

Short-term harvesting of biomass from conservation grasslands maintains plant diversity

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Abstract

High yields are a priority in managing biomass for renewable energy, but the environmental impacts of various feedstocks and production systems should be equally considered. Mixed-species, perennial grasslands enrolled in conservation programs are being considered as a source of biomass for renewable energy. Conservation grasslands are crucial in sustaining native biodiversity throughout the US Upper Midwest, and the effects of biomass harvest on biodiversity are largely unknown. We measured the effect of late-season biomass harvest on plant community composition in conservation grasslands in three regions of Minnesota, USA from 2009 to 2012. Temporal trends in plant species composition within harvested grasslands were compared to unharvested grasslands using mixed effects models. A before-after control-impact approach using effect sizes was applied to focus on pre- and postharvest conditions. Production-scale biomass harvest did not affect plant species richness, species or functional group diversity, nor change the relative abundance of the main plant functional groups. Differences in the relative abundances of plant functional groups were observed across locations; and at some locations, changed through time. The proportion of non-native species remained constant, while the proportion of noxious weeds decreased through time in both harvested and unharvested grasslands at the central location. Ordination revealed patterns in species composition due to location, but not due to harvest treatment. Therefore, habitat and bioenergy characteristics related to grassland plant communities are not expected to change due to short-term or intermittent late-season biomass harvest.

Keywords: bioenergy, cellulosic biofuel, grassland habitat, plant community composition, plant functional groups, prairie, warm-season grass

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Introduction

Displacing 30% of current US petroleum consumption with sustainable bioenergy requires both economic and environmental assessments of potential biomass feedstocks throughout the United States (US Department of Energy, 2011). Studies have measured how biomass yields of dedicated energy crops, such as switchgrass (*Panicum virgatum* L.) and *Miscanthus*, vary related to regional growing conditions (Heaton *et al.*, 2004; Miguez *et al.*, 2009; Wang *et al.*, 2010). Such information is used to predict regional bioenergy production now (Gelfand *et al.*, 2013), and in the future under different climate change scenarios (Behrman *et al.*, 2013). Studies have expanded modeling efforts to not only predict bioenergy potential, but other ecological outcomes of bioenergy cropping systems such as greenhouse gas

mitigation (Gelfand *et al.*, 2013) and avian biodiversity (Robertson *et al.*, 2011). One potential bioenergy system is mixed-species grasslands, which can provide biomass for energy while provisioning other ecosystem services including biodiversity (McLaughlin *et al.*, 2002; Tilman *et al.*, 2006; Gardiner *et al.*, 2010; Robertson *et al.*, 2011).

Managing mixed-species grasslands for bioenergy has benefits over conventional bioenergy crops and grassland plant monocultures. Bioenergy from cellulose of grassland biomass has greater net-energy benefits and greenhouse gas emissions mitigation than biofuels from conventional food crops (Adler *et al.*, 2007; Fargione *et al.*, 2008; Gelfand *et al.*, 2013). Managing grasslands in mixed-species systems rather than in monoculture increases habitat heterogeneity and therefore, benefits biodiversity at both field and landscape scales (Fargione *et al.*, 2009; Meehan *et al.*, 2010; Wiens *et al.*, 2011). Moreover, mixed-species grasslands can be grown on land unsuitable for crop production with relatively fewer inputs than conventional crops, thus avoiding

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land-use conflicts for food or fuel and management-related greenhouse gas emissions (Tilman *et al.*, 2009).

Marginal lands enrolled in state or federal conservation programs and planted to perennial grassland cover at various diversity levels can serve as a source of bioenergy feedstock (Jungers *et al.*, 2013). The Conservation Reserve Program (CRP) promotes soil conservation on easily erodible lands, and provides habitat for grassland wildlife. The voluntary program provides economic incentives for landowners to enroll parcels into the program for contracted periods of 10–15 years. The CRP has been credited with conserving various bird species (Rahmig *et al.*, 2009) and is considered a critical program for the conservation of biodiversity in the US. Recent increases in commodity crop prices coupled with a surge of expiring CRP contracts have raised concerns about the future of the program and grassland conservation (Wiens *et al.*, 2011). Other conservation programs managed by state and federal entities that provide grasslands for wildlife include the US Fish and Wildlife's National Wildlife Refuge System, where public lands and long-term easements are referred to as Waterfowl Production Areas (WPAs). Similarly, some US states like Minnesota maintain Wildlife Management Areas (WMAs). Conservation goals of the CRP, WPAs, and WMAs are set by the managing entity, and most have not been related to renewable energy.

Plant communities influence conservation-related goals of the CRP, WPAs, and WMAs; which include soil protection, habitat enhancement, and carbon sequestration. Managing plant community characteristics – such as species diversity, the composition of plant functional groups, and the relative abundance of non-native species – is necessary for achieving various conservation goals. Disturbance-dependent ecosystems like grasslands are often managed with prescribed burning to control non-native species or maintain a desired proportion of plant species or functional groups (Howe, 1994). However, burning has become increasingly difficult due to urban encroachment and habitat fragmentation, thus alternatives like mowing have been tested to control invasive grasses (MacDougall & Turkington, 2007) and to promote forb establishment (Williams *et al.*, 2007).

It has not yet been determined if harvesting biomass from conservation grasslands, with production-scale equipment in late autumn/early winter, affects management goals set by agency operators. Our objective was to identify changes in plant species composition in conservation grasslands as a result of biomass harvest, and the implications of such changes on plant biodiversity. We tracked possible changes in plant species richness, metrics of plant diversity, relative abundance of plant species and functional groups, and presence/relative

abundance of native, non-native, and state-listed noxious weed species. Results from control plots and baseline conditions (2009) were compared to conditions following up to three consecutive years of biomass harvest (2012).

Materials and methods

Site description and experimental design

Research was conducted at three locations in western Minnesota, an agriculturally dominated region of the Upper Midwest within the historical prairie range (designated as south, central and north locations, Fig. 1). Experimental plots, each about 8 ha, were delineated within previously restored grasslands planted to mixes of perennial warm- and cool-season grasses, legumes, and other forbs. The grasslands were enrolled as WMAs, WPAs, or CRP land and were established at least 5 years prior to the start of our study. Seeding mixtures varied across and within conservation programs, which led to unique plant species compositions across plots at the start of our study. Twenty-eight plots were studied, 8 in each of the north and central locations and 12 in the south. Some plots had been periodically burned prior to the start of the study, but burning did not occur during the study period.

The experiment was a randomized complete block design with four blocks per location. Two harvest treatments were applied in each block. Treatments included (i) harvested (in late fall); and (ii) unharvested (control). One additional harvest plot was added to each block in the south. Due to inclement weather and expiring land contracts, not all plots were harvested or measured during all years of this study (Table 1). Harvest treatments were applied using a self-propelled windrower that cut to a height of about 15 cm. Cut biomass was baled the same day if biomass was considered sufficiently dry by the operator; otherwise biomass was raked into windrows to dry for up to 5 days before baling. For further details on biomass harvest methods and yields, see Jungers *et al.* (2013). Plots were harvested in 2009, 2010, and 2011 from north to south starting in late October and ending in mid December. Plants were senesced at harvest following one or more killing frosts (-3°C).

Plant community measurements

Plant community data were collected before initiation of harvest treatments and each year following biomass harvest from sample quadrats within each plot. The number and size of sample quadrats varied by year due to labor and resource availability (Table 1). Quadrat locations were randomly selected using ArcGIS 9.0 and loaded into hand-held Global Positioning Systems (GPS). Surveyors walked to the random point with the aid of the GPS and used a PVC frame to outline the quadrat. To avoid biased placement of the quadrat, upon reaching the random point, the surveyor turned 180 degrees from the direction of approach to toss the frame over his/her head.

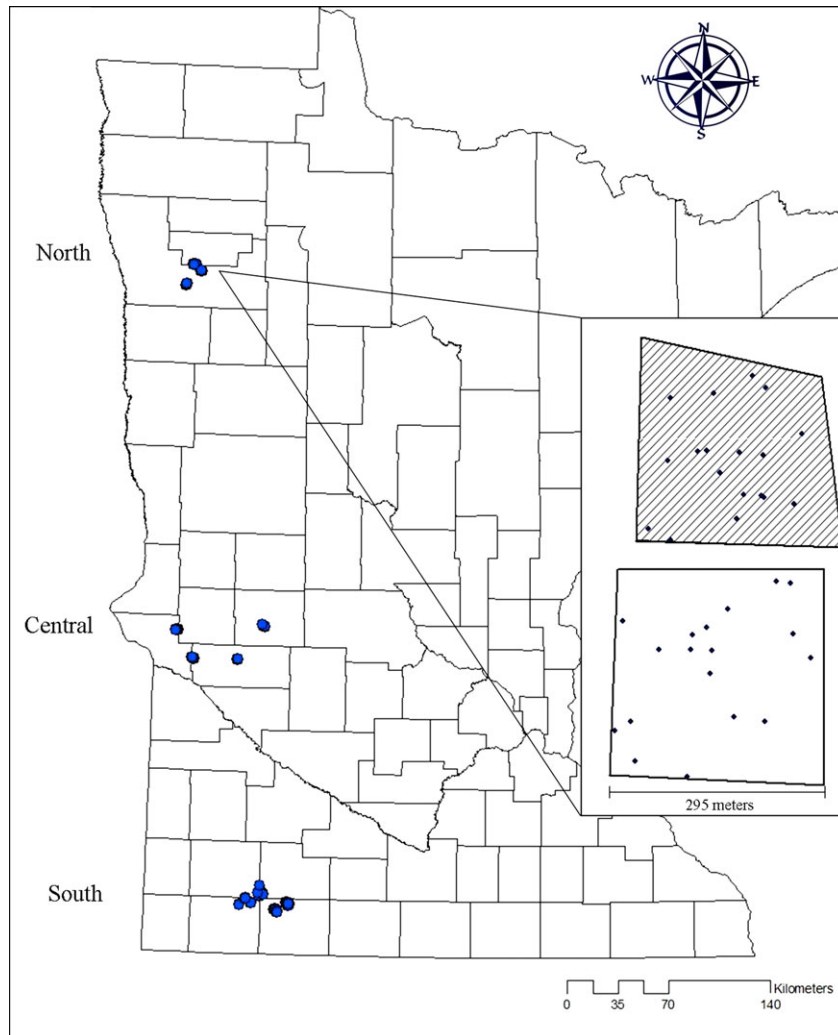


Fig. 1 Map of the study area in Minnesota, USA with each point representing a block of plots. Inset shows one block containing a 100% harvest plot and an unharvested control plot with randomly distributed sample quadrats where plant community composition was measured in 2011.

Table 1 Number of plots sampled, number of quadrats per plot sampled, and size of sample quadrats for determining plant community composition at three study regions of Minnesota, USA

Year	Number of plots sampled			Number of sample quadrats per plot	Size of sample quadrats (m)
	South	Central	North		
2009	12	8	8	2	0.75 × 5.0
2010	12	6	8	24	1.0 × 1.5
2011	9	8	7	12	1.0 × 1.5
2012	11	8	0	12	1.0 × 1.5

Within each quadrat, all unique species were identified using USDA PLANTS names and assigned a score of relative abundance in terms of percent cover. Percent cover was determined as the proportion of aerial coverage by all herbage of the specific species to the nearest percent. Only species rooted within the quadrat frame were counted. Unknown species

were documented and collected when appropriate to be later identified. The percent cover of unidentifiable species was recorded. To avoid misidentification, Goldenrods (*Solidago* spp.) were not identified to species. All species were determined as either native or non-native to the collection site using the USDA PLANTS website (plants.usda.gov). All 'prohibited

noxious weeds were identified according to the USDA PLANTS website for Minnesota state-listed noxious weeds (<http://plants.usda.gov/java/noxious?rptType=State&statefips=27>).

Each plant species was categorized into a functional group on the basis of its growth form. Most plant species in our study sites belonged to one of four primary functional groups: warm-season grasses (C4 grasses), cool-season grasses (C3 grasses), legumes, and nonlegume forbs (forbs). Other groups were sedge, rush, equisetum, woody, and moss. We determined functional groups based on growth form because these can be associated with characteristics that describe habitat. These four major plant functional groups have been used when describing habitat quality in conservation grasslands as it relates to game and nongame birds (Delisle & Savidge, 1997), mammals (Schweitzer *et al.*, 1993), and invertebrates (Doxon & Carroll, 2007).

Within each quadrat, the sum of the cover for all species within each functional group was calculated. Bare ground was assigned when soil was visible in the quadrat, often a result of animal disturbance. The percent cover of litter was recorded. Litter was defined as the layer of dead plant residue from current or previous growing seasons on the ground. Unidentified species were summed together and treated as a separate group. All components summed to 100%.

Statistical analysis

Dissimilarities in plant community composition for harvested and unharvested plots were compared prior to treatment (2009) and following two (north location) or three (central and south locations) years of annual treatment using Non-Metric Multidimensional Scaling (NMDS) ordination based on Bray-Curtis similarity metrics for species cover data. We used the `vegdist` function from the package 'vegan' in R (Oksanen *et al.*, 2013). We plotted vectors illustrating plant community characteristics that were significantly correlated with the NMDS axes. Significance was determined at $P < 0.05$ based on 999 random permutations of the data. We used permutational multivariate analysis of variance (PERMANOVA) to determine differences in plant community composition by location, harvest treatment, and by harvest treatment within each location (Location \times Treatment interaction) at the start and completion of the study. We used the `adonis` function from the package 'vegan' to determine significance at $P < 0.05$ based on 999 random permutations of the data.

The Shannon diversity index ($H' = -\sum p_i \log p_i$) was calculated for each quadrat to determine species diversity, where p_i was the proportion of species i based on percent cover data. Functional diversity was calculated using the Shannon diversity index equation, where p_i was the proportion of functional group i . To compare species richness values across years with different sized quadrats, the number of unique species was determined from both sample quadrats in all plots in 2009. The area of the combined 2009 sample quadrats was 7.5 m² per plot, which was equivalent to the area of five 1.0 \times 1.5 m sample quadrats used during subsequent years. The mean number of unique species was calculated from 100 random samples of

five quadrats in each plot for 2010, 2011, and 2012. The average of each 100 samples was used as the estimated number of unique species per 7.5 m².

Linear mixed effects models were fitted with the 'nlme' package in the program R to account for random variation by plot unique to each year (R Development Core Team, 2010; Pinheiro *et al.* 2013). A global model was constructed to include year, location, and treatment as fixed effects, along with all possible two-way and three-way interactions for all response variables (C4, C3, legume, and forb cover, species and functional group diversity, species richness, and the proportion of non-native and noxious weed species). The global models were reduced sequentially by removing one predictor variable at a time starting with the predictor that was least supported based on t or z statistic. Following the removal of each predictor, a likelihood ratio test was conducted to determine if the removed predictor resulted in a model with worse fit. If the ratio of the negative log-likelihoods of the two models was larger than would be predicted by chance based on a chi-squared distribution with 1 df at an alpha level of 0.05, then the model with a more negative log-likelihood was best supported. Model selection was supported using Akaike's information criteria adjusted for small sample sizes (AIC_c; Table 3). After determining the best-supported model, coefficients from each predictor with a significant P value (0.05) were back transformed and used to discuss the effects of location, harvest, and time.

In some cases, quadrats included only a few individuals of a certain functional group, which resulted in a percent cover of less than two. These values significantly skewed the distribution even after transformations. Therefore, when using mixed effects models to test the effects of year, location, and treatment on the cover of any given functional group, we included only quadrats with 2% cover or more for that functional group in the analysis. The filtered percent cover values were then square root transformed to meet model assumptions. Generalized linear mixed effects models were used to analyze the proportion of non-native and noxious weed species as binomial responses. Logit link functions were applied to binomial data and fit with the Laplace approximation method. Species richness, species diversity, and functional group diversity were not transformed for analysis. Plots of fitted values vs. residuals were used to assess the assumptions for linear mixed effects models.

Filtering observations to include functional groups that consist of more than 2% cover introduces bias to the mixed effects models. To alleviate this bias, we used a Before-After, Control-Impact (BACI) meta-analysis procedure to test if there was an effect of harvest on the relative abundance of plant functional groups. The standardized mean difference (Hedges' g) of percent cover from pre- to posttreatment was used as the effect size (Hedges & Olkin, 1995). A negative effect size indicates that the percent cover of a functional group decreased from pretreatment to either 2 years (north location) or 3 years (south and central locations) posttreatment. Effect sizes were calculated and compared for harvested and unharvested plots at each location. We used 95% confidence intervals to conclude if the effect sizes were similar between harvested and unharvested plots.

Results

Characterization of plant communities

The average percent cover for the main functional groups in sample quadrats was 23% C4 grasses, 19% C3 grasses, 4% nonlegume forbs, 7% legumes and 18% litter, bare ground, or plant species from other functional groups. Big bluestem (*Andropogon gerardii* Vitman), Kentucky bluegrass (*Poa pratensis* L.), goldenrod (*Solidago* spp.), and sweetclover (*Melilotus officinalis* L.) were the most frequently observed species in the C4 grass, C3 grass, forb, and legume functional groups, respectively (Table 2). On average, 69% of the quadrat area was covered by native plants. Averaged across all treatments and years, 15 species were observed per 7.5 m² per plot. The average Shannon diversity index per quadrat was 1.13.

Of the 211 plant species identified, four were noxious weeds in Minnesota. The noxious weeds were Canada thistle (*Cirsium arvense* L.), bull thistle (*Cirsium vulgare* Savi), common sowthistle (*Sonchus oleraceus* L.), and purple loosestrife (*Lythrum salicaria* L.). The two more common weed species, Canada thistle and common sowthistle, were observed in 33% and 7% of all

Table 2 Top five plants in terms of frequency observed and their associated average percent cover for four major functional groups – C4 grasses, C3 grasses, nonlegume forbs, and legumes in Minnesota, USA

Functional group	Species	Rank	Average cover
C4 grass	<i>Andropogon gerardii</i>	1	37
	<i>Panicum virgatum</i>	2	14
	<i>Schizachyrium scoparium</i>	3	16
	<i>Sorghastrum nutans</i>	4	14
	<i>Bouteloua curtipendula</i>	5	3
C3 grass	<i>Poa pratensis</i>	1	20
	<i>Bromus inermis</i>	2	21
	<i>Phalaris arundinacea</i>	3	31
	<i>Agropyron repens</i>	4	11
	<i>Elymus canadensis</i>	5	8
Nonlegume forb	<i>Solidago</i> spp.	1	8
	<i>Cirsium arvense</i>	2	3
	<i>Asclepias syriaca</i>	3	3
	<i>Taraxacum officinale</i>	4	1
	<i>Lactuca scariola</i>	5	1
Legume	<i>Melilotus</i> spp.	1	8
	<i>Dalea purpurea</i>	2	4
	<i>Medicago lupulina</i>	3	3
	<i>Dalea candida</i>	4	4
	<i>Astragalus canadensis</i>	5	5

quadrats, respectively, while bull thistle and purple loosestrife were both observed in less than 0.01%. When present, Canada thistle and common sowthistle covered, on average, 3% and 4% of the quadrat, respectively.

Variation in plant community composition by location

Ordination plots and PERMANOVA tests indicated that plant community types varied by location before (Location: $R^2 = 0.23$, $P < 0.001$) and after (Location: $R^2 = 0.26$, $P < 0.001$) biomass harvest (Fig. 2). Prior to biomass harvest, native species cover and C4 grass cover were negatively correlated with the first NMDS axis (Native: $R^2 = 0.72$, $P < 0.001$; C4: $R^2 = 0.80$, $P < 0.001$), while non-native species cover and C3 grass cover were positively correlated (Non-native: $R^2 = 0.60$, $P < 0.001$; C3: $R^2 = 0.83$, $P < 0.001$). After biomass harvest, native species cover and C4 grass cover remained negatively correlated with the first NMDS axis (Native: $R^2 = 0.31$, $P = 0.015$; C4: $R^2 = 0.48$, $P = 0.002$), while species diversity was positively correlated ($R^2 = 0.34$, $P = 0.007$).

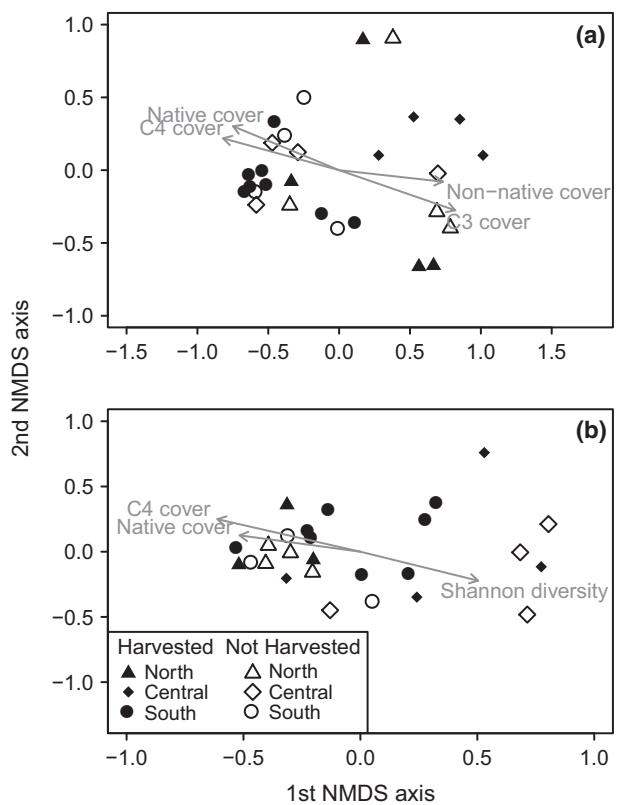


Fig. 2 Nonmetric multidimensional scaling ordination of plant communities in grasslands prior to biomass harvest (a) and following two (North) and three (Central and South) years of biomass harvest (b). Lines represent gradients for metrics of plant community composition, with the length of the line representing strength of correlation with axes.

Throughout the duration of the project, plots from the south location generally resembled plant community types with more C4 grass cover, while plots from the central location were identified with more non-native species cover. After 2 years of harvest, plots in the north location were correlated with higher species diversity (Fig. 2).

Changes in the C4 functional group were explained by the best-supported model which included both a Location \times Year and Location \times Treatment interaction (Table 3). The main effect of location indicated that C4 cover was less in the north compared to the south, but C4 cover increased through time in the north (Table 4; Fig. 3). The Location \times Treatment interaction suggests that, averaged across all years, C4 cover was different between harvested and control plots; but this difference was unique by location (Table 4; Fig. 3). Forb cover was greater in the central location compared to the south (Table 4, Fig. 3g–i), while legume cover was greatest in the south compared to both the central and north locations (Table 4; Fig. 3j–l).

A Location \times Year interaction was retained in the best-supported model for species diversity and weed proportion (Table 3). Averaged across time, species

diversity was similar at all locations, but decreased in the south and north locations (Table 4; Fig. 3). The proportion of noxious weeds was greater in the central location compared to the south, but the proportion of noxious weeds decreased through time in the central location (Table 4). Averaged across time, species richness, functional group diversity, and the proportion of non-native species were similar across locations (Table 3; Fig. 4).

Changes in plant community composition through time

A comparison of the ordination plots from pre- and posttreatment application can be used to identify potential changes in plant community composition due to biomass harvest (Fig. 2). There was no discernible pattern in the distribution of plant community types by harvest treatment in the pretreatment ordination space. Results of PERMANOVA suggest that plant communities in harvested and unharvested plots were similar within each location before biomass harvest (Location \times Treatment: $R^2 = 0.08$, $P = 0.189$) and after 2 or 3 years of biomass harvest (Location \times Treatment: $R^2 = 0.04$, $P = 0.788$; Fig. 2).

Table 3 Model selection results showing parameters from the best-supported, global, and null mixed effects models along with the number of parameters (K), difference in AICc, and model weight (W_i) for plant community composition responses

Response	Model	Parameters*	K	Δ AIC	W_i
C4 cover	Best supported	I + Y + H + L + Y : L + H : L	13	0	0.92
	Global†		16	4.88	0.08
	Null‡		5	27.99	0.00
C3 cover	Best supported	I + Y	6	0	0.86
	Global		16	3.92	0.12
	Null		5	7.14	0.02
Forb cover	Best supported	I + L	7	0	0.76
	Null		5	3.92	0.23
	Global		16	7.14	0.01
Legume cover	Best supported	I + L	7	0	0.87
	Null		5	3.83	0.13
	Global		16	13.21	0.00
Richness	Best supported (Null)	I	5	0	1.00
	Global		16	17.83	0.00
Species diversity	Best supported	I + Y + L + L : Y	10	0	0.99
	Global		16	8.90	0.01
	Null		5	12.78	0.00
Functional diversity	Best supported (Null)	I	5	0	0.98
	Global		16	7.53	0.02
Proportion of natives	Best supported (Null)	I	4	0	0.87
	Global		15	23.88	0.13
Proportion of weeds	Best supported	I + Y + L + L : Y	9	0	0.93
	Global		15	6.18	0.04
	Null		4	6.92	0.03

*I = intercept; Y = year; L = location; H = harvest treatment.

†Parameters for all Global models: I + Y + H + L + Y : L + H : L + Y : H.

‡Parameters for all Null models: I.

Table 4 Parameter estimates, standard errors, *t*-statistics, and *P*-values for best-supported models

Response	Parameters	Value*	SE	<i>t</i>	<i>P</i>
C4 cover	Intercept	5.619	0.486	11.572	<0.001
	Year	0.184	0.142	0.298	0.195
	Harvested	1.168	0.411	2.840	0.010
	Central	0.015	0.769	0.020	0.985
	North	-2.326	0.803	-2.898	0.008
	Year × Central	0.429	0.240	1.784	0.075
	Year × North	0.974	0.297	3.282	0.001
	Harvested × Central	-2.999	0.628	-4.779	0.001
C3 cover	Intercept	5.717	0.315	18.172	<0.001
	Year	-0.340	0.100	-3.389	<0.001
Forb cover	Intercept	2.012	0.090	22.462	<0.001
	Central	0.404	0.140	2.885	0.008
	North	0.194	0.138	1.407	0.172
Legume cover	Intercept	3.975	0.252	15.798	<0.001
	Central	-0.959	0.370	-2.590	0.016
	North	-1.192	0.428	-2.782	0.010
Species diversity	Intercept	1.207	0.066	18.211	<0.001
	Year	-0.115	0.026	-4.380	<0.001
	Central	-0.069	0.109	-0.633	0.533
	North	0.075	0.112	0.674	0.507
	Year × Central	0.132	0.042	3.123	0.002
	Year × North	0.031	0.055	0.565	0.572
Proportion of weeds	Intercept	-3.047	0.189	-16.163	<0.001
	Year	0.154	0.090	1.703	0.089
	Central	0.915	0.285	3.206	0.001
	North	-0.077	0.342	-0.226	0.821
	Year × Central	-0.483	0.135	-3.581	<0.001
	Year × North	-0.103	0.216	-0.477	0.633

*Values not back transformed.

The cover of C3 grasses decreased with time at all locations and in all treatments (Table 3, Table 4). The effect of time on C4 grass cover is explained in terms of the location interaction above, and neither forb nor legume cover changed through time (Table 3). As with the cover of C4 grasses, species diversity and the proportion of weeds changed with time, but uniquely at each location (Table 3). There were no Year × Treatment or Year × Treatment × Location interactions for any response variables (Table 3).

The BACI meta-analysis that included all sample quadrats indicated that the cover of the main plant functional groups might have changed from the start of the experiment to the end (Fig. 5). Legume cover at the central locations decreased in both harvested and control plots. Focusing on the effect sizes by treatment, the 95% confidence intervals of the effect size of time for the control and harvest plots overlapped for all functional groups at all locations (Fig. 5). These data support the results from the mixed effects models that only include quadrats that had more than 2% cover of the tested functional group.

Discussion

Harvesting biomass from conservation grasslands for bioenergy could provide financial resources and incentives to increase the acreage in conservation grassland programs. Before implementing biomass harvest, it is important to know how biomass harvest will affect the primary objectives of conservation grassland programs, including plant and animal diversity. We found that late-season biomass harvest did not affect plant community composition, species richness, functional group relative abundance, or species or functional group diversity after 4 years. We expect that many habitat and bioenergy characteristics related to plant composition will remain the same where late-season biomass harvest is implemented.

No effect of harvest on functional group cover

We did not observe a Treatment × Year, or Treatment × Year × Location interaction for any functional group response variable from the mixed effects model

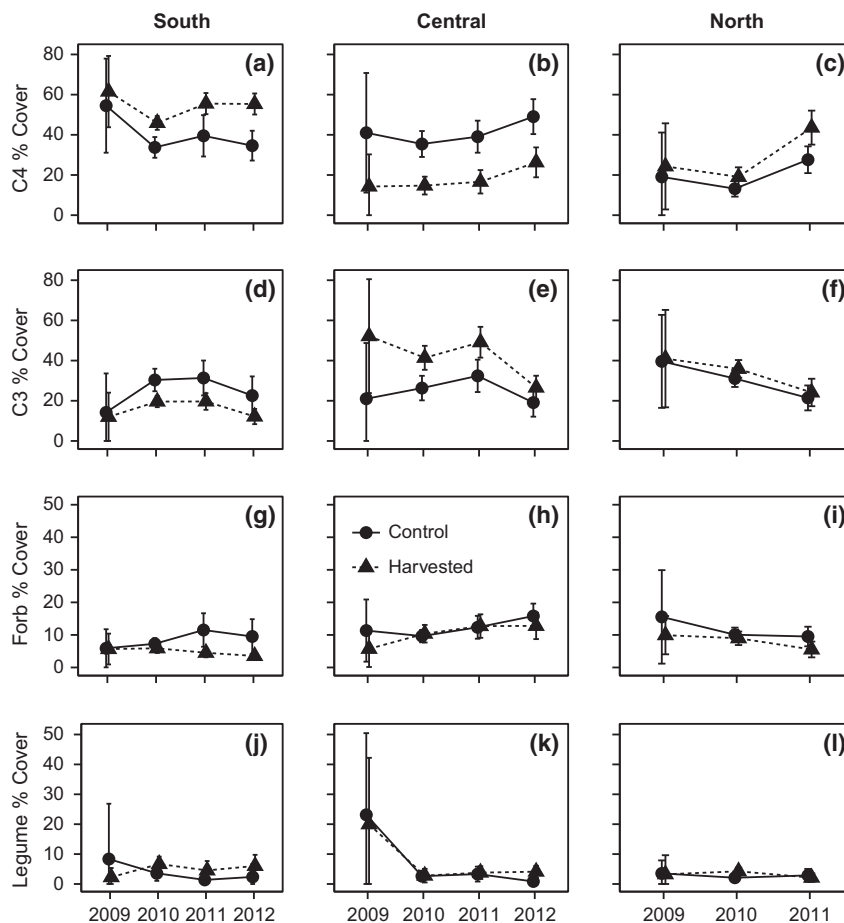


Fig. 3 Average percent cover of the four major plant functional groups in harvested and unharvested plots located in the south, central and north locations from 2009 (pretreatment) to 2012.

results, which we interpret as a lack of effect of biomass harvest. The mixed effects models were useful for testing the effects of time, location, and treatment on response variables that fit certain distributional assumptions. Random effects were also fit to transformed percent cover data for specific functional groups, although the original dataset had to be filtered of high-frequency, low-dominance species to meet model assumptions. Despite the filtering, the mixed effects models of plant functional groups are still useful for identifying differences in relative abundance across locations and through time.

The BACI analysis supported results from the mixed effects models that biomass harvest did not affect the relative abundance of major plant functional groups. The BACI meta-analysis procedure allowed us to include all species data, including those that were filtered from the mixed effects analysis, to determine if biomass harvest altered the trajectory of changing plant functional groups through time. Since there were considerable overlaps of the 95% confidence intervals for

the effect sizes between harvest and control plots for all functional groups at all locations, we determined that biomass harvest did not influence functional group cover. Since there was variation in initial cover of the functional groups across plots, our results suggest that grasslands of varying species compositions can be harvested for up to four consecutive years without altering the relative abundance of major plant functional groups. This is a positive result for land managers who are considering the use of biomass harvest as either a management tool or to produce revenue through bioenergy sales from conservation grasslands.

These results are useful for practitioners who monitor C4, C3, forb, and legume plant functional groups to assess habitat quality. The relative abundance of broad plant functional groups, like those used in this study, may be an easier habitat metric to monitor than plant species diversity or others that require species identification. The use of plant functional group composition has been used to explain the abundance and diversity of some arthropod groups (Symstad *et al.*, 2000),

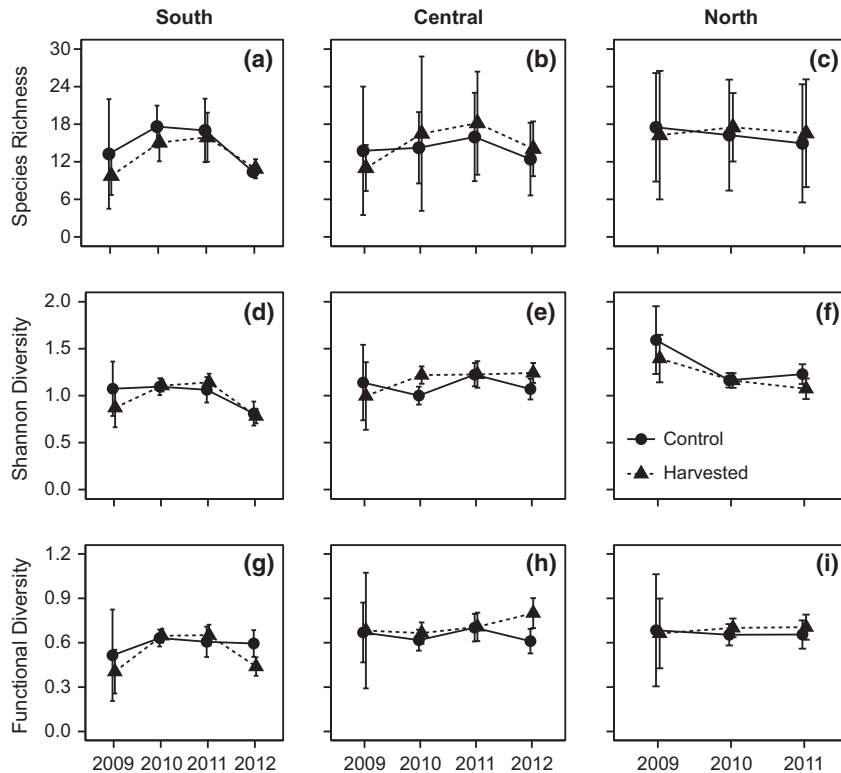


Fig. 4 Average species richness, species diversity, and functional group diversity in harvested and unharvested plots located in the south, central and north locations from 2009 (pretreatment) to 2012.

including pollinators in mixed-species grasslands managed for bioenergy (Robertson *et al.*, 2012). For higher taxonomic levels, legume cover was identified as a useful predictor in explaining variation in waterfowl nest success in prairie pothole grasslands (Arnold *et al.*, 2007). Although plant functional groups are sometimes used to assess habitat quality, habitat variables such as plant litter, vegetation height, and other metrics of structural heterogeneity are also considered (Roth *et al.*, 2005; Arnold *et al.*, 2007). Monitoring plant functional group cover does not provide quantitative metrics to assess structural composition of grasslands, but other studies have found that biomass harvest has similar effects on vegetation structure as prescribed fire in the short-term (Rave *et al.*, 2013). However, monitoring species composition at the coarser scale of functional groups is not sensitive to identifying changes in the abundance of rare plant species. Where the abundance of a specific plant species is of concern, permanent sampling quadrats should be established and monitored annually.

Although our study did not observe any effect of biomass harvest on plant functional group cover, other studies have found varying effects depending on pretreatment community composition. Similar to our results, changes in the relative abundance of native C4

grasses and the non-native C3 Kentucky bluegrass (*Poa pratensis* L.) were the same in harvested and unharvested grasslands following 3 years of biomass harvest in areas dominated by native C4 grasses (Hendrickson & Lund, 2010). However, the same study found that biomass harvest increased the relative abundance of Kentucky bluegrass in grasslands initially dominated by C3 grasses, but not in those initially dominated by C4 species. Questad *et al.* (2011) also observed unique changes in plant composition following harvest in C3 and C4 dominated grasslands, but the responses they observed were opposite those observed by Hendrickson & Lund (2010). Questad *et al.* (2011) reported changes in plant composition as a result of harvest in native C4 dominated grasslands, but not in non-native C3 dominated sites. Inconsistencies in these studies suggest that other factors, other than initial C3 or C4 grass dominance, affect how plant composition responds to harvest.

We observed large variation in legume cover in 2009, especially in the central region (Fig. 3k). This was largely due to the presence or absence of sweet clover (*Melilotus alba* and *Melilotus officinalis*). This tall-statured biennial dominates areas by shading competitors and reducing local diversity. During peak years, this species

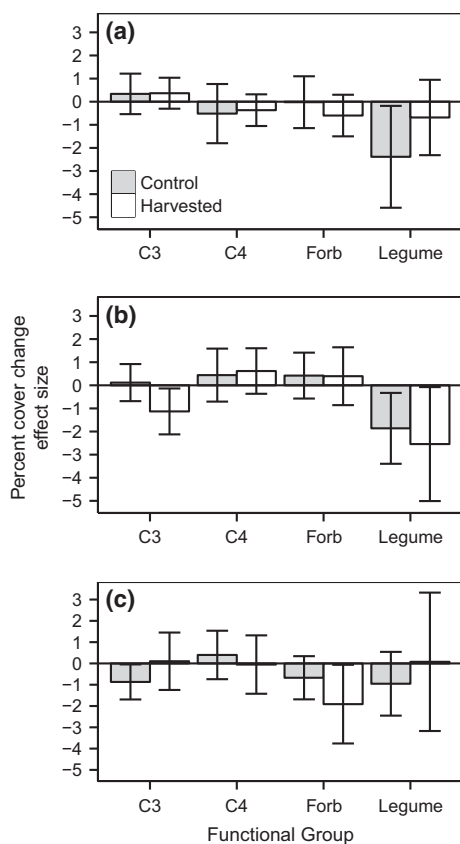


Fig. 5 Effect sizes (Hedges' g) and associated 95% confidence intervals for the change in functional group cover from pre-treatment to final year post-treatment conditions in the south (a), central (b), and north (c) locations in Minnesota, USA.

often covered 100% of our sample quadrats when random points fell in regions dominated by sweet clover. Mean legume cover and variance were large in 2009 due to peak sweet clover abundance combined with the smaller sample size. However, results from the mixed effects models showed that legume cover did not significantly vary through time or by harvest treatment (Table 4). The smaller sample size in 2009 may explain changes in other response variables from 2009 to 2010 when they did not occur in subsequent years.

No effect of harvest on non-native or weed proportions

Harvesting biomass in late autumn did not change the proportion of non-native or weed species for the duration of this experiment. Few studies have investigated the effects of biomass harvest on non-native and weed species in established grasslands in the Upper Midwest. Rave *et al.* (2013) found that the proportion of non-native species was similar between harvested and burned grassland sites in Minnesota. Disturbance intensity, as measured by the number of harvests in one

growing season, did not change the proportion of weed species in polyculture grasslands (Picasso *et al.*, 2008).

Some state and federal agencies recommend mowing grasslands in the spring or summer to decrease annual non-native species populations, if the grassland is not expected to harbor nesting birds (US Department of Agriculture, 2009). This is effective if the non-native plants are mowed before they flower. In grasslands that are harvested for bioenergy, mowing does not occur until after most annual non-natives have set seed. There is some concern that biomass harvest may facilitate non-native species populations (Donald, 2006). Biomass harvest could increase non-native and weed plant populations via two mechanisms. The first is that harvesting biomass could decrease the density of the litter layer, thereby leading to more favorable conditions for species colonization (Tilman, 1993) and establishment (Foster & Gross, 1998). Tarmi *et al.* (2011) observed increased recruitment in harvested grasslands by species in the existing seed bank, as well as species from adjacent ditch habitats. The second is that improperly cleaned harvesting equipment could transport seeds and propagules of non-native and weed species. We implemented an equipment cleaning protocol that was administered between harvests to avoid transporting plant parts between fields.

No effect of harvest on richness, species, or functional group diversity

Late-season biomass harvest did not affect species richness in this study. In other studies, increases in species richness have been observed in harvested plots as soon as 3 years after treatment initiation (Tarmi *et al.*, 2011). Hansson & Fogelfors (2000) observed dramatic increases in species richness in semi-natural grasslands, which was maintained after 15 years of annual harvest. Increased species richness following harvest has been linked to the reduction of litter (Parr & Way, 1988). Reduced litter increases light availability and enhances conditions that promote colonization and seedling establishment (Tilman, 1993). We did not observe a difference in litter cover by year or treatment. Our methods of measuring litter cover did not quantify litter mass or thickness, which are linked to recruitment conditions (Tilman, 1993). Alternatively, we measured how much litter could be observed covering the quadrat, which is more useful as a surrogate for sward density than litter density.

Biomass harvest did not affect species or functional group diversity. Several previous studies have found that biomass harvest has led to positive effects on species diversity. Native grasslands that were annually hayed had higher species and functional group

diversity than unmanaged CRP and cool-season hay pastures (Questad *et al.*, 2011). Especially in more fertile and productive grasslands, biomass harvest increased diversity during most years of a 7 year study (Foster *et al.*, 2009). Similar patterns of increased species diversity as a response to harvest were observed in European grasslands (Antonsen & Olsson, 2005). The lack of an effect of biomass harvest on species diversity in our study could be related to the timing of harvest. The previous studies harvested biomass during peak biomass (June–July) compared to the postsenescence (October–December) harvest time of our study. Midgrowing season harvest could immediately enhance the growing conditions for species that are less dominant; and thus decrease the relative abundance of the more dominant species. For instance, midgrowing season harvest might allow species with later emergence times to establish and better compete with species that typically dominate in early growing season conditions. Since there is little plant growth immediately following late-season harvest, all species will be competing for resources in the spring as usual, only now under slightly different light availability conditions. A direct comparison of plant community dynamics under varying harvest times is needed to validate this hypothesis.

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