Switchgrass

Figure S10. Miscanthus

Figure S11. Native grasses

Figure S12. Poplar

Figure S13. Successional vegetation

Figure S14. Reconstructed prairie

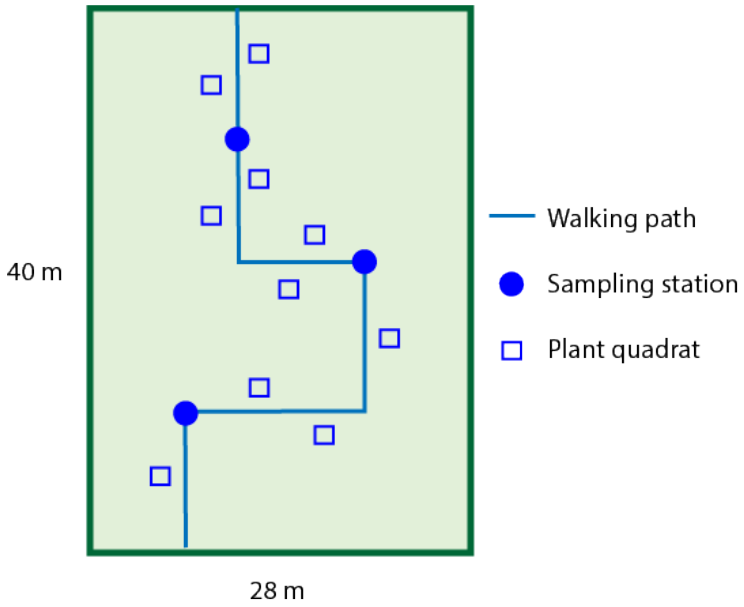

Figure S15. Layout of transect, sampling stations, and plant survey quadrats within a plot.

Figure S16. Example of a pitfall trap deployed for this study with plexiglass rain guard.

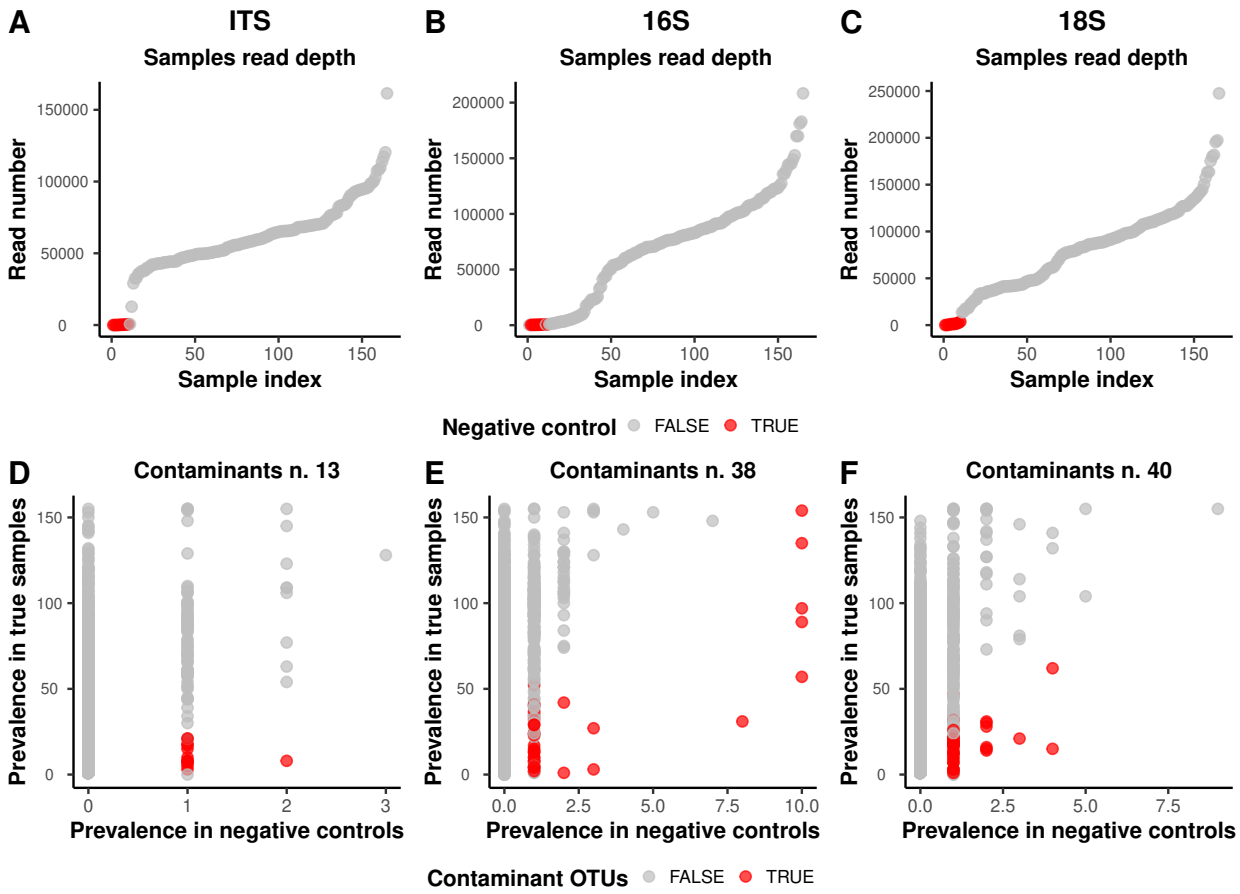

Figure S17. Sequence read number for study (grey points) and control (red points) samples in the ITS (A), 16S (B), and 18S (c) datasets. Contaminant OTUs (red points) detected using *decontam* *R* package and removed from the ITS (D), 16S (E), and 18S (F) datasets.

Table S1. Results of two-way ANOVAs testing if species richness differs by treatment and replicate.

Group	Variable	Df	Sum.Sq	Mean.Sq	*F*	*p*
Ants†	Cropping system	9	9.09	1.01	11.43	<0.01**
	Replicate	4	0.94	0.23	2.65	0.05 •
	Residuals	36	3.18	0.09		
Ground Beetles†	Cropping system	9	9.37	1.04	3.98	<0.01**
	Replicate	4	1.78	0.45	1.70	0.17
	Residuals	36	9.43	0.26		
Bee Groups†	Cropping system	9	21.26	2.36	9.66	<0.01**
	Replicate	4	0.15	0.04	0.15	0.96
	Residuals	36	8.81	0.24		
Bumblebees†	Cropping system	9	32.64	3.63	27.54	<0.01**
	Replicate	4	0.46	0.11	0.87	0.49
	Residuals	36	4.74	0.13		
Butterflies	Cropping system	9	239.28	26.59	16.36	<0.01**
	Replicate	4	4.68	1.17	0.72	0.58
	Residuals	36	58.52	1.63		
Birds	Cropping system	9	95.28	10.59	6.58	<0.01**
	Replicate	4	11.68	2.92	1.81	0.15
	Residuals	36	57.92	1.61		
Small Mammals†	Cropping system	9	2.02	0.22	1.48	0.19
	Replicate	4	0.10	0.03	0.17	0.95
	Residuals	36	5.45	0.15		
Plants†	Cropping system	9	75.78	8.42	52.58	<0.01**
	Replicate	4	1.22	0.30	1.90	0.13
	Residuals	36	5.76	0.16		
Soil Prokaryotes	Cropping system	9	6017148.58	668572.06	4.25	<0.01**
	Replicate	4	2563372.28	640843.07	4.07	0.01*
	Residuals	36	5669826.92	157495.19		
Root Prokaryotes	Cropping system	9	10859223.20	1206580.36	3.84	<0.01**
	Replicate	4	2170565.72	542641.43	1.73	0.17
	Residuals	36	11300525.55	313903.49		
Leaf Prokaryotes†	Cropping system	9	669.57	74.40	5.34	<0.01**
	Replicate	4	25.79	6.45	0.46	0.76
	Residuals	36	501.53	13.93		
Soil Fungi	Cropping system	9	1080828.50	120092.06	23.26	<0.01**
	Replicate	4	34774.60	8693.65	1.68	0.17
	Residuals	36	185907.40	5164.09		
Root Fungi	Cropping system	9	644475.92	71608.44	30.55	<0.01**
	Replicate	4	12439.52	3109.88	1.33	0.28
	Residuals	36	84384.48	2344.01		
Leaf Fungi†	Cropping system	9	65.24	7.25	1.81	0.10
	Replicate	4	25.35	6.342	1.58	0.20
	Residuals	36	144.58	4.02		
Soil Microeukaryotes	Cropping system	9	14954.80	1661.64	1.50	0.18
	Replicate	4	10547.80	2636.95	2.38	0.07 •
	Residuals	36	39823.40	1106.21		
Root Microeukaryotes†	Cropping system	9	100.35	11.15	7.26	<0.01**
	Replicate	4	1.00	0.25	0.16	0.96
	Residuals	36	55.29	1.54		

Leaf Microeukaryotes	Cropping system	9	1617.62	179.74	5.26	<0.01**
	Replicate	4	343.52	85.88	2.51	0.06 •
	Residuals	36	1230.08	34.17		

† Response variable square-root transformed to improve normality. ** $p < 0.01$; * $p < 0.05$; • $p < 0.1$

Table S2. Results of GAMs assessing relationships between plant species richness and richness of other groups across perennial treatments in the experimental array (See Fig 6 in main text). We used k = 3 basis functions in all models and estimated the smoothing parameter using restricted maximum likelihood. The summary statistic '*edf*' describes the degree of nonlinearity of a relationship; values approaching 1 are near-linear while those above 2 are considered highly nonlinear.

Group	***n***	***edf***	***F***	***p***	**Adjusted R^2**
Bee groups	35	1	62.41	<0.01	0.64
Bumblebees	35	1.94	46.92	<0.01	0.73
Butterflies	35	1.34	28.86	<0.01	0.55
Ants	35	1.12	26.29	<0.01	0.49
Ground beetles	35	1.95	10.73	<0.01	0.37
Birds	35	1.81	13.42	<0.01	0.45
Small mammals	35	1	0.283	0.60	-0.02
Microeukaryotes (soil)	35	1.28	2.59	0.07	0.11
Microeukaryotes (root)	35	1.67	2.40	0.07	0.13
Microeukaryotes (leaf)	35	1	19.72	<0.01	0.36
Fungi (soil)	35	1.81	14.58	<0.01	0.43
Fungi (root)	35	1.93	19.41	<0.01	0.51
Fungi (leaf)	35	1	0.29	0.60	-0.02
Prokaryotes (soil)	35	1	0.24	0.63	-0.02
Prokaryotes (root)	35	1.81	2.14	0.14	0.09
Prokaryotes (leaf)	35	1.45	4.68	0.05	0.15

Table S3. Weekly sampling schedule for all taxonomic groups. Boxes marked with a C (= complete) indicate that all 50 plots in the array were sampled that week. For those marked with a P (= partial), we sampled a subset of the plots during that week and the rest of them 1-2 weeks later. Bold boundaries around these boxes indicate a complete sampling event in which all plots were sampled.

		Week															
Group	**Sample type**	**24-May**	**31-May**	**7-Jun**	**14-Jun**	**21-Jun**	**28-Jun**	**5-Jul**	**12-Jul**	**19-Jul**	**26-Jul**	**2-Aug**	**9-Aug**	**16-Aug**	**23-Aug**	**30-Aug**	**6-Sep**
Ants, ground beetles	Pitfall traps		C	C					C	C					C	C	
Small mammals	Sherman traps		P		P					P	P				P	P	
Bees, butterflies	Transects	C	C	C	C			C	C	C	C			C	C	C	C
Birds	Observation walks	C	C	C	C			C	C	C	C			C	C	C	C
Microbes	eDNA								C								
Plants	Quadrats														P	P	