2011
Project Abstract
For the Period Ending June 30, 2014

PROJECT TITLE:	**Prairie Management for Wildlife and Bioenergy - Phase II**
PROJECT MANAGER:	Clarence Lehman
AFFILIATION:	U of MN
MAILING ADDRESS:	1987 Upper Buford Circle
CITY/STATE/ZIP:	Saint Paul, MN, 55108
PHONE:	(612) 625-5734
E-MAIL:	lehman@umn.edu
WEBSITE:	http://www.cbs.umn.edu/wildlife

FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 03g
APPROPRIATION AMOUNT: $600,000

Overall Project Outcome and Results

Many wildlife areas and conservation lands were formerly marginal agricultural fields that have been converted into rich habitats of grasses and flowering plants. That habitat traditionally required maintenance by prescribed burning. However, mowing can be more feasible and can provide future commodity incentives through a carbon-negative energy source.

Our prevailing question was how grassland areas could be harvested annually without upsetting their ability to support wildlife. We organized over 1000 acres into 60 production-size, 20-acre plots spanning the temperature gradient in western Minnesota. The plots were harvested in prescribed intensities and patterns each fall from 2009-2012 after plants had senesced and migratory wildlife left. Each year, surveys of songbirds, gamebirds, small mammals, reptiles, amphibians, insects, and plants were conducted and bioenergy potential calculated.

Our results showed that bioenergy can be harvested sustainably without harming resident wildlife by following simple protocols developed during the project. Specifically, leaving unharvested refuges of 5-10 acres rotated annually in a 20-acre plot minimized significant impact on wildlife, and we recommend such refuges as best practices. Harvesting without any refuge negatively affected some wildlife, specifically prairie and meadow voles, a shrew, sedge wren, common yellow throat, clay-colored sparrow, swamp sparrow, waterfowl nesting, and potentially native bees. Deer mice, grasshopper sparrows, common grackles, spiders, flies and beetles increased with harvest. Plant cover and biomass did not change significantly during our harvesting tests. We cut and analyzed over 3,000 tons of biomass with yields ranging from 0.6-1.8 tons/acre and projected ethanol yields averaging 108-gallons/ton. Recommendations for best harvesting equipment are low weight-to-tire-width ratio, easily repaired, and readily cleaned between fields.

The broad consensus among wildlife experts is that diverse ecosystems offer habitat that is superior for a spectrum of wildlife, The overall significance of this project is that it

identified and tested better methods for maintaining such habitat on public and private grasslands of Minnesota.

Project Results Use and Dissemination

During this six-year project the Environmental Trust Fund and other substantial federal and local funds have resulted in two graduate theses, 26 publications, posters and presentations, five outreach events and newspaper articles, nine symposia, a website, a publically available dataset carrying the raw data and metadata supporting our conclusions, and a draft Best-Management-Practices document.

That draft document has been formatted professionally for publication, with release scheduled this calendar year. Some managers in the Minnesota DNR have begun using harvesting as a grassland management tool on Wildlife Management Areas and through Cooperative Farm Agreements, and we expect that this can expand and become routine as project results, including the Best-Management-Practices document, are published and disseminated broadly.

Dissemination will be ongoing for some time, with new scientific papers in preparation and continuing presentations at conferences.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2011 Work Plan Final Report

Date of Report: 8/15/2014
Final Report
Date of Work Plan Approval: 6/23/2011
Project Completion Date: 6/30/2014
Is this an amendment request? No

Project Title: Prairie Management for Wildlife and Bioenergy - Phase II

Project Manager: Clarence Lehman
Affiliation: U of MN
Address: 1987 Upper Buford Circle
City: St Paul **State:** MN **Zipcode:** 55108
Telephone Number: (612) 625-5734
Email Address: lehman@umn.edu
Web Address: http://www.cbs.umn.edu/wildlife

Location:
Counties Impacted: Statewide
Ecological Section Impacted: Lake Agassiz Aspen Parklands (223N), Minnesota and Northeast Iowa Morainal (222M), North Central Glaciated Plains (251B), Northern Minnesota and Ontario Peatlands (212M), Northern Minnesota Drift and lake Plains (212N), Northern Superior Uplands (212L), Paleozoic Plateau (222L), Red River Valley (251A), Southern Superior Uplands (212J), Western Superior Uplands (212K)

Total ENRTF Project Budget:
ENRTF Appropriation $: 600,000
Amount Spent $: 600,000
Balance $: 0

Legal Citation: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 03g

Appropriation Language:
$300,000 the first year and $300,000 the second year are from the trust fund to the Board of Regents of the University of Minnesota to research and evaluate methods of managing diverse working prairies for wildlife and renewable bioenergy production. This appropriation is available until June 30, 2014, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Prairie management for wildlife and bioenergy: Phase II

II. FINAL PROJECT SUMMARY: This project forms part of a broad effort to sustain Minnesota resources while improving the rural economy and contributing to our energy independence. The project tested best management practices to maintain grassland habitat for wildlife while generating bioenergy. The work continued and completed the monitoring of wildlife responses and other ecological responses to harvesting prairies for bioenergy, as started from a 2008 ENRTF project., allowing sufficient time for the significance of results to be tested. A major objective of the project was to identify biomass harvesting patterns that could maintain wildlife populations by leaving distinct size and shapes of refuges within the grassland, but doing so while harvesting the greatest sustainable amount of biomass from the sites. Over 1000 acres of restored grasslands across western Minnesota were divided into 20-acre plots. The plots were organized in three locations spanning the temperature range of Minnesota---in the region of Windom, Morris and Crookston. Wildlife surveys encompassed birds, small mammals, and insects, with special attention to pollinators. Bioenergy surveys monitored changes in plant communities and differences in bioenergy potential from production-scale harvests. Results are being used to develop guides for landowners and to produce standard protocols for bioenergy and wildlife evaluations. Best management reports are being produced for state land-managing entities to improve management efficiency and potentially reduce costs of habitat management.

III. PROJECT STATUS UPDATES:

Project Status as of (January 1, 2012):
This project, which began in July 2008, was selected for continued funding so Phase II began in July 2011. As that was in the middle of field season, surveys continued throughout the remainder of the summer and into Fall 2011. A full harvest and the associated surveys (such as bale cores) occurred in two of the three regions. It was decided to focus efforts and funds on the southwest and west central location and

discontinue harvest in the northwest region. Field season data is being entered into spreadsheets and will be processed further throughout the winter. An upcoming staff and PI meeting will review protocols, interim data, outreach, and future research directions.

Amendment Request (May 24, 2012)**:**
We are requesting the reallocation of funds between the budget categories Travel Expenses and Professional/Technical Contracts. This change would move $25,000 from the latter category into three activities of the travel budget. This is in response to the unexpectedly high cost of travel throughout the field plots for wildlife and biomass surveying, re-projecting costs for future travel, and of paring down harvest. Specifically, we request to move $12,000 into Activity 1 of Travel, $7000 to Activity 2, and $6000 to Activity 3. We do not expect this shift to significantly alter the deliverables of the Activities.

Amendment request approved by LCCMR – 6/11/12.

Project Status as of *(July 1, 2012)*:
Field work for summer 2012 is underway. Surveys that have begun include plant flower blooms, songbird, herpetafauna, insect sweepnets, bees and pollinators, and insect pitfalls. Data from the previous field season has been entered into spreadsheets and processed. A meeting was held in January 2012 which included faculty, PIs, and staff. Paper assignments and data for papers were divided and ideas for future studies were exchanged. We continue to review and update protocols; release interim data as appropriate; facilitate outreach, including preparing papers for publication and materials for dissemination; plan future research directions in response to results so far; and apply for supplemental funding.

Project Status as of *(January 1, 2013)*:
Fall harvest was completed in late November, following a summer of drought throughout the region. Bale and harvest data were collected, as in previous years. Wildlife and vegetation surveys continued throughout the summer as well. Project members met in early November to review current project status, discuss available data, identify subprojects, assign members to these subprojects, and discuss ongoing funding options. Twelve distinct subprojects were identified: one has been published, one submitted, and an additional eight are in various stages of completion. Subprojects appear below under the appropriate Activities.

Project Status as of *(July 1, 2013)*: A paper titled "Energy potential of biomass from conservation grasslands in Minnesota, USA" has been published and is open access at *http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0061209*

Songbird surveys were conducted and data are being entered into digital formats. Two papers on these results are nearing completion. Data from 2012 plant and animal surveys has been entered. Data analysis is underway for waterfowl nest success, songbird abundance and diversity, small mammal abundance and diversity, and the effect of biomass harvest on plant composition and diversity. Manuscripts are in preparation. A meeting was held between UMN researchers and DNR staff to report preliminary findings from the study. A paper summarizing the design and intent of the project was published, following a related Sun Grant Conference presentation funded by a supplemental UMN travel grant (Williams et al. 2012, see citation below in Dissemination, Section V).

Project Status as of *(January 1, 2014):* A manuscript reporting the effects of biomass harvest on pheasant and duck nest survival and density was accepted with revisions by the *American Midland Naturalist* journal. Revisions were made and are now in review by the journal editors. A poster was prepared and presented at the 98th Ecological Society of America (ESA) annual meeting. At the ESA conference, a special Ignite session based on ecosystem complementarity highlighted work from this project. A presentation summarizing the preliminary results was delivered for the UMN Conservation Biology Seminar Series. Two manuscripts from insect surveys are nearing submission. Data from bee surveys is being analyzed and preliminary results have been discussed with the MN DNR to aid in their best management practice development. Specific results are discussed in individual sections below.

Amendment Request (January 21, 2014)**:**
We are requesting a retroactive amendment to reallocate funds between travel, contracts, and personnel. Specifically, we expect to save $8,000 from Activity 4, reducing technical contracts from $75,000 to $67,000. Because complete field surveys were not planned for Summer 2014, we did not need to not apply the full harvest treatment in Fall 2013. This also reduced travel costs in Activity 2 (determining biomass productivity and harvest yields) from $8,000 to $5,215. We are requesting that the $10,785 be transferred to the Personnel category in Activity 5 (analyses, reporting, and publications). Moving funds to this category is for analysis by staff, faculty, and technicians.

Approved by the LCCMR February 24, 2014

Amendment Request (June 13, 2014)**:**
We are requesting two sets of amendments. The first request is for expertise necessary to complete two specific tasks. The second request reflects some redistributions between categories that will help more efficiently complete this study.

Proposed increase 1. We need to contract with an expert in bee identification for the final stages in species identification and to do so need to move $375 to the Contracts section of the budget in Activity 3. We would also like to employ the statistical consulting expertise at the University to assist in insect data analysis. As this is employing internal resources at the University and falls under the Other category, we would like to move $180 to the Other category in Activity 5. This is a proposed increase of $555 for these categories.

Proposed increase 2. The majority of the remaining work includes writing and analysis falling under Activity 5. These tasks require personnel time resulting in a change to the Personnel budget from $58,785 to $65,687. We would also move $46 to the Equipment budget for Activity 3, changing the Equipment budget from $2,900 to $2,946. The $46 would pay for curation supplies to prepare the insect collection for long term storage. This is a proposed increase of $6948 for these two categories.

Proposed reductions. As we have nearly completed (a) Travel for Activities 1, 3, and 4, (b) Equipment needs for Activities 1, 2, and 4, and (c) Other for Activity 3, we propose the balance in other areas to be provided from these three categories. Specifically we propose to reduce budgets for the following categories: Travel: Activity 1 ($19,500 to 16,318), Activity 3 ($6,000 to 3,979), Activity 4 ($1,500 to 978); Equipment: Activity 1 ($4,000 to 3,339), Activity 2 ($100 to 83), Activity 4 ($1,000 to 835); Other: Activity 3 ($2000 to 1065). This amounts to a total reduction of $7503.

In summary, the total Personnel budget would change to $497,687 by adding $6,902, the Contracts budget to $67,375 by adding $375, the Equipment budget to $7,203 by subtracting $797, the Travel budget to $26,490 by subtracting $5,725 and the Other budget to $1,245 by subtracting $755. These changes balance the project through its completion date.

Approved by the LCCMR June 24, 2014

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Wildlife Sampling

Description: Survey crews will sample songbirds, small mammals, and insects. Bird surveys will be conducted in the spring using transect counts. Insects will be sampled at least once during the summer in all plots with sweep nets and potentially pit-fall traps. Small mammal surveys will take place in all plots in the fall of each year using catch-and-release live traps.

Summary Budget Information for Activity 1:	ENRTF Budget:	$ 210,157
	Amount Spent:	$ 210,157
	Balance:	$ 0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Determine relative abundance of small mammals in various harvesting regimes.	**Spring 2014**	**$54,500**
2. Determine relative abundance of song birds in various harvesting regimes.	**Spring 2014**	**$54,500**
3. Determine biomass of insects of various size classes and functional groups.	**Spring 2014**	**$101,083**

Activity Status as of *(January 1, 2012):* Small mammal trapping was performed in Fall 2011 following the protocols of previous years. A total of fifty-nine plots in all three regions were successfully surveyed for four consecutive nights. QuIST insect sampling, which had begun in Summer 2011 during Phase I of the project, was completed in the southwest region. Songbird surveys were completed before July 1, 2011 and so were reported with the previous phase. Data for these surveys has been entered into spreadsheets and will be further formatted this winter.

Activity Status as of *(July 1, 2012):* The wildlife sampling season began in early April. Plot preparation (flagging boundaries and survey points) for the song birds, insect, and small mammal surveys began on April 30, 2012. *Song bird surveys* began on May 5 and finished on June 14. Song bird data entry began in late May and will be further formatted this summer. Preliminary analysis shows that species richness was significantly less in 2011 than in the other three years of study. In both 2011 and 2012, species richness was significantly less in full harvest plots than in controls; this was a decline of 39.8% in 2011 and 23.0% in 2012. Preparation for the August *small mammal surveys* began in mid April and will continue through July. Random *insect* GPS sweep points were generated for all survey periods and plots (March-May). The first round of insect sampling was performed in

the southwest site from May 30th to June15 on all plots and in the west central site between June 18-22 on all plots. Insect pit-fall traps were installed (May 2012) on nine plots in the southwest site and the first round of sampling was performed on June 22-25. Bee bowl sampling posts were installed (April 2012) and surveys were run on April 26-27, May 3-4 with another sampling scheduled for late June or early July. Bloom transects were laid out on nine plots, and quadrat locations flagged on all transects (Early April). Weekly bloom survey began on April 17 2012, data spreadsheet entry began on June 13.

Re-installation of herpetafauna (reptile and amphibian) trap arrays was started on April 3rd and was completed on April 12. Trapping of herpetafauna began on April 12. Data entry into spreadsheets for the summer-long herpetafauna survey started on June 13.

Activity Status as of *(January 1, 2013)*: The wildlife sampling season was completed in early September. Survey markers and equipment, including herpetofauna arrays, insect sampling posts, and small mammals markers, were removed in preparation for harvest. Subprojects that have emerged from Activity 1 include

- *Small mammals*: A preliminary manuscript has been written, covering three years of small mammals survey data. It will be expanded to include 2012 survey results. Preliminary abundance analysis was performed for two small mammals: Microtus (genus-level) abundance declined and short-tailed shrews (*Blarina brevicauda*) abundance did not change. Occupancy analysis (presence/ absence, as opposed to abundance) was performed for seven small mammals: percent harvest affected the presence of both Microtus and masked shrews (*Sorex cinerea*) negatively (i.e. the species/genera was less likely to be found in plots with increasing percent harvest). The magnitude and significance of these changes and subsequent analysis will be discussed in the completed small mammal manuscript.
- *Song birds:* Four years of data are being incorporated into a manuscript. No differences in preliminary analysis were observed in species richness or total number of species, but no abundances increased with harvest. Preliminary results are that four of ten birds surveyed were affected by harvest, including the sedge wren, which is of conservation concern.
- *Game bird nesting:* Daily survival rates were calculated for waterfowl and pheasant nests in harvested and un-harvested areas within the study. Preliminary results suggest that biomass harvest does not change the probability of a nest surviving. The nest density is being compared among harvest and un-harvested regions. Preliminary results show that nest density is higher in the uh-harvested areas, but that this does not translate to fewer nests in harvested areas. Preliminary manuscript is underway.
- *QuIST:* The Quantitative Insect Sampling Technique is a new method for assessing and calibrating standard insect collection methods. Data acquisition is complete and preparation of a manuscript is underway.

- *Herpetofauna*: Reptile and amphibian survey data were identified at a recent project status meeting as being robust and warranting a manuscript. Data is assembled and will be analyzed by project members in spring 2013.

Activity Status as of *(July 1, 2013)*: Insect field survey were complete as of the last field season and the Quist data from those surveys is being analyzed. Two scientific papers are in preparation, one on best management practices for sweep netting and calibrating the resulting data with quantitative exhaustive samples, the other applying those methods.

Activity Status as of *(January 1, 2014)*: Quist data has been analyzed. Two manuscripts related to the Quist method are nearing submission, one on the formula for calculating the volume swept by a net in radial and forward motion, and correcting an erroneous formula that has been in the scientific literature, another on calibrating sweep net data with quantitative exhaustive samples. Manuscripts that result from this quantitative work will show the fraction of each major arthropod group that is captured by sweep netting, which turns out to average only a few percent. Bee and Bloom data has been entered and formatted and data analysis has begun and manuscript has been initiated. A master's thesis by Robert Dunlap on birds and small mammals in the study is nearing completion, with results from that thesis previewed under birds and mammals below.

Birds. We observed a total of 57 bird species in our plots over five years. 11 of which were frequent enough for analysis of abundances. All but two of these were characteristic grassland birds. Red-winged blackbirds and common grackles are not grassland specialists but are generally common in agricultural lands, and are included in the analysis. The common species are Sedge Wren, Common Yellowthroat, Clay-colored Sparrow, Savannah Sparrow, Grasshopper Sparrow, Le Conte's Sparrow, Swamp Sparrow, Dickcissel, Bobolink, Red-winged Blackbird, and Common Grackle. Sedge wren and grasshopper sparrow are designated as Partners in Flight conservation priority species. Le Conte's Sparrow and Dickcissel were only regionally abundant.

Overall, heavier harvesting slightly decreased the number of bird species occupying the sites, but not substantially (Figure 1.1). Individual bird species responded differently, with grasshopper sparrow and common grackle increasing in abundance with heavier harvesting, sedge wren, common yellowthroat, clay-colored sparrow, and swamp sparrow declining with heavier harvesting. and savannah sparrow, Le Contes sparrow, dickcissel, bobolink, and red-winged blackbird remaining unaffected by the intensity of harvesting.
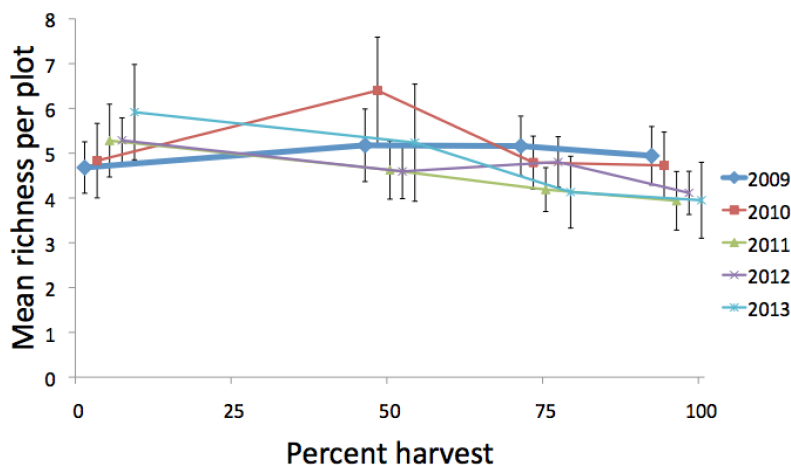
Figure 1.1. The pre-harvest 2009 trend is fairly flat, with slightly more species in harvested plots. This changes somewhat in 2010 with more species present in 50% harvest plots. But for the most part in 2011, 2012, and 2013, the trend was slightly decreasing abundance with greater harvest.

In exploratory models, the amount of grassland and wetland within a quarter kilometer of each plot was important to most species. An interesting discovery from our exploratory models is that harvest pattern (various size blocks and strips) did not seem important to any species in our study, but the number of species was slightly reduced for strip patterns.

Mammals. We observed a total of 11 species/genera of small mammal in our plots over four years of small mammal surveys. Of these, three were common enough to allow analysis of abundance---voles, deer mice, and northern short-tailed shrews. Four other species occurred in lesser numbers---the meadow jumping mouse, thirteen-lined ground-squirrel, short-tailed weasel, and masked shrew. For those we applied presence-absence analyses. Only a few times we observed the northern grasshopper mouse, too infrequently for analysis.

Overall, heavier harvesting does not seem to affect the number of small mammal species occupying the sites (Figure 1.2). Northern short-tailed shrews, meadow jumping mice, thirteen-lined ground-squirrel, and short-tailed weasel were not affected by the intensity of harvest. Voles and masked shrews were reduced and the case was not clear for deer mice, due to low abundance in some years. Population cycles with large fluctuations in abundance are common for many small mammals. No small mammals in our study appeared to be affected by the pattern of harvest.

Voles were most abundant in the northwest region and northern short-tailed shrews were most common in the southwest. However, our species-richness analysis did not identify the region as a significant variable, suggesting that similar communities of small mammals are present throughout the study area.
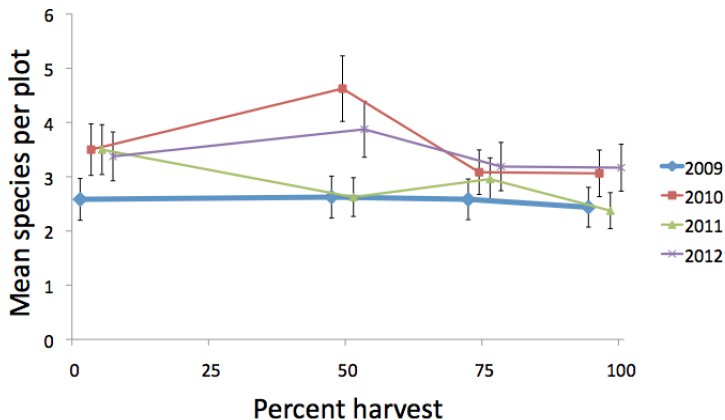

Figure 1.2. The number of species of small mammals is not substantially reduced by increased intensity of harvest.

Final Report Summary: (August 15, 2014):

Small Mammals. Small mammal catch-and-release trapping was performed in Fall 2011 and fall 2012 following the protocols of previous years. A master's thesis by Robert Dunlap on birds and small mammals in the study was completed in 2014 and detailed analysis is presented in there.

In summary, we observed a total of 11 species/genera of small mammal in our plots over four years of small mammal surveys. Of these, three were common enough to allow statistical analysis---voles, deer mice, and northern short-tailed shrews. Four other species occurred in lesser numbers---the meadow jumping mouse, thirteen-lined ground-squirrel, short-tailed weasel, and masked shrew. For those we applied presence-absence analyses. We observed the northern grasshopper mouse a limited number of times, too infrequently for analysis. A note for future researchers is that masked shrews have very high metabolism and catch-and-release traps must be checked frequently to ensure their well-being. Voles were most abundant in the northwest region and northern short-tailed shrews were most common in the southwest. However, our species-richness analysis did not identify the region as a significant variable, suggesting that similar communities of small mammals are present throughout the study area.

Population cycles with large fluctuations in abundance are common for many small mammals, but overall, heavier harvesting did not seem to affect the number of small mammal species occupying the sites (Figure 1.2). Northern short-tailed shrews, meadow jumping mice, thirteen-lined ground-squirrel, and short-tailed weasel were not affected by the intensity of harvest. Voles and masked shrews were reduced as the fraction of the plot harvested increased. The case was not clear for deer mice, due to low abundance in some years. No small mammals in our study appeared to be affected by the shape of harvested area.

These results are significant to grassland management because small mammals occupy a central role in the natural food web, and with appropriate biomass harvesting, such as leaving an unharvested refuge each year within the grassland, that role should not be adversely affected. These results have been incorporated into a best management practices document. Methods used in this portion seem appropriate to questions being asked.

Songbirds. Songbird surveys were conducted in May-June 2012 according to protocols of previous years. A master's thesis by Robert Dunlap on birds and small mammals in the study was completed in 2014 and detailed analysis is presented there.

In summary, we observed a total of 57 bird species in our plots over five years. Eleven species were common enough for analysis of abundances. All but two of these were characteristic grassland birds. Red-winged blackbirds and common grackles are not grassland specialists but are generally common in agricultural lands, and were included in the analysis. The common species are Sedge Wren, Common Yellowthroat, Clay-colored Sparrow, Savannah Sparrow, Grasshopper Sparrow, Le Conte's Sparrow, Swamp Sparrow, Dickcissel, Bobolink, Red-winged Blackbird, and Common Grackle. Sedge wren and grasshopper sparrow are designated as Partners in Flight conservation priority species. Le Conte's Sparrow and Dickcissel were only regionally abundant.

Overall, heavier harvesting slightly decreased the number of bird species occupying the sites, but only by two species or fewer. Individual bird species responded differently, with grasshopper sparrow and common grackle increasing in abundance with heavier harvesting, sedge wren, common yellowthroat, clay-colored sparrow, and swamp sparrow declining with heavier harvesting. And savannah sparrow, Le Conte's sparrow, dickcissel, bobolink, and red-winged blackbird remaining unaffected by the intensity of harvesting.

In statistical models, the amount of grassland and wetland within a quarter kilometer of each plot was important to most species. An interesting result is that harvest pattern

(various size blocks and strips) did not seem important to any species in our study, but the number of species was slightly reduced for strip patterns.

Results have been incorporated into best management practices document which further discusses their implications. Generally, if certain songbirds are targets of management, then their response to harvest in this study should guide management to either leave more or less area as unharvested refuge. Many grassland birds use areas larger than our plot size and this is something to consider in future studies.

Insects. Quist insect sampling, which had begun in Summer 2011 during Phase I of the project, was completed in the southwest region in July and August 2011. Insect sweeps were performed in all sites from May-August 2012. Insect pit-fall traps were run in June July and August 2012 on nine plots (control and full harvest) in the southwest site. Bees were sampled in control and full harvest plots using bee bowls five times between April and August 2012. Bee sampling formed a valuable contribution given the current state of bee decline worldwide.

One manuscript related to the Quist method has been submitted, on the formula for calculating the volume swept by a net in radial and forward motion, and correcting an erroneous formula that had been in the scientific literature. Another on calibrating sweep net data with quantitative exhaustive samples is pending local review and submission. Manuscripts that result from this quantitative work will show the fraction of each major arthropod group that is captured by sweep netting, which turns out to average only a few percent. A calibration table for use with sweep netting is presented in the best management practices document.

June vegetation height (sward height) in unharvested plots appeared to increase through subsequent years as compared to fully harvested plots. This in later months heights appeared equal between treatments. However statistical analysis did not show the June difference to be significant statistically. Therefore sward height simply became a covariate in biomass analysis.

Insect response to harvesting was measured in biomass. Dry weights were taken from each arthropod taxon. Arthropod biomass was affected by harvest, with harvested plots displaying slightly but statistically significantly higher arthropod biomass. Among taxa, this effect is significant for spiders, beetles and flies, but not for true bugs, ants bees and wasps, larvae, or grasshoppers.

Certain functional groups of invertebrates---that is, groups defined by their roles in the ecosystem---are beneficial to humans. Pollinators enable seed and fruit production, and natural enemies (parasites and predators) help control certain crop pests. We counted individuals of these groups from sweep net samples and they showed no degradation due to harvest. Pollinator numbers actually showed a slight statistically significant increase in

the full harvested plots by the last year. (However see next section on bees.) Insects that are natural enemies of other potentially harmful insects showed no significant difference between control and full harvest plots. Study in this area will continue, and continued study by future investigators is suggested. Pitfall data has not been fully analyzed due to time and funding, but will be analysed in future funding. Sometimes grasslands could harbor insect pests or insects that are vectors for plant diseases, but that information is not available from our study.

Bee bowl data has been analyzed and our data do not show that total bee abundance and abundance of small bees and stem nesting bees were not significantly affected by full harvest as compared to control plots. However there is a trend in small stem nesting bees that suggests possible negative impact recommending caution in suggesting that harvest is completely benign. One flaw with this data set is that it did not include the pre-harvest bee community, due to its start date, so we are unable to make that comparison. Bee populations fluctuated greatly during the study, highlighting the benefits of long term monitoring for bee research.

Bee bowls are an efficient way to sample bees. Though other researchers are showing it does not sample the full community, it can still show effects of an experimental treatment. Sweep netting is also efficient but vegetation height turns out to be important. Although valuable and necessary to this project, Quist is time consuming and instead we recommend our calibration table or including vegetation height in sweep net analysis wherever possible. Any research involving insects would benefit from focused goals and significant time or funds allocated to processing samples and identifying insects.

Herpetafauna. Reptiles and amphibians were surveyed from April to August in 2011 and 2012 following the successful methods of previous years. This was continued because survey data were robust and warranting future publications. Data analysis was not within the scope of this project but has begun with other funding, and future results will be distributed. We found the arrays we used to be more effective than lone bucket traps. The following reptiles and amphibians identified in our southwest study area.

Rana pipiens	Northern Leopard Frog	55%
Bufo americanus	American Toad	19%
Thamnophis radix	Plains Garter Snake	11%
Thamnophis sirtalis	Common Garter Snake	6%
Ambystoma tigrinum	Tiger Salamander	4%
Pseudocris triseriata	Western Chorus Frog	3%
Eumeces septentrionalis	Prairie Skink	1%
Bufo cognatus	Great Plains Toad	<1%
Chrysemys picta	Painted Turtle	<1%

Game bird nesting. Game birds surveys were completed during the first phase of this project and analyzed during the current phase. and are incorporated in a publication currently in review.

Biomass harvest can affect nesting biology in at least two ways (1) If harvested areas are less suitable for nesting, nest density would decrease. (2) If harvested areas are less suitable for nesting but waterfowl still nest there, nest predation could increase. We found that the probability of a nest surviving is the same for nests initiated in harvested areas and unharvested areas. Nest predators in the region of this study were not more or less likely to find and consume nests in harvested areas. However, waterfowl preferred to nest in the unharvested regions. Nest density was lower in the harvested regions. It is important to note that there was a similar number of nests initiated prior to the first harvest and following harvest, but that the nests were more concentrated in the unharvested regions.

We found more nests in plots with taller grass and also in those plots that had more abundant wetlands within a 500 meter radius from the plot center (Jungers et al., in review). Waterfowl preferred nesting in upland grassland sites that were near wetlands, and these nests had a better chance at surviving compared to those further from wetlands. Therefore, some regions of upland habitat within conservation grasslands should be left standing if managed for bioenergy, and these unharvested regions should be located near wetlands if possible. This selection strategy should not only help maintain waterfowl populations during harvest, but may also limit harvest inefficiencies due to wet ground.

ACTIVITY 2: Vegetation and soil sampling

Description: Survey crews will measure plant species richness and diversity in all plots. Summer vegetation will be sampled to determine standing biomass stocks. Soil cores will be collected in all plots. Bale cores will be collected in all plots for chemical analysis and dry matter calculations to be used for determining harvest yield. Stubble height will be measured in all plots during harvest.

Summary Budget Information for Activity 2:	ENRTF Budget:	$ 44,199
	Amount Spent:	$ 44,199

	Balance:	$ 0

Activity Completion Date:

Outcome	Completion Date	Budget
Determine biomass productivity and harvest yields.	**Spring 2012-2014**	**$ 44,199**

Activity Status as of *(January 1, 2012):* Vegetation cover surveys were performed in Fall 2011 in all three regions on a total of fifty-eight plots. Biomass samples from approximately twelve points on each of the fifty-eight plots were sampled and weighed. Stubble height was measured at randomized plot locations following harvest in the southwest and west central regions. These data have been entered into spreadsheets and will be further formatted this winter.

Activity Status as of *(July 1, 2012):* Data from the previous biomass samples were analyzed and will be reported in prepared manuscripts (reported under Activity 5). Preparation is underway for 2012 vegetation sampling which will start in late July. Preparation includes generating random sampling points for each of the forty-four plots, training staff, assembling survey equipment, and finalizing data sheets.

Activity Status as of *(January 1, 2013):* Vegetation sampling, including bloom surveys, percent cover, and vegetation transects, were completed in summer 2012, as in previous years. Three subprojects were identified under Activity 2 and will be prepared in separate manuscripts. These include:
- *Bioenergy potential*: This manuscript has been submitted for publication and is currently under peer review. A result of this data is that fall harvest during the first four years of this project had no statistically significant effect on bioenergy potential. That is, four years of harvest did not diminish grassland yield potential.
- *Plant community percent cover*: A preliminary result of this analysis is that no change was observed in composition of plant functional group with harvest. This manuscript is in preparation.
- *Blooms and pollinators*: These data include number and abundance of blooms for 2011 and 2012. Data are assembled and analysis is expected to begin in spring 2013. The working hypothesis for this subproject is that dead litter is cut out (i.e. harvested) leaving space for increased blooms.

Activity Status as of *(July 1, 2013):* Collection of vegetation data from all years, now completed, were compiled, audited, and analyzed. A publication reporting the effect of biomass harvest on the relative abundance of plant species functional groups and diversity was initiated.

Activity Status as of *(January 1, 2014):* A publication reporting the effects of biomass harvest on plant composition was completed, to be submitted to the journal Biological Conservation pending co-author approval. This paper describes that biomass yields from conservation grasslands in the south location averaged 4 Mg ha^{-1}, only marginally less than first-year harvest yields from high-diversity mixtures in experimental plots at a nearby agricultural research center. Biomass quality from mixed-species grasslands not managed for bioenergy is similar to dedicated energy feedstocks, in terms of theoretical ethanol conversion efficiency and biomass nitrogen. Almost all of the variation in land ethanol yield was based on biomass yield, and therefore efforts in managed grasslands should be focused on maximizing biomass yield rather than biomass quality, where ethanol yield is a factor. A combination of climate, soil fertility, and plant community factors influence overall bioenergy potential. The effect of forbs and legumes on biomass yield and tissue nitrogen, respectively, were different in the south compared with the central and north locations. A greater proportion of warm-season grasses increased ethanol conversion efficiency. After three continuous years of harvest, with a portion of standing biomass within the harvested area left unharvested, yield did not decrease with number of harvests. Tables and figures below, available in the paper, provide additional detail.
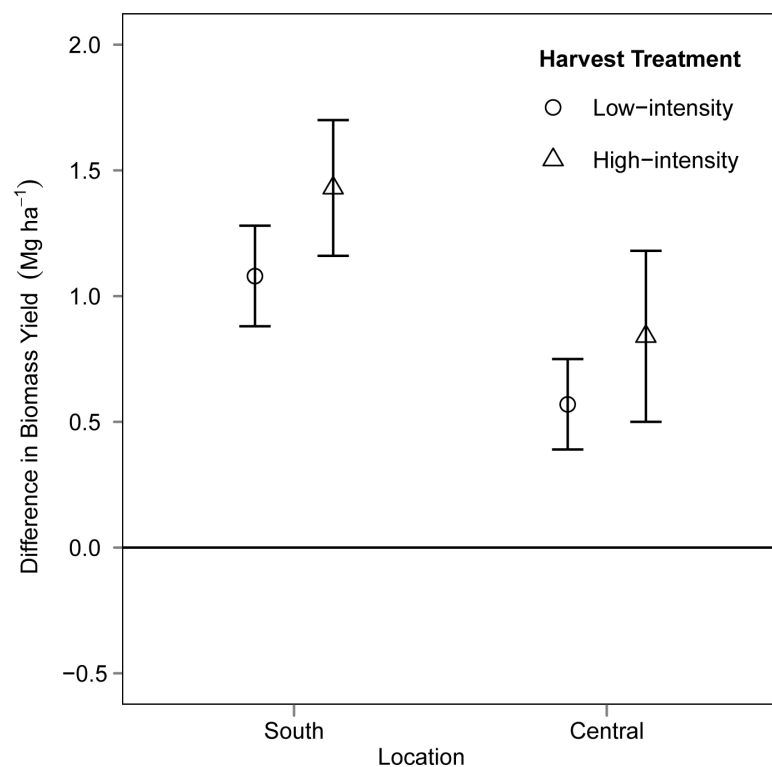
Table 1. Mean biomass yields (Mg ha^{-1}).

Location	2009	2010	2011	2012	Average
South	2.7	2.5	4.0	2.8	3.1
Central	1.6	1.6	2.2	-	1.8
North	1.3	1.6	-	-	1.6

Table 2. Estimated maximal biomass yields (Mg ha^{-1}) from hand-clipped samples.

Location	2009	2010	2011	Average
South	7.1	3.3	4.5	5.2

Central	3.6	2.9	4.0	3.6
North	3.1	2.7	-	2.9

Figure 2.1. Leaving some parts of the field unharvested for wildlife refuges, rotated in different parts of the field each year, does not reduce biomass when those parts are harvested. Fields with refuges of standing biomass (circles) have similar biomass yields through time as those that are completely harvested annually (diamonds). Error bars overlap, indicating no significant differences.
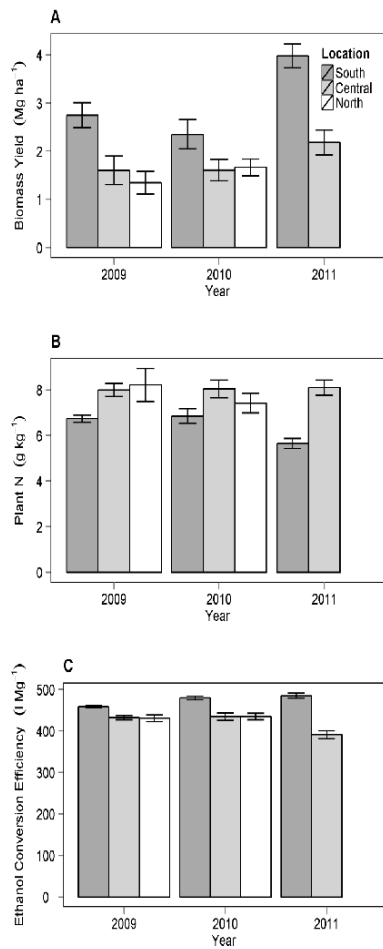
Figure 2.2. Black, gray and white bars are mean values from plots harvested in south, central and north locations respectively. Biomass yield (A) on conservation grasslands is almost double in the south versus the north, plant tissue nitrogen (B) has the opposite trend, and ethanol conversion efficiency (C) does not vary strongly across the region.
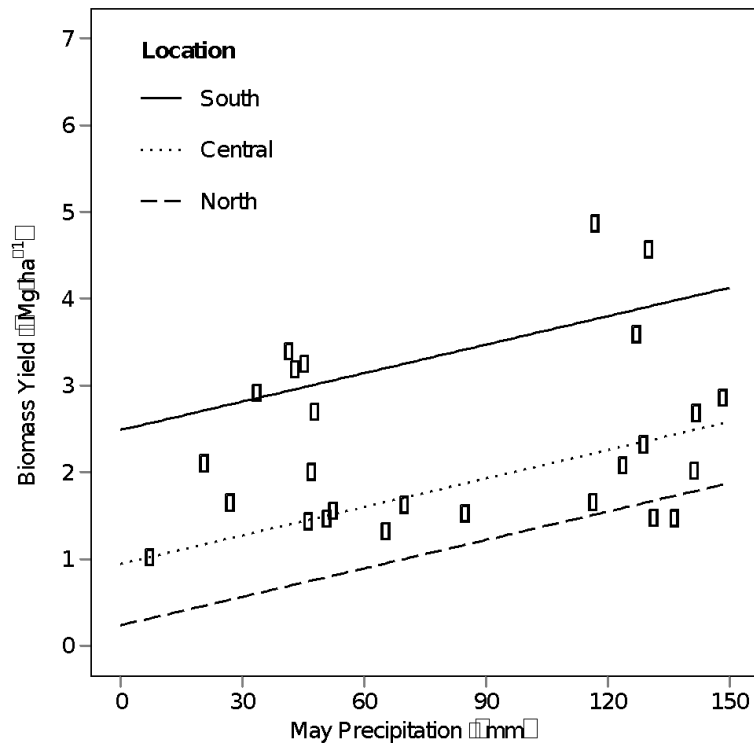
Figure 2.3. May precipitation is an important part of biomass yield. Dots represent average measured biomass yield and May precipitation values. Lines are model estimates for bioenergy yield, with all other things equal at their average values.

Plant community percent cover: It is important to know how biomass harvest will affect the primary objectives of conservation grassland programs, including plant and animal diversity. We have found that late-season biomass harvest did not disturb plant community composition, number of species present, abundance of functional group of plants, or diversity, after four years of harvest. We expect that many habitat and bioenergy characteristics related to plant composition will remain the same where late-season biomass harvest is implemented, as depicted in the figure below.
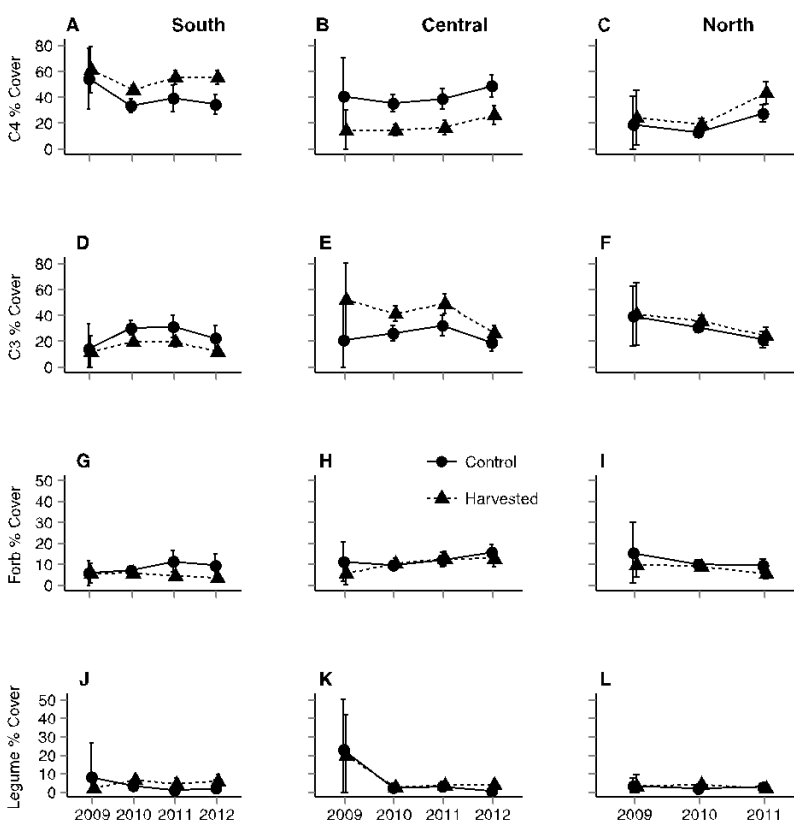
Figure 2.4. Biomass harvest does not significantly affect the composition of conservation plant communities, as shown by the similar trends for harvested (diamonds) and unharvested plots (circles).

Blooms and pollinators: The data include number and abundance of blooms for 2011 and 2012. Data are assembled and analysis is expected to begin in spring 2013. The working hypothesis for this subproject is that dead litter is cut out (i.e. harvested) leaving space for increased blooms.

Final Report Summary (August 15, 2014)**:**
Vegetation cover surveys, biomass samples and stubble height were performed in 2011 and 2012. Soil cores are planned for late summer 2014, using supplemental funding obtained from the University of Minnesota. A new vegetation measure, blooming plant surveys, were conducted once a week during the summers of 2011 and 2012 to correlate with bee abundance data. Transects and quadrats were set up in 9 control and full harvest plots in the southwest region. Species of plants in bloom were counted on transects, and number of blooms per species were counted in quadrats.

A publication reporting on biomass yields and energy potential was completed, Jungers, J. M., J. E. Fargione, C. C. Sheaffer, D. L. Wyse, and C. L. Lehman. (2013). Energy potential of biomass from conservation grasslands in Minnesota, USA. *PLoS One*. 8(4): e 61209. This paper describes that biomass yields from conservation grasslands in the south location averaged 4 Mg ha^{-1}, only marginally less than first-year harvest yields from high-diversity mixtures in experimental plots at a nearby agricultural research center. Biomass quality from mixed-species grasslands not managed for bioenergy is similar to dedicated energy feedstocks, in terms of theoretical ethanol conversion efficiency and biomass nitrogen. Almost all of the variation in land ethanol yield was based on biomass yield, and therefore efforts in managed grasslands should be focused on maximizing biomass yield rather than biomass quality, where ethanol yield is a factor. A combination of climate, soil fertility, and plant community factors influence overall bioenergy potential. The effect of forbs and legumes on biomass yield and tissue nitrogen, respectively, were different in the south compared with the central and north locations. A greater proportion of warm-season grasses increased ethanol conversion efficiency. After three continuous years of harvest, with a portion of standing biomass within the harvested area left unharvested, yield did not decrease with number of harvests. Theoretical ethanol yields suggest that southwesten Minnesota has enough biomass to support a production scale ethanol facility. Tables and figures to this effect are in previous updates and available in the paper.

Plant community percent cover: A paper is in press on this topic, Jungers, J.M., J. E. Fargione, C. C. Sheaffer, D. L. Wyse, and C. L. Lehman. (In Press). Short- term harvesting of bioenergy from conservation grasslands maintains plant biodiversity. *Global Change Biology: Bioenergy*. It is important to know how biomass harvest will affect the primary objectives of conservation grassland programs, including plant and animal diversity. We have found that late-season biomass harvest did not disturb plant community composition, number of species present, abundance of functional group of plants, or diversity, after four years of harvest. We expect that many habitat and bioenergy characteristics related to plant composition will remain the same where late-season biomass harvest is implemented.This is an important result for managers because it means that they can interrupt their normal disturbance schedule to harvest biomass from conservation grasslands without affecting the plant community. Land managers continued some of their usual weed control measures, such as spot spraying thistles, during the study.

Bloom surveys. Data do not show that full harvest significantly reduced or increased bloom abundance or timing. Thus it is likely that fall harvest will have little impact, though harvested

areas did green up sooner in the spring. Full analysis is forthcoming. Bloom survey methods were reasonably practical, though it is difficult to say with present information what quantity of resources for bees various blooming species represent.

ACTIVITY 3: Sample Analysis

Description: Biomass collected from the bale cores will be analyzed for nutrient and sugar concentrations. Sub-samples will be combusted to determine ash and energy content. Soil cores will be analyzed for carbon/nitrogen ratios and other nutrient concentrations. Sweep net and pitfall samples of insects will be sorted to functional group and size, in part to estimate biomass value as food for other wildlife. Insect samples will be sorted by year and collection method.

Summary Budget Information for Activity 3:	ENRTF Budget:	$ 193,465
	Amount Spent:	$ 193,465
	Balance:	$ 0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Determine ethanol, gasification and other energy potential of biomass.	**Spring 2012-2014**	**$6,000**
2. Identify trends in nutrient stocks in soil and biomass to understand ecosystem nutrient sustainability.	**Spring 2012-2014**	**$6,000**
3. Biomass of insects as food source for waterfowl, game-birds, and songbirds.	**Spring 2014**	**$181,465**

Activity Status as of *(January 1, 2012)*: Bale cores from all harvest treatments in the west central and southwest region were taken at harvest and analyzed for sugar ratios, macro- and micro-nutrient concentrations. Results from the chemical analyses are being entered into spreadsheets. Insects collected by QuIST and sweepnet methods in Summer 2011

(project Phase I) are being analyzed by project members in labs at the U of M and Cedar Creek.

Activity Status as of *(July 1, 2012):* Biomass samples from 2011 have been analyzed. Data have been processed, grouped, and assigned to project members, and results are being written into project publications. Sorting of 2011 insect samples was completed in January and the data was entered. Analysis and manuscript preparation of insect data from the QuIST absolute sampling method was begun February-March. Data from insect sweepnet, pitfall and bee bowls from 2011 has been entered and prepared for the analysis which will be conducted after the 2012 season. Processing of 2012 insect samples began in June 2012. No new information on trends in insect data are available at this stage. 2012 samples appear comparable to previous years, with perhaps fewer bees captured in 2012. Small mammal data from 2009-2011 was analyzed over the winter and will be written up once 2012 data is collected. Data will continue to be analyzed by project members in labs at the U of M and Cedar Creek.

Activity Status as of *(January 1, 2013):* The emphasis of Activity 3 is determining harvesting effects on *insect biomass*. Insects collected via sweep nets and pitfalls during summer 2012 were classified and tallied by project members and a team of undergraduate researchers at Cedar Creek Ecosystem Science Reserve. Data were incorporated with previous years and analysis and manuscript preparation is underway. Preliminary analysis found insect biomass, as length-weighted counts, increased after one harvest. Also, from zero (control plot) to full harvest there is a statistically significant harvest effect.

Activity Status as of *(July 1, 2013)***:** A summary of theoretical ethanol potential based on bale core analysis is published in the peer-reviewed article "Energy potential of biomass from conservation grasslands in Minnesota, USA" published in the open-access journal *PLoS One.*

Activity Status as of *(January 1, 2014)***:** No further sample analysis was performed in this time period, pending information from the final field season.

==Final Report Summary== (August 15, 2014)**:**
Bale cores from all harvest treatments in the west central and southwest region were taken at harvest in 2011 and analyzed for sugar ratios, macro- and micro-nutrient concentrations. A summary of theoretical ethanol potential based on bale core analysis is given in the final report summary of Activity 2 and in published in the peer-reviewed article "Energy potential of biomass from conservation grasslands in Minnesota, USA" published in the open-access journal *PLoSOne.*

Insects were analyzed by project members in labs at the U of M and Cedar Creek. Sorting and processing was completed in January 2013. Insects of each taxa division and length class were dried and weighed and a chart produced in 2013 to calculate insect biomass. In June 2014 an expert in bee identification was contracted to identify more difficult species. Results of this data are discussed in Activity 1.

ACTIVITY 4: Production-scale biomass harvest

Description: Each plot will be harvested using farm-grade harvesting equipment. Each plot will have an assigned harvesting regime, which includes a precise size and shape of refuge. Refuges will be left as 50%, 25%, and 0% of the plot and left as either a block or a set of equally distributed strips. Refuges will rotate annually within the plot. Harvesting will take place after the primary nesting season when plants have senesced, yet before spring green-up. This category covers transportation of the biomass.

Summary Budget Information for Activity 4:	ENRTF Budget:	$ 86,313
	Amount Spent:	$ 86,313
	Balance:	$ 0

Activity Completion Date:

Outcome	Completion Date	Budget
1. *Provides treatment effects for experiment*	**Fall 2014**	**$ 86,313**

Activity Status as of *(January 1, 2012)*: Harvest for the southwest and west central regions began at the end of September 2011 in the west central region, and was completed in early November 2011 in the southwest region. All plots in these regions were dry and able to be harvested per their refuge regime. A total of 1537 bales were created in the southwest plots and 479 on the west central plots. No harvesting was done in the northwest region in Fall 2011 due to expense, although ruts from the wet 2010 harvest were repaired.

Activity Status as of *(July 1, 2012)*: Land-use contracts between project management and landowners are in development. Negotiations between project management and harvest contractor are in progress, and a contract is expected to be signed by mid-July.

Activity Status as of *(January 1, 2013)***:** Harvesting was completed in late fall by the same harvesting contractor, Minnesota Native Landscapes, as in previous years of the project. This lends consistency year to year and reduces the "learning curve" of locating fields and performing the correct harvest pattern. A drought occurred during summer 2012 in Minnesota and throughout the Midwest. Data from bale cores and harvest outcomes are not yet analyzed, though from preliminary observations in the field, less baled biomass is expected. To maximize efficiency in the field, contractors did not harvest fields where little to no biomass could be harvested due to lack of moisture. Harvest was performed only in the southwestern region, though wildlife and vegetative surveys were conducted in both the west-central (Morris, MN area) and southwestern region (Windom, MN area).

Activity Status as of *(July 1, 2013)***:** Biomass yield data from all years, now complete, were electronic-ally entered and formatted for analysis and archival storage. A publication on energy potential of biomass from conservation grasslands was prepared for submission. See a summary of results under Activity 2.

Activity Status as of *(January 1, 2014)***:** Biomass harvesting for the project was completed in the late fall of 2012. No harvest was planned or performed in 2013 because there will be no subsequent sampling in 2014, due to project completion.

==Final Report Summary== (August 15, 2014)**:**
The southwest region was harvested in fall 2011 and 2012 and west central region was harvested in 2011. In 2011 plots were generally dry and harvest went according to plan with 1537 bales from southwest plots and 479 on the west central plots. No harvesting was done in the northwest region in Fall 2011 due to difficulties in that region, and due to expense, although ruts from the wet 2010 harvest were repaired. The same harvesting contractor, Minnesota Native Landscapes, was used throughout the project. This lent consistency year to year and reduced the learning curve of locating fields and performing the correct harvest pattern. In 2012 contractors did not harvest fields where little to no biomass could be harvested due to lack of moisture. A publication on energy potential of biomass from conservation grasslands was prepared for submission, see Activity 2. No harvest was planned or performed in 2013 because there will be no subsequent sampling in 2014, due to project completion. Harvest logistics and recommendations can be found in the best management practices document related to this project. Much of project knowledge of harvesting was learned by examination differing methods. Conservations

grasslands are not as uniform as agricultural fields are and require adaptability and ecological knowledge on the part of harvesters.

ACTIVITY 5: Reports and dissemination

Description: Results will be distributed in the form of academic publications, public reports, project web site pages, local newsprint, and other forms of media. Substantial funds are allocated to this activity because multiple publications are needed for a variety of audiences to disseminate the results of this broad study.

Summary Budget Information for Activity 5:	ENRTF Budget:	$ 65,867
	Amount Spent:	$ 65,867
	Balance:	$ 0

Activity Completion Date:

Outcome	Completion Date	Budget
1. Economic and logistic analysis report of harvest feasibility	**Spring 2014**	**$23,000**
2. Final report for DNR explaining ecological impacts of harvesting for BMP	**Spring 2014**	**$23,000**
3. Multiple peer-reviewed publications on impacts of harvesting prairies for energy	**Spring 2014**	**$19,687**

Activity Status as of *(January 1, 2012)*:   Project investigator, Clarence Lehman, was interviewed by Scott Rall, Worthington (MN) Daily Globe's outdoors columnist for an article published January 6, 2012. The article discusses how this project may benefit hunters and sportsmen in that area. Dr. Lehman was also interviewed by Carol Davit, Missouri Prairie Journal editor about his work on native prairie. The article, titled "Carbon Storage, Ecological Stability, and Epiphany at Bluebird Prairie," can be found in volume 32, numbers 3 & 4 of the journal. In addition, this interview is linked from the project website (http://ww.cbs.umn.edu/wildlife).

Activity Status as of *(July 1, 2012):* Two manuscripts have been prepared and will be submitted for publication this summer. The first is titled "Energy potential of biomass from conservation grasslands in Minnesota," and is intended for a journal like *PLoS One,* and the second is titled "Short-term harvesting of bioenergy from conservation grasslands maintains plant biodiversity," and is intended for a journal like *Biological Conservation*. These papers will be sent to LCCMR staff as soon they are accepted for publication, or earlier upon request. Manuscript preparation for QuIST absolute insect sampling method, small mammal trapping and bird surveys has begun.

A best management practice report has been prepared and will be updated as necessary throughout the project. Additional modes of dissemination are discussed in the section below.

Activity Status as of *(January 1, 2013)***:** Three subprojects were identified under Activity 5. One has been published, the second is submitted, and the third will be prepared at the completion of the project:

- *First year summary of harvest:* Jungers J, Lehman C, Sheaffer C, and Wyse D. Characterizing Grassland Biomass for Energy Production and Habitat in Minnesota, *Proceedings of the 22nd North American Prairie Conference*, 2010. 168-171.
- *Summary of methods:* A manuscript was submitted to the proceedings of the *2012 Sun Grant Initiative Conference* under the title "Bioenergy From Reserve Prairies in Minnesota: Methods for Measuring Harvest and Monitoring Wildlife." All project participants are authors.
- *System integration and dynamics:* This planned manuscript will accompany the final report the LCCMR, as a synthesis of project results and analyses.

Activity Status as of *(July 1, 2013)***:** A paper titled "Energy potential of biomass from conservation grasslands in Minnesota, USA" has been published in *PLoS One* and is available for free at
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0061209

Activity Status as of *(January 1, 2014)***:**
A paper titled "Effects of grassland biomass harvest on nesting ducks and pheasants" was submitted and accepted with revisions to the journal *American Midland Naturalist*, and revisions of been submitted.

A poster summarizing the effects of biomass harvest on plant community composition was prepared and presented by Jacob Jungers at the *Ecological Society of America* annual meeting in August 2013.

A summary of the preliminary results from plant, songbird, waterfowl, and small mammal surveys was presented by Jacob Jungers at the UMN Conservation Biology Seminar in December 2013. Data from this presentation are included under Activity 2 status.

Two manuscripts on Quist method are nearing completion and manuscript on bee and bloom data has been initiated.

Preliminary results of bee data have been reviewed with the Minnesota DNR to aid in their development of best management practices.

Final Report Summary (August 15, 2014)**:**

For the Final Report Summary of Activity 5, please see the related Final Report Summary in Section V, Dissemination, immediately below.

V. DISSEMINATION:

Description: The results of this project will be distributed to a wide range of audiences, from industry to academia. Results will be distributed in the form of academic publications, public reports, project web site pages, local newsprint, and other forms of media. Substantial funds are allocated to this activity to disseminate the results of this broad study in multiple publications for a variety of audiences.

Status as of *(January 1, 2012)*: Project investigator, Clarence Lehman, was interviewed by Scott Rall, Worthington (MN) Daily Globe's outdoors columnist for an article published January 6, 2012. The article discusses how this project may benefit hunters and sportsmen in that area. Dr. Lehman was also interviewed by Carol Davit, Missouri Prairie Journal editor about his work on native prairie. The article, titled "Carbon Storage, Ecological Stability, and Epiphany at Bluebird Prairie," can be found in volume 32, numbers 3 & 4 of the journal. In addition, this interview is linked from the project website (http://www.cbs.umn.edu/wildlife).

Status as of *(July 1, 2012)*:   The project website has been continually updated to reflect seasonal information and photos. Data relevant to land managers has been sent to them.

These include the raw data and results of wildlife surveys on their respective properties. Project member Jake Jungers presented information at the Third Crop Producers Meetings in April. Project member Colleen Satyshur presented information on the project to Cedar Creek personnel and interns in June 2012. An abstract to present information on the effects of grassland biomass harvest on nesting waterfowl was accepted for The Wildlife Society 19th Annual Conference.

Status as of *(January 1, 2013)***:** Subprojects discussed under Activities 1-5 are summarized here:

Subproject	Activity
QuIST: Insect calibration	1
Small mammals	1
Birds	1
Game bird nesting success	1
Herpetofauna	1
Blooms and pollinators	2
Bioenergy potential	2
Plant community percent cover	2
Insect biomass	3
First year synthesis	5
Summary of methods	5
System integration and dynamics	5

The project website continues and photos are added throughout the year. The survey and harvest data will be publicly shared on the website as reports and papers are published. Project participants actively seek additional funding and recently secured an Institute on the Environment mini-grant which will be used to share and coordinate prairie and wetland research currently being done at the UMN. In addition, project participants continue to coordinate with Minnesotan and outstate groups that are interested in pursuing grassland biomass as a renewable bioenergy.

A poster summarizing project methods was presented at the 2012 Sun Grant Initiative Conference held in New Orleans, LA. The poster shared a title with the submitted paper: "Bioenergy From Reserve Prairies in Minnesota: Methods for Measuring Harvest and Monitoring Wildlife."

Preliminary results from the waterfowl and pheasant nest surveys was presented in the form of a poster at The Wildlife Society Annual Conference in October 2012. The title of the poster was "Managing conservation grasslands for bioenergy and wildlife: Measuring the effects of biomass harvest on waterfowl and pheasants". Copies of the posters are available on the project website.

Status as of *(July 1, 2013):*

Project design and logistics were presented by S. Williams in a poster at the Minnesota Society for Conservation Biology annual meeting on March 16 at Dodge Nature Center in West Saint Paul, MN.

Project status was presented at a special DNR meeting for the purpose on March 26 in Hutchinson MN, titled "Grassland Biomass Harvest Wildlife Impacts, Project Update." Presented by C. Lehman, J. Jungers, C. Satyshur, and S. Williams.

A project overview was given to participants at the DNR Prairie Partnership meeting on June 26 in Spicer, MN. Presented by C. Lehman and S. Williams. Travel paid by a supplemental UMN IonE mini-grant.

A project overview paper was published in the Sun Grant proceedings, S. Williams, J. Jungers, K. Johnson, C, Satyshur, M. DonCarlos, R. Dunlap, T. Mielke, J. Schaffer, D. Tilman, D. Wyse, R. Moon, T. Arnold, and C. Lehman, 2012. "*Bioenergy from reserve prairies in Minnesota: Measuring harvest and monitoring wildlife.*" Proceedings from Sun Grant National Conference: Science for Biomass Feedstock Production and Utilization, Volume 2, Chapter 5, New Orleans. Online at http://sungrant.tennessee.edu/NR/rdonlyres/78D6D6DF-1610-4A79-A04C-A9278C860C0D/3742/Williams_Shelby.pdf).

A data management paper relevant to the project was published in the computing literature, C. Lehman and A. Keen, *"Using the Centinel Data Format to Decouple Data Creation from Data Processing in Scientific Programs."* International Conference on Scientific Computing, Proceedings, CSC'13. Journal link

http://world-comp.org/p2013/CSC.html , article link
http://world-comp.org/p2013/CSC7282.pdf .
This paper and one other it references describes the data archival system we developed for this and other projects, and used for this project. Publication funded by other sources.

Status as of *(January 1, 2014):*
Project concepts were presented by J. Jungers at the August 4-9 national meeting of the Ecological Society of America (ESA) at an Ignite session organized by S. Williams. Funded by a supplemental UMN EEB travel grant.

Wildlife and biomass aspects of the project were presented by S. Williams at the national ESA meeting in a special biomass session. Funded by a supplemental UMN EEB travel grant.

Design and status of the duck and pheasant nesting aspects of the project were presented by J. Jungers in a poster at the National ESA meeting. Funded by a supplemental UMN ConsBio student grant.

Results from the project are guiding a new effort, the Midwest Conservation Biomass Alliance (MBCA), combining government, academia, industry, and other organizations. C. Lehman and J. Jungers presented and participated at MCBA meetings in Wisconsin, Missouri, and Iowa, funded by supplemental UMN and external resources. In additional, a UMN IonE mini-grant has been secured for the spring meeting of this group in St. Paul, which will further highlight, promote, and perpetuate the goals of this project.

A second UMN IonE mini-grant has been secured to fund an initiative to connect UMN interests with DNR, TNC, USFWS, and other agencies concerning the broader goals of this project. A preliminary meeting at DNR Headquarters in Saint Paul on July 15, with C. Lehman, J. Jungers, and S. Williams kicked this off.

An overview of the entire project and specific data management practices used in the project were presented to the USGS at the Northern Prairie Wildlife Research Center in North Dakota on October 28 by C. Lehman. They may be interested in adopting or adapting this project's data management practices for broader use. Funded by supplemental UMN grants.

A summary of the preliminary results from plant, songbird, waterfowl, and small mammal surveys was presented by J. Jungers at the UMN Conservation Biology Seminar in December 2013. Attended by LCCMR staff.

A project paper summarizing theoretical ethanol potential based on bale core analysis, J. Jungers, J. Fargione, C. Sheaffer, D. Wyse, and C. Lehman, "Energy potential of biomass from conservation grasslands in Minnesota, USA" was published in the open-access journal *PLoS One.*

A project paper on game birds and biofuel harvest, J. Jungers, T. Arnold, C. Lehman, "*Effects of grassland biomass harvest on nesting ducks and pheasants*" was submitted and accepted by the *American Midland Naturalist* journal, pending revisions that have been submitted.

Final Report Summary (August 15, 2014):

Dissemination of results was maintained throughout the project and continues after its completion. In summary of what is detailed elsewhere in this report, project results were communicated through (1) nine presentations to groups such as UMN, USGS, and MN DNR, (2) two presentation to participating state land managers, (3) two press interviews, (4) three posters at the national conferences, (5) a session of six presentations at the Ecological Society of America's annual meeting, (6) one masters and one doctoral thesis completed, (7) six scientific papers published, in press, or in revision, with others in preparation, (8) contributions to two new emerging organizations, (9) project data communicated and reviewed with the Minnesota DNR to help in their development of best management practices for pollinators, (10) a report of best management practices for managing grasslands for wildlife under harvest being formatted for general publication, and (11) an ongoing project website,

In particular, supporting the above summary, (1) J. Jungers presented information at the Third Crop Producers Meetings in April 2012. (2) C. Satyshur presented information on the project to Cedar Creek personnel and interns in June 2012. (3) Project status was presented at a special DNR meeting for the purpose on March 26 2013 in Hutchinson MN, entitled Grassland Biomass Harvest Wildlife Impacts, Project Update. Presented by C. Lehman, J. Jungers, C. Satyshur, and S. Williams. (4) A project overview was given to participants at the DNR Prairie meeting on June 26, 2013 in Spicer, MN. Presented by C. Lehman and S. Williams. Travel paid by a supplemental UMN IonE mini-grant. (5) Project concepts were presented by J. Jungers at the August 4-9 national meeting of the Ecological Society of America (ESA) at an Ignite session of eight speakers organized by S. Williams. Funded by a supplemental UMN travel grant. (6) Wildlife and biomass aspects of the project were presented by S. Williams at the national ESA meeting in a special

biomass session in 2013. Funded by a supplemental UMN travel grant. (7) Design and status of the duck and pheasant nesting aspects of the project were presented by J. Jungers in a poster at the National ESA meeting in 2013. Funded by a supplemental UMN ConsBio student grant. (8) An overview of the entire project and specific data management practices used in the project were presented to the USGS at the Northern Prairie Wildlife Research Center in North Dakota on October 28 2013 by C. Lehman. They may be interested in adopting or adapting this projects data management practices for broader use. Funded by supplemental UMN grants. (9) A summary of the preliminary results from plant, songbird, waterfowl, and small mammal surveys was presented by J. Jungers at the UMN Conservation Biology Seminar in December 2013. Attended by LCCMR staff. (10) A poster summarizing project methods was presented at the 2012 Sun Grant Initiative Conference held in New Orleans, LA. The poster shared a title with the submitted paper: Bioenergy From Reserve Prairies in Minnesota: Methods for Measuring Harvest and Monitoring Wildlife. Funded by a supplemental UMN travel grant. (11) The waterfowl and pheasant nest surveys was presented in the form of a poster at The Wildlife Society Annual Conference in October 2012. The title of the poster was Managing conservation grasslands for bioenergy and wildlife: Measuring the effects of biomass harvest on waterfowl and pheasants. Copies of the posters are available on the project website. (12) Project design and logistics were presented by S. Williams in a poster at the Minnesota Society for Conservation Biology annual meeting on March 16 2013 at Dodge Nature Center in West Saint Paul, MN. (13) A project overview paper was published in the Sun Grant proceedings, S. Williams, J. Jungers, K. Johnson, C, Satyshur, M. DonCarlos, R. Dunlap, T. Mielke, J. Schaffer, D. Tilman, D. Wyse, R. Moon, T. Arnold, and C. Lehman, 2012. Bioenergy from reserve prairies in Minnesota: Measuring harvest and monitoring wildlife. Proceedings from Sun Grant National Conference: Science for Biomass Feedstock Production and Utilization, Volume 2, Chapter 5, New Orleans. (14) Two data management papers relevant to the project were published in the computing literature by C. Lehman, S. Williams and A. Keen, Adrienne in 2012 and 2013. These papers describe the Centinel data archival system we developed for this and other projects, and used for this project. (15) A project paper summarizing theoretical ethanol potential based on bale core analysis, J. Jungers, J. Fargione, C. Sheaffer, D. Wyse, and C. Lehman, Energy potential of biomass from conservation grasslands in Minnesota, USA was published in the open-access journal PLoS One in 2013 (16) A project paper on game birds and biofuel harvest, J. Jungers, T. Arnold, C. Lehman, Effects of grassland biomass harvest on nesting ducks and pheasants was submitted in 2013 and accepted by the American Midland Naturalist journal, pending revisions that have been submitted. (17) A project paper on calibration of sweep netting results by C. Lehman and C. Satyshur was submitted and is in revision. (18) Two papers describing the archival database system used in the project by C. Lehman, S. Williams, and A. Keen are published in the computing literature. (19) A

masters thesis by R. Dunlap was completed in 2014, entitled "Responses of Songbirds and Small Mammals to Harvests of Native Grasslands for Biofuels in Western Minnesota," T. Arnold advising. (20) A doctoral thesis by J. Jungers was completed in 2014, entitled "Managing Conservation Grasslands for Bioenergy and Wildlife," C. Lehman advising. (21) Project participants continue to coordinate with Minnesotan and outstate groups that are interested in pursuing grassland biomass as a renewable bioenergy. (22) Results from the project are guiding a new effort, the Midwest Conservation Biomass Alliance (MBCA), combining government, academia, industry, and other organizations. C. Lehman and J. Jungers presented and participated at MCBA meetings in Wisconsin, Missouri, Iowa, and Minnesota, funded by supplemental UMN and external resources. In additional, a UMN IonE mini-grant has been secured for the spring meeting of this group in St. Paul, which will further highlight, promote, and perpetuate the goals of this project. (23) Project participants secured an Institute on the Environment (IonE) mini-grant in 2013 being used to share and coordinate prairie and wetland research currently being done at the UMN with DNR, TNC, USFWS, and other agencies concerning the broader goals of the University and of of this project. Meetings within the University and at DNR Headquarters in Saint Paul have been conducted, with S. Williams organizing. (24) A second IonE mini-grant has been secured that funded a regional meeting of the MCBA in Minnesota, with J. Jungers organizing. (25) A third IonE mini-grant has been secured that will fund a training session for biological staff of University and agencies to learn identification of bees, in connection with ongoing pollinator aspects related to this project, with C. Satyshur organizing.

VI. Project Budget SUMMARY:

Funds will employ 9.3 FTE technicians, managers, students and interns to survey and analyze results of wildlife and bioenergy potential from harvested grasslands.

A. ENRTF Budget:

Budget Category	$ Amount	Explanation
Personnel:	$ 497,687	Two full-time research coordinators (3 FTE), project manager (0.2 FTE), 60 months of intern work (5.1 FTE), and a graduate student (1 FTE).
Professional/Technical Contracts:	$ 67,390	Contract to harvest experimental plots: MN Native Landscapes Inc.(Selected after competitive evaluation before a panel of forage experts. This company has proved reliable and will continue to be contracted, if quality persists,

		at equal or less cost to maintain consistency in treatments)
Equipment/Tools/Supplies:	**$ 7,188**	Field equipment: Replacement bamboo poles: $75/fifty, flagging tape, replacement small mammal traps: $15 each, microscope parts, bale coring supplies (drill battery: $60, parts for corer: $25, ATV maintenance), sorting supplies, materials for disposable insect pit-fall traps, blades for clippers: $132/six, sample bags: $0.10/bag, cleaning chemicals/tools for small mammal traps, safety equipment for field interns (gloves: $12/pair, hip-boots: $30/pair, safety glasses: $9/pair)
Travel Expenses in MN:	**$ 26,490**	Travel and lodging between St. Paul, Windom, and Morris MN-Based on standard University compensation rates. About 6500 miles/year at $0.51 / mile standard UM reimbursement rate.
Other:	**$ 1,245**	Chemical Analysis: Biomass-150 samples: Mineral analysis ($14/sample), Carbon/Nitrogen ($3/sample) and sugar analysis ($15/ sample). Soil- 195 samples: Carbon/Nitrogen ($4/sample), p.H. Organics, N,P,K ($20/sample)
TOTAL ENRTF BUDGET:	**$ 600,000**	

Explanation of Use of Classified Staff: NA

Explanation of Capital Expenditures Greater Than $3,500: NA

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation: 9.3

VII. PROJECT STRATEGY:

A. Project Partners: Minnesota DNR, USDA-NRCS, Minnesota citizens.

B. Project Impact and Long-term Strategy: Bioenergy production in Minnesota and around the globe has the potential to improve conditions for wildlife species, but if not properly done, could make conditions markedly worse. The broad consensus among wildlife experts is that diverse ecosystems, such as prairie grasslands or diverse woodlands, offer habitat that is superior for a wide spectrum of wildlife, in comparison with simplified habitats like cornfields or brome grass (Fargione et al., 2009). The present project focuses on such habitats of high biodiversity that can be useful for bioenergy and beneficial to wildlife. It is well understood that biodiversity is good for wildlife, and this project provides information of how management methods can best provide bioenergy production, wildlife protection, and other services to society. Practical question such as how much refuge in a bioenergy system must be maintained as wildlife cover have been part of the experimental examination, necessary biomass industries ramp up to a large scale.

C. Spending History:

Funding Source	M.L. 2008 or FY 2009	M.L. 2009 or FY 2010	M.L. 2010 or FY 2011	M.L 2011 or FY 2012	M.L. 2012 or FY 2013
ENRTF subd. 3(q)	$750,000				
National Fish and Wildlife Foundation 2009-2011	$300,000				
USDA-Conservation Innovation Grant 2009-2011		$500,000			
UMN-College of Biological Sciences 2010		$60,000			
UMN-Institute on the Environment PWP, SW					$2,000
UMN-Institute on the Environment MCBA, JJ					$2,000

UMN-Institute on the Environment Training, CS				$3,000
UMN-Office of Vice President of Research				$30,000

VIII. ACQUISITION/RESTORATION LIST:NA
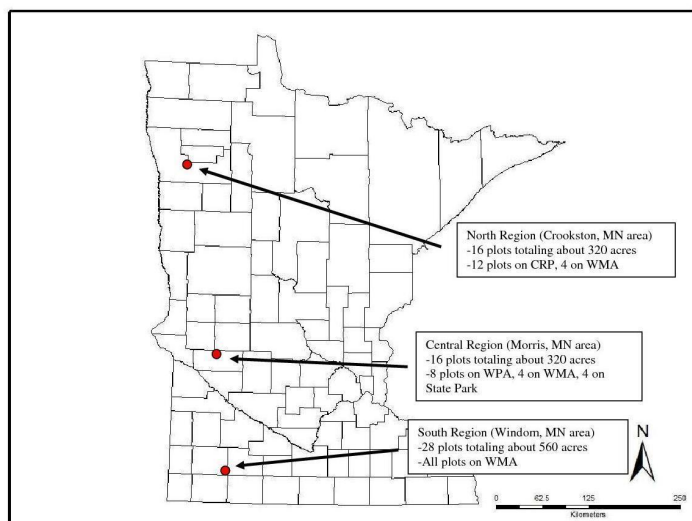
IX. MAP(S):

X. RESEARCH ADDENDUM:

XI.   REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than Jan 1, 2012, July 1, 2012, Jan 1, 2013, July 1, 2013, Jan 1, 2014. A final report and associated products will be submitted between June 30 and August 15, 2014 as requested by the LCCMR.

Attachment A: Budget Detail for M.L. 2011 (FY 2012-13) Environment and Natural Resources Trust Fund Projects

Project Title: Prairie management for wildlife and bioenergy: Phase II
Legal Citation: M.L. 2008, Chap. 367, Sec.[2], Subd. 3(q)
Project Manager: Clarence Lehman
M.L. 2011 (FY 2012-13) ENRTF Appropriation: $ 600,000
Project Length and Completion Date: June 31, 2014
Date of Update: Aug 15, 2014

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Balance	Activity 2 Budget	Amount Spent	Balance	Activity 3 Budget	Amount Spent	Balance	Activity 4 Budget	Amount Spent	Balance	Activity 5 Budget	Amount Spent	Balance	Total Budget	Total Balance
BUDGET ITEM																	
Personnel (Wages and Benefits)																	
Field Coordinator 1: 2 FTE. Organize and manage field sampling of birds, mammals, and vegetation. Oversee vegetative sampling and harvest operation. Help with data for reports. **Field Coordinator 2:** 1 FTE. Organize and manage field sampling of insects. Organize and manage lab sorting of insects, voucher collection, and data. **Project Manager:** 0.2 FTE to manage expenses, work programs, and field coordinators. **Interns:** 4 seasonal interns for 15 months to collect data. 5.1 FTE **Graduate Student:** 1 FTE. Manage data sets. Design floral surveys and harvest collection protocols. Author manuscripts of vegetation and harvest data.	190,500	190,500	0	38,900	38,900	0	185,100	185,100	0	17,500	17,500	0	65,687	65,687	0	497,687	0
Professional/Technical Contracts Contract for bee identification with an expert in the field, $1.50/specimen identified. Contract to harvest experimental plots: MN Native Landscapes Inc.(Selected after competitive evaluation before a panel of forage experts. This company has proved reliable and will continue to be contracted, if quality persists, at equal or less cost to maintain consistency in treatments)	0	0	0	0	0	0	390	390	0	67,000	67,000	0	0	0	0	67,390	0
Equipment/Tools/Supplies Field equipment: Replacement bamboo poles: $75/fifty, flagging tape, replacement small mammal traps: $15 each, microscope parts, bale coring supplies (drill battery: $60, parts for corer: $25, ATV maintenance), sorting supplies, materials for disposable insect pit-fall traps, blades for clippers: $132/six, sample bags: $0.10/bag, cleaning chemicals/tools for small mammal traps, safety equipment for field interns (gloves: $12/pair, hip-boots: $30/pair, safety glasses: $9/pair)	3,339	3,339	0	83	83	0	2,931	2,931	0	835	835	0	0	0	0	7,188	0
Travel expenses in Minnesota Travel and lodging between St. Paul, Windom, and Morris MN-Based on standard University compensation rates. About 6500 miles/year at $0.51 / mile standard UM reimbursement rate.	16,318	16,318	0	5,215	5,215	0	3,979	3,979	0	978	978	0	0	0	0	26,490	0
Other Statistical consulting at the University of Minnesota, $90/hour, for 2 hours. Chemical Analysis: Biomass-150 samples: Mineral analysis ($14/sample), Carbon/Nitrogen ($3/sample) and sugar analysis ($15/ sample). Soil- 195 samples: Carbon/Nitrogen ($4/sample), p.H. Organics, N,P,K ($20/sample)	0	0	0	0	0	0	1,065	1,065	0	0	0	0	180	180	0	1,245	0
COLUMN TOTAL	$210,157	$210,157	$0	$44,199	$44,199	$0	$193,450	$193,450	$0	$86,313	$86,313	$0	$65,867	$65,867	0	600,000	$0

Best Management
of
Minnesota Native Grasslands
for
Wildlife and Ecosystem Services

2014

(NOTE: This is a manuscript version of the document presently being prepared professionally for general publication.)

Table of Contents

Forward

This collection of best management practices grew from an intensive six-year project to learn how natural grasslands could be managed to provide services for society at the same time as they provided habitat for wildlife.

A main idea was to obtain bioenergy from grasses mowed late in the fall, after the plants had senesced and drawn nutrients from their leaves back into their roots, and after migratory wildlife had left the area. The project covered a thousand acres along the temperature gradient of western Minnesota. We were told that at the outset that the project was very ambitious, and heard that there was no way that harvesting bioenergy would also support wildlife. We learned otherwise.

With generous support from our state and federal funding agencies, we harvested, baled, and transported many thousands of tons of biomass from public and private grasslands while conducting scientific studies of the effects on wildlife, including mammals, birds, reptiles, amphibians, insects, and spiders. We also surveyed the plants that formed the habitat.

Most scientific studies look for significant effects of some treatment. But we were looking for the opposite. Here our treatments were harvesting or not, and harvesting in various patterns. We were looking for no significant effects---for methods of harvesting that on average would not harm wildlife. If such methods could be found, then simplified management and the possibility of income from harvested biomass could help increase the acreage of wildlife lands, and thereby could significantly help wildlife.

This booklet outlines what we learned and recommends practices that can be followed to manage grasslands in the Upper Midwest to promote the well-being of the resident wildlife. We offer these practices as our contribution the ongoing process of understanding how to live on our planet gracefully and harmoniously with the other species we share it with.

---CL, Saint Paul, Fall 2014

Project staff and students

Colleen Satyshur, Jacob Jungers, Joseph Schaffer, Kevin Johnson, Melissa DonCarlos, Robert Dunlap, Shelby Williams, Troy Milke

Project investigators

Clarence Lehman, David Tilman, Donald Wyse, Roger Moon, Todd Arnold

Funding agencies and supporting partners

Legislative Citizen Commision on Minnesota Resources: Environment and Natural Resources Trust Fund *(insert logo_ENRTF.jpg).*

National Fish and Wildlife Foundation *(insert logo_NFWF.gif)*

US Department of Agriculture: Natural Resource Conservation Service *(insert logo_NRCS.jpg)*

Minnesota Department of Natural Resources *(insert logo_DNR.gif)*

Long Term Ecological Research at Cedar Creek Ecosystem Science Reserve *(insert logo_LTER.jpg)*

The Nature Conservancy *(insert logo_NC.jpeg)*

College of Biological Sciences, College of Food, Agricultural and Natural Resource Sciences, University of Minnesota *(insert UMN logos here?)*

Case IH Corporation

For more information and contact information, see project website:
http://www.cbs.umn.edu/wildlife

Summary of Management Practices

The body of this document explains our six-year effort at learning how to manage grasslands for both biomass production and wildlife habitat, covering experimental results, literature references, and recommendations. The present section is a quick summary of the practical ideas we learned.

In broad outline, we found that harvesting for biomass can be done in ways that will not significantly affect overall wildlife populations nor plant species diversity. To help insure this, the following practices can be observed.

General
1. Consider harvesting and removing grassland biomass as an easier, less expensive, and less dangerous alternative to prescribed fire for managing wildlife habitat.
2. Consider planting new grassland habitat for bioenergy harvests as bioenergy markets develop.
3. Leave a portion of the area unharvested each year as wildlife refuges; this portion can rotate and occupy a different part of the area in different years.
4. Employ workers trained and familiar with land stewardship, or so train them.
5. Use shape of harvested areas to an advantage, since shape did not adversely affect wildlife populations in this study. For example, let unharvested areas follow the lay of the land for aesthetic benefits. However, consider contiguous unharvested patches rather than strips for some songbird species
6. Include simple adaptive-management experiments where possible as part of each project, to learn which practices applied are most effective.
7. In analyzing harvests, use before-after statistics to detect effects of harvests.
8. Continue examining publications for new information on best management practices as technology and science on the topic progresses.

Planning and Logistics
1. Consider future expected climate conditions when choosing sites for bioenergy and wildlife.
2. Study special regulations that may apply, such as federal or state restrictions on earliest harvesting date.
3. Plan harvests after the growing season but before the snow. In Minnesota this usually means October to November.
4. Plan some catch-up harvests in spring, for not all available acres will be able to be harvested each fall.
5. Cluster harvest areas together for efficient transport.
6. Recognize that wet or rocky fields will not be fully used each year. This will affect yield calculations but unharvested areas will be additional refuges good for wildlife.

Harvest

1. Harvest at most once per year to avoid the times of most active use by wildlife and when plants are growing and flowering.
2. Abandon ideas for long straight harvesting rows, but rather harvest to follow the lay of the land and to avoid obstacles.
3. Use four-wheel drive tractors wherever possible.
4. Maintain large tire-area-to-weight ratios to help on wet fields and soft soil.
5. Allow for repairing unexpected ruts other field damage in wet conditions.
6. Favor equipment that is easy to repair. Common brands and lower-tech systems can help.
7. The previous point notwithstanding, do consider innovative ways of improving the equipment to suit the conditions for bioenergy harvest.
8. Plan for incidental equipment repair on rocky or wet fields.
9. Consider disc-bine cutting heads with multiple small spinning heads for the type of cutting that will be encountered.
10. Minimize water content in the harvest by careful timing of cutting and baling.
11. Equip tractors with tines on the front and/or rear for moving bales.
12. Outfit transportation equipment with on-board air compressors and clean all equipment before leaving an area.
13. Use GPS devices on tractors to monitor area harvested and calculate yields.

Bioenergy potential

1. Consider biomass harvest as a management tool to reduce costs over other methods such as prescribed burning.
2. Consider periodic over-planting of harvest areas with warm season grasses and legume mixtures for best biofuel production.
3. In new plantings, include sufficient early successional warm-season grasses in the seed mix for better biomass in the first years.
4. In over-plantings or new plantings, consider adding trace quantities of seed for every native species suitable for the region and conditions, in case that particular species finds its ideal habitat there and can spread to form habitat and biofuel.

Ecological considerations

1. Situate sites near wetlands for ecological benefits to waterfowl and other wetland species.
2. Keep harvested areas under eighty contiguous acres each year to accommodate flying distances of the smallest typical pollinators.
3. Do not harvest all of an area in any year, since complete harvests can lead to population reductions of some species. Be aware that some birds may decrease in abundance with harvesting while others increase, as described in the body of this document. For greatest benefit to stem-nesting bees, keep refuges intact for three consecutive years.

4. Maintain bloom abundance to provide food for pollinators by avoiding harvesting until flowering is completed for the year. However, since each field is distinct, consider methodical photography to ascertain timing and document changes in blooms.
5. Preserve nesting sites for pollinators. Some bare ground will attract ground nesting bees if it is well drained. Maintaining shrubby field borders and rotating within-field refuge areas every three years will make stems available.
6. In monitoring harvesting projects, conduct surveys before any management starts to identify potential pre-existing bias between sites, for use in later analysis.
7. Include vegetation height in analysis of any sweep net data used for monitoring, as described in the body of this document.
8. Follow general guidelines for pollinator management and monitor the fast growing pollinator research sector. For example: (www.xerces.org.)

Section 1
Background and Introduction

(banner picture here: prairie clear sky.jpg
or harvest vs not fall Harvesting09.gif)

1.1) Background: New possibilities in renewable energy
Minnesota restored prairies reliably produce resources for bioenergy that largely go untapped. The figure below conceptualizes the benefits that a restored and managed prairie field may offer.

(Foley figure.png)
Above: Ecosystem services comparing agricultural row crops (A), wholly natural systems (B), and cropland with restored and mixed ecosystem services, such as in this project (C). Water quality, carbon sequestration, and wildlife habitat are highlighted here. Others include forest production, flood and drought mitigation, and air quality control (Foley et al. 2005).

Minnesota has large areas of non-agricultural land --- from native remnant prairies to abandoned cropland that resembles reconstructed prairie. Conservation of these lands is a value held by many, and in Minnesota over 1.5 million acres---almost three percent of the state---are held in CRP (Conservation Reserve Program) alone. WMAs (Wildlife Management Areas), WPAs (Waterfowl Production Areas), and the CRP are vehicles for rejuvenating and maintaining the quality of soil, water, and habitat. They follow the prairie-forest border diagonally through the state and are relatively evenly dispersed throughout the grasslands, with the Red River Valley's fertile cropland a notable exception.
(map MN map green dots.jpg)
Above: This map shows in green land held in these three reserve programs.

CRP is a program that takes land out of crop production for 10-15 years while appropriate grasses and cover are re-planted. Participants who enroll entire fields in the program account for half of participants. They commonly rely on incomes outside of farming, while the remaining participants report farming as their primary source of income and prefer conservation techniques that allow for continued crop production (Lambert et al. 2006).

Farm managers must maintain financial sustainability of their operation but non-monetary factors of land management are important as well. Protecting open space and helping future generations are examples of common motivations for undertaking conservation plans, while tax and monetary benefits provide incentives which make them possible (Lambert et al. 2006).

Large swaths of CRP-enrolled land were up for renewal in 2012 and were not renewed, in part because of higher commodity crop prices (Rashford et al 2011). Land managers seeking incentives to support conservation-minded plans therefore can use new ways to add value to those choices. Bioenergy derived from harvested, mixed prairie grasses could be part of the solution by offering a regional fuel source, environmental services, and rural economic development.

Conditions for wildlife in Minnesota could be enhanced by proper bioenergy practices using diverse native plant communities, especially in comparison with conditions that would prevail if management steps are not taken. Without natural or managed disturbance such as fire, grazing, or mechanical biomass removal, trees can invade Minnesota grasslands and make the habitat unsuitable for prairie wildlife.

The recommendations in this report are primarily based on results from a six year project and a consolidation of our experience and understanding, supplemented with references to various studies and information from other sources. We report novel information on many aspects of grassland harvest for renewable energy. However, we could not monitor all possible species and conditions. Therefore, this is a summary of results from one research project, and implications of biomass harvest for representative plant and wildlife species. We present this information with hopes that can be a valuable resource for land managers to consider throughout Minnesota, and in neighboring regions.

1.2) Introduction to the research that lead to this document

We designed a working-scale project in western Minnesota to identify management practices that will promote (1) wildlife conservation and habitat biodiversity and (2) crop production of low input, high diversity mixed prairie plants (Tilman et al 2006). This is part of a broad effort to sustain Minnesota resources while improving the rural economy and contributing to energy independence.

A major objective of the working-scale project was to identify biomass harvesting patterns that maintain wildlife populations by leaving distinct sizes and shapes of refuges within the grassland, but doing so while harvesting the greatest sustainable amount of biomass from the sites. This project covered over 1000 acres of previously restored grasslands, which were organized into 20-acres plots located near the communities of Windom, Morris, and Crookston, MN. This study used re-established prairies that were under an existing fire and weed management strategy. We measured bioenergy potential from grassland biomass harvested in late fall from each plot, and monitored wildlife throughout the project. Wildlife surveys included birds, small mammals, and insects. Full description of project methods may be found in Williams et al (2012)

(map of sites and harves trts fig.tif)
Above: Research plots were chosen throughout western Minnesota to sample a north-to-south spectrum of temperature. The inset is the experimental harvest patterns used in the project.

This project presented an innovative way to simultaneously promote renewable bioenergy and wildlife habitat---namely, make bioenergy lands into wildlife habitat. It leveraged the powers of federal, state, academic, and non-governmental agencies to solve an urgent need of global significance.

Please see our project website (www.cbs.umn.edu/wildlife) to access other reports and information, such as working protocols for bioenergy and wildlife evaluation that we established for this project. Read on for our thoughts and recommendations on how to manage grasslands for both wildlife habitat and bioenergy production.

Section 2) Planning & Logistics

(Banner picture: Bale and Shadow.JPG)

2.1) Field selection

An important factor in the feasibility and productivity of harvesting grassland biomass is initial field selection. When selecting fields, consider characteristics such as field history and topography.

Rocks and other debris, such as abandoned fence posts, fencing, and woodpiles, are obstacles that interfere with harvesting and may cause damage equipment. Soil moisture may be a factor in most fields, especially if they do not have drainage tiles. This is one challenge in selecting appropriate non-agricultural land, which is often marginal, and considerations of harvest equipment and timing, as well as total harvest acreage should take field moisture into account. We found a number of otherwise productive wet fields difficult to completely harvest, at times impractical to drive heavy equipment on, and needing more time for the material to dry for baling. A few of our plots in northern Minnesota were located on CRP fields that were relatively flat and contained saturated soils. A two-wheel drive tractor used during the first harvest frequently got stuck and dug ruts in wet fields. This delayed harvest and sometimes broke equipment. The ruts were repaired the following spring, which interfered with other farm tasks. However, moisture conditions during harvest time varied by years. Combined with drier conditions, equipment operators learned when to drive on certain fields that were susceptible to wet conditions, therefore harvest efficiency increased throughout the duration of the study. The proportion of acres harvested of those available for harvested by not accessed was 82% in 2009 but increased to 87% in 2012. Wetland area are best excluded when computing expected harvest acreage.

(wet NW2010-harvest-ruts.png)
Above: Wet conditions during the 2010 harvest in the northwest study region. Wet ground and harvesting equipment could result in muddy ruts and lost time.

Detailed maps that include soil and elevation parameters, wetland delineation, and land cover from satellite images are becoming increasingly available (e.g. Gu et al. 2012), and LiDAR will further enhance maps and help optimize placement of bioenergy fields.

2.2) Site distribution

(map areal of plots harvest actual Timber 2011.jpg)
Above: Plots within one region are chosen to minimize travel between plots. Also shows actual area harvested from each plot in 2011.

Land must be chosen carefully on a regional scale as well as local. Generally, prairie grasslands in Minnesota are located in a wedge from the northwestern corner to much of the southwestern corner, thus these areas are best suited for bioenergy grasslands. See biomass yield section for comparison of yields between regions of the state. Factors for predicting high bioenergy yields include climatic indicators, previous performance in crop production, and clustering. The proximity of appropriate lands to each other is important when moving harvest equipment between fields and transporting the biomass to where it will eventually be consumed for energy.

Thus land managers with numerous harvest locations should consider the geographical distribution of the fields. On a large-scale basis, spreading sites out over distances greater than 4 or 5 miles requires additional planning and equipment. Also, the distribution of harvested plots within fields must considered. As plots get further away from roads into fields, removing the bales from the plots becomes more difficult and expensive.

To minimize transportation of invasive or unwanted plant species between fields, equipment should be cleaned between fields. See Equipment section for more details.

2.3) Growing degree day implications

**Use highres version of this picture**
GDD is a measure of air temperature and is a primary predictor of the rate or timing of plant development. However the quantity of the biomass produced is influenced by soil quality, precipitation events, and nutrients, especially nitrogen. These three factors result in a difference in biomass yield between the two northern study sites and the southwest site. GDD is an indicator of plant development (Frank and Hofmann, 1989; Frank and Ries, 1990) and is one of the predictors of harvest for these prairie plants. This study is not able to correlate GDDs with the optimum harvest schedule because land manager restrictions forbid harvest at potentially peak opportunities. This confounding factor prevents using GDD as a predictor of harvest timing in this study.

Certain regions of Minnesota will respond differently because of differences in growing seasons. The northern portion of the state generally receives fewer growing-degree days (GDDs) in a

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season, thus less energy for biomass production---although this may be changing as number of growing degree days increases throughout Minnesota. The figure shows the movement of sample growing degree days over the previous century (www.cbs.umn.edu/climatetracker). For instance, the yellow track shows that the number of GDDs experienced west of the Twin Cities, MN in 1900 is now experienced further north, near Wadena, MN.   The east-to-west movement is indicative of precipitation change -- a drying trend in the 1930s moved the track east, and has since become wetter, thus moving west, ending generally west of the 1900 point. GDDs have been hovering since 1966.

2.4) Modelling the future

Minnesota has the potential to produce biofuels decades into the future, although the specific growing areas may be on the move. Both panels of the figure on the left highlight areas that should theoretically produce high yields on bioenergy based on climatic indicators, previous performance in crop production, and clustering. The lighter areas on the first panel indicate that the tri-state area of North Dakota, South Dakota, and Minnesota could potentially generate high yields of biomass. The second panel of the figure projects climate conditions 30 years in the future, where increased areas of northwest and southwest Minnesota are included in potential biomass production areas. Planning for future biomass production should take possible climate changes into account.
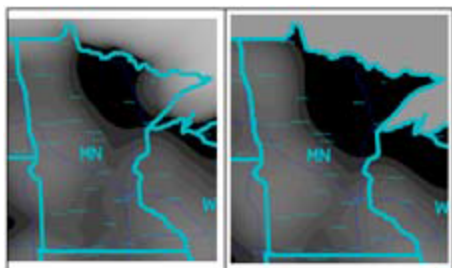
Insert high-res images

Section 3) Harvest

3.1) Harvest timing

Our harvests were initiated after the first hard frost, usually in mid- to late-October. At this time, plants were past the senescence stage. Waiting until post-senescence reduces biomass moisture content and allows the perennial plants to transfer nutrients from shoots to roots, to be used in subsequent growing seasons. To minimize impact on wildlife, harvest was delayed on DNR experimental plots until November. Consequently, there was a short window of time to harvest before the first snowfall. With variable Minnesota weather, snow can come as early as late October and terminate harvest efforts for the year. Fall weather in the harvested region can also be tricky for proper drying of biomass. Short days, cool temperatures, and snow or rain require careful planning for harvest in late autumn/early winter.

We could begin harvesting CRP plots in the northwest part of the state in mid-October, followed by selected plots in west-central areas one to two weeks later. We began harvest in southwest areas the first week of November and ended in early December. Wet conditions prevented complete fall harvests in the northwest plots in one year and harvest was completed in April of the following spring. Early heavy snow prevented a complete harvest in the southwest in another year and part of that were harvested in May of the following year.

(snow on harvest 2.JPG, birds chick.png, wet flooded.jpg)
Above: Timing the harvest to avoid wildlife nesting periods and adverse weather can be a challenge in the upper midwest.

3.2) Harvest equipment

3.2.1) Cutting
(eqp swath type cutter head.png)
Left: A disc bine cutting head consists of multiple small spinning heads and works well for the type of cutting encountered in this project.

We used standard haying and baling equipment for harvesting grassland biomass. This project served as a "proof-of-concept" demonstration that grassland biomass can harvested with common equipment. A discbine cutting head was used to harvest biomass. This consists of multiple small spinning heads that hold cutting blades, as opposed to a sickle-type cutter. After the discs cut the material, it is run through a roller-conditioner to form the windrow. The discbine head works well for cutting the various grasses and forbs encountered on the project, and allows for cutting wet or dry material. It also allows a faster ground speed if the landscape permits. The main disadvantage to running a disc-bine header is that it is expensive and time consuming to repair if damaged during harvest, as by unseen rocks or other debris. We frequently

encountered rocks and obstructions on the marginal lands where our experimental plots were located.

In the first year, the disc bine head was mounted on a self-propelled swather. This was an effective setup, but had some qualities that made it suboptimal for this kind of harvest. It is a difficult machine to load for transport and requires a special trailer due to its wide wheel base. Because it is two-wheel-drive it does not handle wet ground well and can get stuck. It required a significant amount of time to move between harvest sites.

In subsequent years, the discbine cutter was mounted on a four-wheel drive tractor. This configuration is recommended because it solved many of the problems associated with the self-propelled swather---it is easily loaded, can be driven on roads, and does not get stuck as easily. It was also convenient to have a tractor available instead of the swather since it is more versatile.

(SWATHER: eqp swather 09.jpg, TRACTOR: eqp tractor with cutter.JPG
Above: Disc bine head on swather (left) and tractor (right).

3.2.2) Raking biomass

A high capacity wheel V rake was used to merge two windrows of cut biomass into one windrow and to turn the material to expedite drying. This type of rake worked well when biomass was too wet for immediate baling. Raking two windrows together sped up the baling process and reduced the number of passes the baler had to make on the field, thus reducing rutting and fuel use.

(eqp tractor with rake.JPG)
Above: A tractor raking biomass.

3.2.3) Baling biomass

(bales square and round.JPG)
Above: Both round and square bales were used. Below, Round baler (left) and square baler(right)
(eqp baler-round backlit close.JPG, eqp baler-square.jpg)

We tested two primary baling systems, round and square balers. In the first year we used a large square baler which produced 4'x 4'x 8' bales that were tied with twine. These bales weighed about 1,000 pounds at 15% moisture. Advantages of large square bales include efficient stacking, hauling, and transport compared to round bales. Also, there is no danger of the bales rolling on slopes. The square baler was efficient to operate and handled most of the material and conditions. The one we used was a research-level bailer being evaluated for production and had three issues that could be improved. One was that is was relatively heavy in relationship to its tire size, compared with a conventional round baler. Also it was difficult to load onto a semi-trailer for transport, and it had to be protected from rain. These issues may be specific to our

equipment and all can be overcome by through mechanical engineering, and square balers may be the recommended choice for the future.

In subsequent years we switched to a round baler for comparison and for utility. It produced a 4' wide by 6' high bale wrapped with a plastic net, that also weighed about 1,000 pounds. This bale size is appropriate because of the relative ease hauling them by truck to their final destinations. However, attention is necessary so that the round bales do not roll on steeper slopes. The round, net-wrapped bales can be left out in the elements without having to be covered for up to three years or more without losing significant quantity or quality of biomass. This introduces the important possibility of storing the bales in the field where land costs are low, giving farmers more control over their commodity, and allows for more time to be spent on the harvest.

3.2.4) Material handling
The best method we found for transporting bales from the field uses tractors with front and/or rear mounted bale spikes. Properly equipped, a single tractor can remove up to six bales from the field on each trip. This speeds up the process and minimizes traffic on the field. Bales can be placed in a staging area near the field for future transport or loaded directly onto trucks.

(eqp tractor with bale spikes loaded.JPG)
Above: tractor with bale spikes hauling bales.

3.2.5) Summary of equipment used in this project

Equipment type	Equipment details	Picture	When Used	Advantages	Disadvantages
Cutters	disc bine head	*(eqp swath type cutter head.png)*	all years, on different tractors	Works well for cutting grass and forbs, wet or dry.	As with all equipment, it can have expensive breakdowns on rocks. Check for local part availability
	two-wheel-drive, self-propelled, swather	*eqp swather 09.jpg*	2009, carried disc bine	Designed for biomass harvest specifically, can have high ground speed.	Difficult to load on a trailer, requires special trailer to fit wheel width. 2wd gets stuck in wet ground
	discbine mounted on a	*eqp tractor with cutter on trailer.jpg*	2010 and on	easily loaded, does not get stuck	Check fuel usage. May operate at

	four-wheel drive tractor			as quick as 2wd above. Can drive on roads. Tractor can be used for other purposes	slower speeds than 2wd above.
Racking	high capacity wheel V rake	*eqp tractor with rake.JPG*		Good for combining 2 windrows to 1 and flipping biomass for quicker drying	Adds an additional pass over field, requiring additional fuel and time.
Bailers	large square baler produced 4'x 4' x 8' twine-tied bales; 1,000 pounds at 15% moisture	*eqp baler-square.jpg*	2009 onward	Stack and transport better than round bales. Baler was efficient and handled most materials	Weight:tire size rather large, suboptimal in wet conditions. difficult to load on semi. Must be protected from rain.
	round baler produced a 4' x 6' bale wrapped with plastic net	*eqp baler-round backlit close.JPG,*	2010 onward	Round bales can be left on field for up to 3 years	Bales may roll. Harder to load on truck and transport
Material handling	tractor with rear and front mounted bale spikes	*eqp tractor with bale spikes loaded.JPG*	all	6 bales/trip in field	no major disadvantages to note

Used a different cutter also in 2012, have to look up it's name don't know much about how it worked.

3.3) Minimizing spread of invasive and unwanted plants
(eqp cleaning tractor.JPG, includes 2 of Joe Schaeffers employees)
Plots to be harvested can be located some distance apart and managed by different agencies or organizations. When moving equipment from site to site it is critical to maintain equipment in a sanitary condition, to avoid the transport of unwanted plant propagules such as weed seeds. To

accomplish this, transportation equipment should be outfitted with on-board air compressors and all equipment cleaned before leaving an area. In addition to using an air compressor to blow off vegetation, was necessary to use a paint scraper to scrape off caked biomass from our equipment. This process took about half an hour each time and should be factored into harvest timing. Individual landowners will see this practice is in their best interest as well, since it protects their sites from unwanted settlers.

3.4) Personnel

Having people trained and familiar with land stewardship and harvesting equipment operation is of utmost importance. In our project, the variability of sites and differences among landowners required that harvesting personnel know what is acceptable and what is not for each field, there being more to the harvest than just getting biomass from the field. Integrity of the prairie ecosystem that supports the biomass, of the wildlife that occupy it, of the services to society it provides, and the ethics in managing it are necessary to ensure sustainable opportunities in grassland biomass harvest.

3.5) GIS and tracking harvests

Because land allocated to reserve programs and wildlife conservation tends to be marginal for agriculture, it may not be possible to harvest in long straight rows, or even to harvest all of the area. For that reason, we found it useful to track our harvest with an on-board GPS (Geographic Position Sensor). Many farm operations already use this type of equipment, and if not, handheld versions can be a good investment at relatively low costs. Recording the harvest area gave a better idea of potential bioenergy production for each place.   All calculation of yield and harvested area in our study were derived from actual area taken from the GPS, which gave a more accurate output. Similar hand-held GPS devices were used in wildlife surveys, including when we walked transects for bird surveys.

Section 4) Bioenergy potential

(banner picture: Bales on semi square side.JPG…. or: Bales on semi square front.JPG, bales on semi round loading.JPG, bales on semi round diagonal.JPG)

The amount of energy that can be produced from a grassland is based on two characteristics, biomass quantity and biomass quality. Biomass quantity is often referred to as 'biomass yield' --- the amount of biomass that can be harvested in a given area. Biomass quality refers to the amount of energy that can be produced from one unit of biomass, such as from one ton. We measured both characteristics to learn how they will vary across Minnesota.

4.1) Biomass yield.

We assessed biomass yield northwest, west central, and southwest Minnesota. This assessment was unusual in that biomass yields were derived from production-scale harvests. Once a plot was harvested and baled, the number of bales left in the field were counted. This number was multiplied by the average weight of a bale to estimate the total amount of biomass harvested from the plot. Total plot biomass was then divided by the area that was cut to determine biomass yield.

(bales in field 1.JPG)
Above: bales produced per plot were counted

It is important to minimize water content in the bales, because (1) biomass that is wet is prone to decomposition, which decreases the energy potential of the biomass, (2) trucking wet biomass to conversion facilities is limited by weight, so damper biomass means that not as much can be moved per trip, (3) and decomposing biomass can be a fire hazard and has been known smoulder and even combust.

We measured biomass moisture content by collecting and weighing samples of cut biomass from bales, drying the same sample to remove all water, and then re-weighing the same samples to determine how much of the initial weight was actually dry biomass. This is called "dry matter determination". Probes are available to producers that give immediate estimates of moisture content.

The timing of harvest and duration of windrow drying prior to baling affect final bale moisture content. In the south region, we consistently lowered average moisture content each year of the study, from 23% in the first year to only 10% in the last. Biomass yields reported here are on a dry-matter basis, which means that the values have been adjusted for 0% moisture content. This is common practice so that values represent actual biomass weights and do not include variability added due to moisture content.

Table X. Average biomass yield (tons per acre) from plots in three study regions in western MN from 2009 to 2012.

Region	2009	2010	2011	2012	Average

South	1.2	1.1	1.8	1.3	1.4
Central	0.7	0.7	1.0	-[1]	0.8
North	0.6	0.7	-	-	0.7

[1] Plots in this location were not harvested.

Average biomass yields in the southwest were about 60% greater than yields in the west-central and northwest (*Jungers et al 2013*). There was no difference in yields between the west-central and northwest regions. We found that fields with high biomass yields also had a greater abundance and cover of warm season grasses (C4 grasses), such as switchgrass (*Panicum virgatum L.*), big bluestem (*Andropogon gerardii* Vitman), and indiangrass (*Sorghastrum nutans (L.) Nash*). This correlation is not surprising because warm season grasses are 40% more efficient in accumulating carbon (Beadle and Long, 1985), require half of the water (Long et al. 1990), and use nitrogen more efficiently (Brown 1985) than cool season grasses (C3 grasses). Identifying fields with abundant stands of warm season grasses, or overplanting them with warm season grasses, could be a good recommended method for high biomass yield.

Other related studies have shown that a significant legume population also contributes to biomass yield (Fornara and Tilman, 2008).

4.2) Subsequent harvests.
A concern among land managers is that repeated biomass harvest could lead to decreasing yields during future harvests. We studied this by comparing the change in biomass yield in fields that were harvested every year with fields that were rested a year between harvests. Our results suggest that biomass yields do not decline with annual harvest for up to three years. It has been long known that biomass yield does not decline with annual burning (Collins et al 1998).

4.3) Biomass quality.
A standard metric for the quality of biomass for energy conversion is the higher heating value (HHV) of the material. HHV is used for comparing other fuel sources and is not based on conversion method (e.g. ethanol, gasification, combustion). We determine HHV by bomb calorimetry. There was little variation in HHV in our samples from the various regions, and the average value was 17.3 megajoules per kilogram (MJ/kg) and a standard deviation of 0.19)

(bale-coring.jpg, bale with horsetail.JPG)
Above: bale coring (right) and a bale with significant percentage of horsetail (left)

We also predicted how much ethanol could be produced by measuring the concentration of fermentable sugars within the biomass. The analysis estimates ethanol production if all available fermentable sugars are consumed. The result is a metric called "theoretical ethanol potential", which is measured in liters per metric tonne (L/Mg). (Multiplying these values by 0.23 will convert the values to gallons per short ton.)

The figure below shows the average ethanol potential from biomass harvested in the south, central, and north locations during the first three years of the project (Jungers et al., 2013). Averaged across all sites and years, ethanol potential was about 450 L/Mg. Ethanol potential was greater in biomass harvested from the south, which is also likely related to the higher abundance and cover of warm season grasses there. That could be remedies in the north and central by emphasizing them in plantings. In general, however, there was little variation in ethanol potential among different regions.
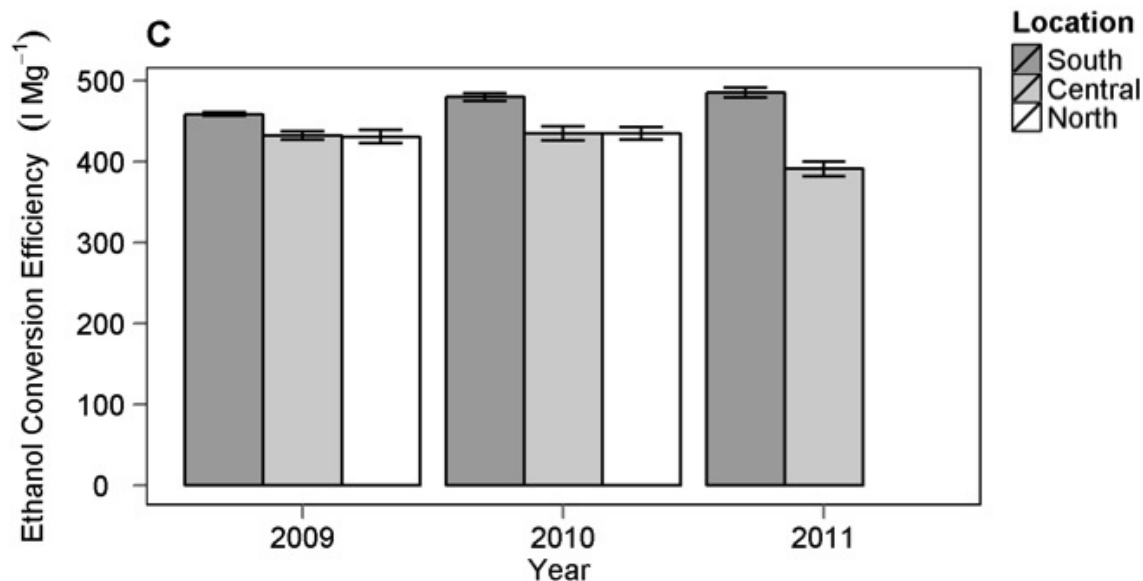
With biomass yield and ethanol potential being greater in the south, it makes sense to consider that location first as a potential location for a renewable energy production facility. If we combine biomass yield and ethanol potential, we can predict how much energy can be produced per unit of land; or land ethanol yield (gallons of ethanol per acre). Approximately 150 gallons of ethanol could be produced per acre of conservation grasslands in the southwestern region of Minnesota. If we can harvest biomass from half of the available acres in conservation grasslands within an area that's profitable for biomass transportation, there is enough biomass to produce nearly 20 million gallons of cellulosic ethanol. That is enough biomass to support a production-scale cellulosic ethanol facility.

Section 5) Ecological implications

5.1) Effects of biomass harvest on plants

Historically, natural disturbances such as fire and grazing maintained plant species composition in prairies. Without disturbance, woody plants can invade and outcompete prairie plants, which shifts the ecosystem to something other than prairie. Land managers prescribe fire, grazing, and mowing as a disturbance to maintain the prairie plant community. One goal of this project was to track how biomass harvest influenced the plant community --- for example, would harvest change plant diversity, the abundance of dominant plant species, and the abundance of non-native species.

Our results show no effects of biomass harvest on plant species composition, diversity or the relative abundance of non-native and noxious species following three years of harvest. Some changes were observed through time at each location, but these changes occurred in both harvested and unharvested plots (Jungers et al., in press). This is a good result for managers because it means that they can interrupt their normal disturbance schedule to harvest biomass from conservation grasslands without affecting the plant community. This is also a sign that the equipment cleaning protocols we implemented worked, and that non-native species and noxious weeds did not increase in plots where biomass harvesting equipment was used. It should be noted that landowners were allowed to continue their normal weed control measures, such as spot spraying thistle.

5.2) Small mammals

Trends suggest that harvesting can increase overall abundance of some small mammals if equal areas are left as a refuge. Others are reduced. Small mammals occupy a central place in the prairie food web, so these trends can be expected to have an effect up and down through the ecosystem.

Small mammals were surveyed in each region using grids of Sherman live traps. The mammals were briefly processed, marked for recapture, then released at the point of capture. Results and analyses were reported in Dunlap (2014). Almost 4500 small mammals were captured during the study, and over half were *Microtus* species. Approximately one-quarter of captures were northern short-tailed shrews (*Blarina brevicauda*), followed by fewer deer mice (*Peromyscus* spp.), short-tailed weasels (*Mustela erminea*), thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), and masked shrews (*Sorex cinereus*). We also caught fewer than ten northern grasshopper mice (*Onychomys leucogaster*), plains pocket mouse (*Perognathus flavescens*), western harvest mouse (*Reithrodontomys megalotis*), and house mouse (*Mus musculus*).

Microtus species, which may include the prairie vole (*M. ochrogaster*) and the meadow vole (*M. pennsylvanicus*) were most abundant in the northwest and least abundant in the southwest. The *Microtus* genus were the only small mammals with sufficient captures for analysis which declined with increased harvest percentage. Literature in Dunlap (2014) notes that removing grassland vegetation is generally correlated with fewer *Microtus*, thus allowing unharvested vegetation to stand in fields is supportive of greater number of *Microtus* in grasslands harvested for bioenergy. No edge effect of harvested fields was found in our study, so the pattern of harvest does not appear to affect *Microtus* species. Thus, to manage for the *Microtus* genus, harvesting that include up to 75% of standing vegetation, in either strip or block pattern, are unlikely to cause significant declines in populations. However, full harvest led to population reductions in our study. Dunlap (2014) recommends that land managers work with biofuel harvesters to retain unharvested sanctuaries where small mammals such as *Microtus* species can persist despite biomass harvest.

In contrast to *Microtus* species, the northern short-tailed shrew remained relatively constant over the course of the study and deer mice seemed to increase in abundance with increased vegetation harvest. Short tailed shrews were captured with less frequency in higher percent harvest plots. Other species of small mammals on our study plots seemed to be unaffected by amount or pattern of biomass harvest (Dunlap, 2014).

5.3) Reptiles & amphibians
(herp array.jpg, herp sal2.png, herp toad.png)

We surveyed reptiles and amphibians (herpetofauna) in four consecutive years, using catch-and-release trap arrangements that incorporated funnel and pit-fall live traps . We tested techniques for capturing herpetofauna in Minnesota grasslands and developed a method for a fenced array that works well. Frogs, toads, and garter snakes made up over 90% of species identified, with salamanders, skinks, turtles, and and other herpetofauna constituting the remainder. Approximately 2000 individual reptiles and amphibians were identified, measured, and weighed in the process. Preliminary analysis reveals an average of 28.3 individuals identified on average per array per year in the non-harvested plots and 28.7 in the fully harvested plots---not significantly different. As of this writing, effects of harvesting are being analyzed with continuation funds supplied by the University of Minnesota and will be made available in updates to this document, but the preliminary results show no adverse effects of harvesting on herpetofauna.

Proportional abundance of reptiles and amphibians identified in the southwest study area.

Rana pipiens	Northern Leopard Frog	55%
Bufo americanus	American Toad	19%
Thamnophis radix	Plains Garter Snake	11%
Thamnophis sirtalis	Common Garter Snake	6%

Ambystoma tigrinum	Tiger Salamander	4%
Pseudocris triseriata	Western Chorus Frog	3%
Eumeces septentrionalis	Prairie Skink	1%
Bufo cognatus	Great Plains Toad	<1%
Chrysemys picta	Painted Turtle	<1%

5.4) Waterfowl and Pheasants
(birds nest.png, birds ducklings.jpg)

Some conservation grasslands, such as state owned WMAs (Wildlife Management Areas) and federally owned WPAs (Waterfowl Production Areas), include the management objective of sustaining populations of waterfowl and game birds for hunting. Game bird hunting is an important economic activity for many rural communities, and hunting license fees help pay for the acquisition and management of conservation grasslands. Therefore, it is important to understand how waterfowl and pheasants respond to biomass harvest.

We focused on monitoring the nesting biology of waterfowl, since they utilize upland grasslands as nesting sites near wetlands. We searched for nests using the chain drag method (Klett et al. 1986) and monitored nest development. During sampling, pheasant nests were also found and included in the analysis. Nest searches and monitoring was conducted in spring prior to biomass harvest in the first and year following first harvest in the subsequent year, in southwestern Minnesota.

Biomass harvest can affect nesting biology in at least two way, (1) if harvested areas are less suitable for nesting, nest density would decrease, and (2) If harvested areas are less suitable for nesting but waterfowl still nest there, nest predation could increase. We found that the probability of a nest surviving is the same for nests initiated in harvested areas and unharvested areas. Nest predators in the region of this study were not more or less likely to find and consume nests in harvested areas. However, waterfowl preferred to nest in the unharvested regions. Nest density was lower in the harvested regions. It is important to note that there was a similar number of nests initiated prior to the first harvest and following harvest, but that the nests were more concentrated in the unharvested regions.

We found more nests in plots with taller grass and also in those plots that had more abundant wetlands within a 500 m radius from the plot center (Jungers et al., in review). Waterfowl preferred nesting in upland grassland sites that were near wetlands, and these nests had a better chance at surviving compared to those further from wetlands. Therefore, we recommend that some regions of upland habitat within conservation grasslands be left standing if managed for bioenergy, and that these unharvested regions be located near wetlands if possible. This selection strategy should not only help maintain waterfowl populations during harvest, but may also limit harvest inefficiencies due to wet ground.

5.5) Songbirds

(Birds path_2-4_Color KJ 1dec10.jpg)
Above: the path walked for songbird point count surveys)

Two important measures of harvest effects are total songbird abundance and number of species recorded. We surveyed plots from mid-May to late June each year to more or less coincide with the breeding period of most grassland birds. The first year of surveys in 2009 represented the pre-harvest conditions of the plots, and all years after represent the post-harvest conditions. We used area-based search methods to survey birds in our plots. We began surveys 30 minutes after sunrise, and finished by noon at the latest. We conducted two rounds of our southern plots and one round each of our west central and northwest plots. The second round in the southern plots was conducted because some species such as the dickcissel (*Spiza americana*) arrive later in the spring. Two observers independently surveyed each plot per round, and each plot was only surveyed once each round by the same observer.

We observed a total of 57 species in our plots over the five years of our study. Of these species, we identified 11 that provided us with enough data to analyze abundances. These were: sedge wren (*Cistothorus platensis*), common yellowthroat (*Geothlypis trichas*), clay-colored sparrow (*Spizella pallida*), savannah sparrow (*Passerculus sandwichensis*), grasshopper sparrow (*Ammodramus savannarum*), Le Conte's sparrow (*Ammodramus leconteii*), swamp sparrow (*Melospiza georgiana*), dickcissel (*Spiza americana*), bobolink (*Dolichonyx oryzivorus*), common grackle (*Quiscalus quiscula*), red-winged blackbird (*Agelaius phoeniceus*). Additionally, two of our common species—sedge wren and grasshopper sparrow—are designated as Partners in Flight conservation priority species, and thus the data we collected on them was of particular importance as these species have shown significant declines throughout their ranges and may be at risk of further declines without other conservation initiatives in place.

Analysis and results are presented in Dunlap (2014). In summary we found that four species—sedge wren, common yellowthroat, clay-colored sparrow, and swamp sparrow—showed declines in abundance following harvesting. Two species —grasshopper sparrow, and common grackle—actually increased in abundance following harvesting. Additionally, we found that species richness declined significantly with increasing percent of plot harvested but that the difference was very slight, 2 species or less (Dunlap, 2014). Species richness measures total number of species, without taking into account if some species are leaving, but are replaced by new species. Results became more pronounced as the number of years of fall harvest increased. Some of these results are in accord with other research on grassland birds and haying, although haying often occurs during the summer. Some birds such as grasshopper sparrows seems to prefer shorter vegetation, while others such as sedge wren and prefer taller denser vegetations.

Our results suggest that overall songbird community is amenable to fall biomass harvest as a management technique. Especially if biomass harvest provides incentives to keep grasslands

from being converted to crop production. However, our study shows the importance of having initial data on songbird occupancy and of long term monitoring. If funds are limited, surveying the year following the first harvest may be omitted. In our study summer after the first harvest showed less effect of harvest than later years, possibly due to territory fidelity of birds returning from the previous year. Also if management goals prioritize any of the species negatively affected by harvest in our study, leaving at least 50 percent unharvested on 20 acre parcels is advisable. If goals include species positively affected by harvest, then higher percent harvests on 20 acre parcels may help. There was a slight preference in species richness for block over strip harvest, but not in any other group. Therefore harvest shape could be adapted to harvest logistics and landscape features, while generally leaving unharvested areas in contiguous chunks where possible.

5.6) Predator cameras
We had hoped for more information from automatic field cameras, but did not get it. There were few sightings on predator cameras, too few to be analysed statistically. We believe the concept is still sound, but either more cameras or more sampling dates should be used. Other survey methods may be more effective.

5.7) Deer surveys
Pellet count surveys were conducted in February of the second year to determine use of the bioenergy plots by deer. Although we found a trace number of deer pellets in the survey, deer did not appear to be using plots, probably because of deep snow. Deer use of bioenergy plots may need to be assessed by observation from blinds or other methods.

5.8) Arthropods & Pollinators
(Insects-sweeps-amanda.JPG)
There were three main components to our arthropod research, absolute quantitative calibration for sweepnets, arthropod biomass measured by sweepnetting, and bee abundance measured by bee bowls.

5.8.1) Quantitative sampling
(Insects Quist 2010 1.jpg)
Above: QuIST is a new technique to assess insect sweep net collections, using a small "tent" and vacuum equipment.

Because fall harvest could affect vegetation height, we designed a new process, called "Quantitative Insect Sampling Technique" (QuIST), for assessing the comprehensiveness and efficiency of sweep net collection. QuIST is an enclosed screened "tent" in which we work to capture all insects in its interior with clipping and vacuum equipment. We obtained enough information on six taxa to calculate their capture efficiency at different vegetation heights. Coleoptera (beetles) and Hymenoptera (ants wasps and bees) both appear to be captured at the same rate, no matter what the vegetation height. Vegetation height did influence capture of the other four groups Diptera (flies) Araneae (spiders), Hemiptera (true bugs, eg. aphids, stink

bugs), and larvae (designated as young insects which are in the form of a caterpillar, or "grub"). These groups were captured at a increasing efficiency until vegetation height reached the diameter of the sweepnet. At this point the trend reversed and the arthropods were captured at a diminishing rate as vegetation height increased above the diameter of the net. Generally speaking, one bug in a net in tall vegetation represented a larger total population than one bug in a net from shorter vegetation.

Absolute quantitative sampling such as we did is comprehensive but labor intensive, and we do not recommend it on a regular basis. Instead, it should also be ascertained if a management practice or experimental treatment in question affects height of the vegetation. If it does then vegetation height should be measured along with any methodical sweepneting and height should be included in the analysis. Our formulae for calibrating sweepnet catch are being finalized and will be released in upcoming publication (Satyshur et al, in prep). In the mean time this table of approximate calibration amounts can be used.

Table 4: Unpublished data: Quick reference table displaying a correction coefficient which can be multiplied by number of the appropriate arthropod group in a sweepnet sample to obtain an estimate of total arthropods based on height and our best fit models. Correction coefficient is the inverse of capture efficiency.

Vegetation height(cm)	Araneae	Coleoptera	Diptera	Hemiptera	Hymenoptera	Larvae
10	141.3	72.5	17.7	71.3	116.3	110.7
20	112.6	72.5	14.1	56.8	116.3	88.2
30	106.6	72.5	13.3	53.8	116.3	83.5
40	119.7	72.5	15	60.4	116.3	93.7
50	149.6	72.5	18.7	75.5	116.3	117.2
60	179.5	72.5	22.4	90.6	116.3	140.6
70	209.4	72.5	26.2	105.7	116.3	164
80	239.3	72.5	29.9	120.7	116.3	187.4
90	269.2	72.5	33.7	135.8	116.3	210.9
100	299.2	72.5	37.4	150.9	116.3	234.3

110	329.1	72.5	41.1	166	116.3	257.7
120	359	72.5	44.9	181.1	116.3	281.2
130	388.9	72.5	48.6	196.2	116.3	304.6
140	418.8	72.5	52.4	211.3	116.3	328
150	448.7	72.5	56.1	226.4	116.3	351.5
160	478.7	72.5	59.8	241.5	116.3	374.9
170	508.6	72.5	63.6	256.6	116.3	398.3
180	538.5	72.5	67.3	271.7	116.3	421.8

5.8.2) Overall arthropod response
(insects, sorting.JPG)

Insects and spiders are an important food source for songbirds, small mammals, and other animals in grasslands. We surveyed insects and spiders in unharvested and fully harvested plot using sweep nets. Eight transects were selected from each plot and were sampled three times in each growing season, in June, July, and August. Insect samples were frozen and then sorted into taxonomic groups by laboratory specialists. June vegetation height in unharvested plots appeared to increase through subsequent years as compared to fully harvested plots. This in later months heights appeared equal between treatments. However statistical analysis did not show the June difference to be a significant difference. Therefore sward height was simply entered as a covariate in biomass analysis.

Overall insect response to harvesting was measured in biomass. Dry weights were taken from each arthropod taxa. Arthropod biomass was affected by harvest, with harvested plots displaying slightly, but significantly, higher arthropod biomass. Among taxa, this effect is significant for spiders, beetles and flies, but not for true bugs, ants bees and wasps, larvae, or grasshoppers.

5.8.3) Beneficial Insects

Certain functional groups of invertebrates---that is, groups defined by their roles in the ecosystem---are beneficial to humans. Pollinators enable seed and fruit production, and natural enemies (parasites and predators) help control certain crop pests. We counted individuals of these groups from sweepnet samples and they showed no degradation due to harvest. Pollinator numbers actually showed a slight, though significant, increase in the full harvested plots by the last year. However see the next section on bees. Natural enemies showed no significant difference between control and full harvest plots. As evidence is slight, continued study is suggested.

Sometimes grasslands harbor insect pests or insects that are vectors for plant diseases. That information is not available from our study.

5.8.4) Bees
(p38 sunflower bee-2.JPG)

There are between 300 to 400 species of bees in Minnesota, of which the managed honey bee is one and about eighteen others bumblebees. About 70% of the remaining bees nest in small tunnels in the ground and 30% nest in holes in wood or in hollowed stems. All of these feed their young nectar and pollen. Many are solitary nesters and generally depositing this food supply in their nests and then leave their young to develop on their own over the winter. The next generation emerges from their nest in the spring. Social bee species care for their young throughout the summer and may have several generations in a year all living together. Still, in most cases, only a queen survives the winter and a new colony is formed each year.

A study of bees and floral abundance was begun in the third year of the project and conducted five times a year for two years. Bees were sampled by placing an elevated ring of white, blue and yellow bowls, five meters apart, in the center of each plot. Bowls were filled partway water on one evening, and bees trapped in the water were collected the next evening.

Bee foraging distance relates to body size, and small bees would probably depend more on the floral resources in our plots and thus be more likely to be impacted by harvest one way or the other. We measured the body size of our bee specimens and using the formulas in Greenleaf et al. (2007), computed that 80 acres would cover the maximum foraging area of the smallest stem nesting-bees. The actual flight distances of these bees may be half to one-quarter of this size.

Young of stem nesting bees may spend the winter in grassland plant stems and could be removed by harvesting in the fall. Some stem nesting bee use shrubs with pithy stems such as rose, blackberry, raspberry, sumac and, by our size measurements, may also use grassland forbs with stems at least five millimeters in outer diameter---such as goldenrod or Monarda. Small stem nesting bees might be most responsive to harvesting.

Our study did not show that fall harvest affected total bee abundance in bowl traps. The more sensitive subgroups were analyzed also and while trends are suggestive of a negative impact on the small stem nesting bees, this was not verified with statistical analysis. However, relatively few small stem nesting bees were collected and at this point we do not consider the data to be conclusive. To be useful to a bee for nesting, a dead stem must persist through the two springs after its initial growing season. In the first spring, the stem is available for nesting, in the second spring the next generation of bees would emerge. Thus refuge areas that are mowed every third year cycle would provide nests.

Along with sampling bees, we measured abundance of blooming forbs in our plots. Analysis of data did not show significant difference in bloom abundance between control and full harvest.

Thus it is likely that fall harvest will have little impact though fully harvested plots did green up sooner in the spring. However, as each piece of land is different, it is well to understand the initial floral composition of a field intended for bioenergy harvest. Methods such as those used in this project for surveying plant species and counting blooms in 10-20 small squares of a field during the summer before a the first harvest would accomplish this. Photographing set points from the same angle and height on a regular schedule .This could be quicker to carry out in the field and done in each year. Then later if personal observation of review of photographs suggests that number blooms may be decreasing or increasing the photos may be useful in confirming suspicions, provided the photographs are of good quality and reliably taken.

(Bale rolling DSCN2003.JPG)

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PLOS ONE

Energy Potential of Biomass from Conservation Grasslands in Minnesota, USA

Jacob M. Jungers[1]*, Joseph E. Fargione[2], Craig C. Sheaffer[3], Donald L. Wyse[3], Clarence Lehman[4]

1 Conservation Biology Graduate Program, University of Minnesota, Saint Paul, Minnesota, United States of America, 2 The Nature Conservancy, Minneapolis, Minnesota, United States of America, 3 Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, Minnesota, United States of America, 4 College of Biological Sciences, University of Minnesota, Saint Paul, Minnesota, United States of America

Abstract

Perennial biomass from grasslands managed for conservation of soil and biodiversity can be harvested for bioenergy. Until now, the quantity and quality of harvestable biomass from conservation grasslands in Minnesota, USA, was not known, and the factors that affect bioenergy potential from these systems have not been identified. We measured biomass yield, theoretical ethanol conversion efficiency, and plant tissue nitrogen (N) as metrics of bioenergy potential from mixed-species conservation grasslands harvested with commercial-scale equipment. With three years of data, we used mixed-effects models to determine factors that influence bioenergy potential. Sixty conservation grassland plots, each about 8 ha in size, were distributed among three locations in Minnesota. Harvest treatments were applied annually in autumn as a completely randomized block design. Biomass yield ranged from 0.5 to 5.7 Mg ha^{-1}. May precipitation increased biomass yield while precipitation in all other growing season months showed no affect. Averaged across all locations and years, theoretical ethanol conversion efficiency was 450 l Mg^{-1} and the concentration of plant N was 7.1 g kg^{-1}, both similar to dedicated herbaceous bioenergy crops such as switchgrass. Biomass yield did not decline in the second or third year of harvest. Across years, biomass yields fluctuated 23% around the average. Surprisingly, forb cover was a better predictor of biomass yield than warm-season grass with a positive correlation with biomass yield in the south and a negative correlation at other locations. Variation in land ethanol yield was almost exclusively due to variation in biomass yield rather than biomass quality; therefore, efforts to increase biomass yield might be more economical than altering biomass composition when managing conservation grasslands for ethanol production. Our measurements of bioenergy potential, and the factors that control it, can serve as parameters for assessing the economic viability of harvesting conservation grasslands for bioenergy.

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* E-mail: junge037@umn.edu

Introduction

Perennial biomass is an alternative to conventional starch-based biofuel feedstocks such as corn. It may improve land-use efficiency, reduce greenhouse gas emissions, promote biodiversity, and support other components of sustainability [1–3]. Research comparing ecosystem services of various native and non-native perennial bioenergy crops in the Upper Midwest indicates that bioenergy systems with more plant species support greater avian diversity [4], abundance and diversity of beneficial arthropods [5], carbon storage and complexity of belowground food webs [6]. In many regions of North America, diverse grasslands have not produced as much gross biomass as dedicated energy crops grown in monoculture such as switchgrass [7]. This has initiated questions regarding the economic viability of diverse grassland bioenergy, yet few studies have quantified bioenergy yields from diverse perennial plantings over multiple years. Only recently have studies compared the bioenergy potential of mixed-species grasslands harvested with production-scale techniques in various regions of the Upper Midwest [8].

Growing biomass on land unsuitable for commodity crops transforms the economic outlook for bioenergy systems. Bioenergy production from feedstocks grown on marginal or underutilized land, such as land enrolled in the Conservation Reserve Program (CRP), can provide immediate greenhouse gas benefits [9] while avoiding competition for land between food and energy crops [10]. One idea is to harvest biomass from CRP land as revenue to supplement government subsidies, potentially incentivizing renewal of CRP contracts and offsetting recent trends in expiring CRP acreage [11]. Current CRP regulations do not allow biomass harvest from land enrolled in the program. If economic opportunities from bioenergy initiate new regulations that allow biomass harvest, these regulations should be designed to support the original intentions of the CRP, including improved wildlife abundance [12], an important component of biodiversity.

Other conservation lands managed for wildlife by state, federal, and non-profit agencies have been planted with mixtures of perennial grassland species. These may serve as biomass sources for energy production. Studies are underway to determine the effects of biomass harvest on resident wildlife in various types of conservation grasslands [13]. If research concludes that conserva-

tion grasslands can be managed for bioenergy and biodiversity simultaneously, then the quality and quantity of harvested biomass from conservation lands should be considered before bioenergy management is implemented.

The amount of bioenergy from conservation grasslands depends on both biomass quantity and quality. One means of measuring biomass quantity is to multiply yields from CRP fields in different regions of North America by estimates of available acreage [8,14–16]. These yields can then be extrapolated to estimate biomass from land not currently enrolled in, but eligible for conservation programs. Another important component of predicting bioenergy potential is biomass quality, often defined by the mineral and sugar concentrations of the biomass. Mineral concentrations are used to predict conversion efficiency for thermochemical energy production. High concentrations of alkali metals in post-combustion ash lead to slagging and fouling in thermochemical systems [17], while high concentrations of N, S, and other elements pose issues of oxide emissions and possibly nutrient removal from soils in long-term harvested systems [18]. Predicting the efficiency of biofuel production with biochemical technologies requires measuring the plant sugar and carbohydrate concentrations. High values of cellulose and hemicellulose relative to lignin results in greater liquid biofuel potential [19].

Variation in the quantity and quality of grassland biomass with respect to energy production–hereafter called bioenergy potential– can occur due to variation in plant species composition, geographic location, and management activities. Plant composition influences bioenergy potential with studies indicating positive relationships between (i) biomass yield and planted species richness [2] and (ii) relative cover of warm-season grasses (C4) and lignocellulose ratios that favor ethanol production [14]. In southern Iowa, spatial variation in biomass yield and elemental composition was greater within fields than between fields and was correlated to individual species within cool-season (C3) grasslands [20]. A broad-scale analysis of switchgrass yields across the Great Plains indicated that within-field variation is small enough to consider the mean biomass yield of a field for modeling purposes [21]. Di Virgilio *et al.* found correlations between switchgrass yields and both soil fertility and moisture, which were interpreted as sources of within-field variation [22].

Management activities, including harvest, also affect bioenergy potential. Harvesting biomass after senescence allows for plants to translocate nutrients to belowground tissues, but harvesting post-senescence means that vegetation is removed after peak biomass and lodging have occurred. In Oklahoma and South Dakota, delaying harvest until October increased yields and decreased N and ash concentrations in CRP biomass compared to pre-peak biomass harvests [16,23]. Harvesting switchgrass-dominated CRP lands every year compared with alternate years increased yields [24], while deferring harvest to more than two year intervals lowered bioenergy potential in Canadian conservation grasslands managed for wildlife [25].

In the present study, we modeled bioenergy potential of conservation grasslands based on three response variables related to quantity and quality: biomass yield, theoretical ethanol conversion efficiency, and plant tissue N. We used data collected from large-scale plots distributed across three locations of western Minnesota and harvested with commercial-scale tools and techniques. Our objectives were (i) to determine biomass yields, theoretical ethanol conversion efficiency, and plant tissue N content from conservation grasslands, (ii) to measure the variability of bioenergy potential along a latitudinal gradient in western Minnesota, and (iii) to understand what factors affect bioenergy potential by modeling the three response variables with data on plant communities, soil fertility, precipitation, and management activities while accounting for space and time. Two harvest treatments were used to determine if yields from completely harvested plots followed similar trends through time as yields from plots that included previously unharvested regions of biomass. Our results are intended to aid policy and land-management decisions regarding the use of conservation grasslands for bioenergy production in the Upper Midwest, USA.

Methods

Experimental design

In 2008, we located and delineated 60 plots within existing grasslands enrolled in a conservation program. Plots were distributed among three locations (hereafter north, central, and south locations) spanning a latitudinal gradient in western Minnesota, USA (Figure 1). Soils of the south are glacial till, the north are laucustrine, and the central has regions containing both. Forty plots were located on conservation grasslands managed by the Minnesota Department of Natural Resources (DNR), eight plots managed by the US Fish and Wildlife Service, and 12 plots managed by private landowners as part of the CRP. Each plot was about 8 ha (20 acres; mean = 8.1 ha, SD = 0.5 ha) in size and contained a mixture of grasses and forbs. All plots were established more than five years prior to the project start date. Three of 12 CRP plots were planted with perennial introduced grasses and legumes (CP1) and the rest with perennial native grasses (CP2). The DNR plots were established with different species, but all were categorized as "restored/planted tall grass prairie". A list of the most frequently observed species is in Table S1. Plots were managed periodically for woody species with prescribed fire and/or mechanical harvest prior to the project start date. Fire was not implemented on our plots during the duration of the study. Occasional spot-spraying of herbicides was done in the south location to control invasive species.

Within each location, treatments were replicated in four blocks (Figure 1). Each block contained a control (no harvest) and three harvested plots. Since the control plots were not harvested, this analysis does not include data from those plots. Plots were randomly assigned a harvest treatment, and, for this analysis, were considered either a high- or low-intensity harvest. High-intensity treatments involved a complete harvest of the assigned plot while low-intensity treatments involved a partial harvest so that the plot contained a refuge of standing vegetation of 2 or 4 ha. The harvest treatments were designed to maintain other uses of the grassland, such as habitat for wildlife. In low-intensity harvest treatments, the refuge moved annually within the fixed plot area so that each year, a portion of the harvested area contained biomass that was not harvested the previous year. At all three locations, each block included one control plot, one high-intensity treatment, and two low-intensity treatments with refuges of 2 ha. A separate sub-study allowed the establishment of extra plots in the south location. Blocks in the south location included one extra high-intensity treatment plot and two extra low-intensity treatment plots (totaling seven plots per block). The extra low-intensity treatment plots had refuges of 4 ha. Twenty four plots were scheduled to be harvested in the south and twelve in each the central and north locations. Weather prevented the harvest of certain plots each year. No plots were harvested in the north in 2011 due to expiring land contracts.

Field and laboratory methods

A single operator harvested the plots between late October and mid December in 2009, 2010, and 2011. No plots were harvested after the first significant snowfall. Vegetation was harvested to a
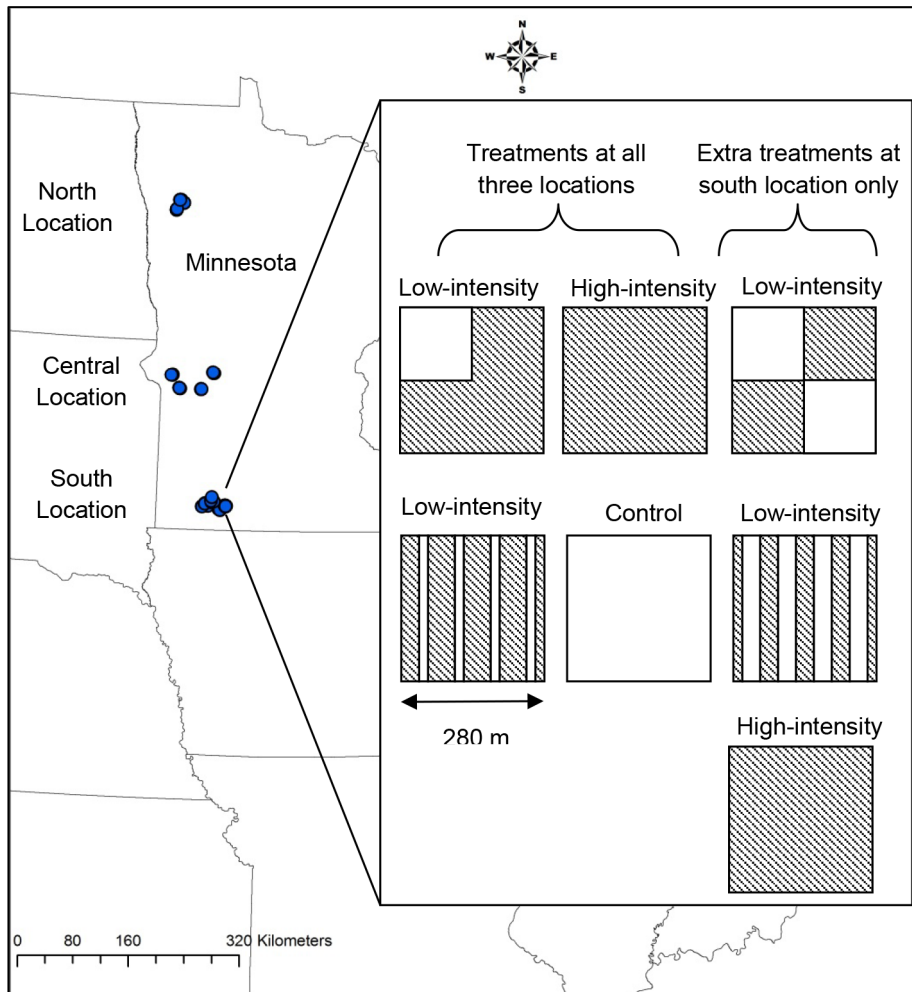
Figure 1. Study areas in Minnesota, located in the Upper Midwest, USA. Research blocks are indicated by circles within the outline of Minnesota in north, central, and south locations. Inset outlines treatments within blocks.
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target height of 15 cm with a self-propelled windrower with a mounted disc cutter. When conditions were deemed dry enough by the operator, the cut biomass was immediately baled using a large round baler. If the cut biomass required drying, it was raked into larger windrows and left to dry before being baled. Due to time constraints and landowner regulations, bales were removed from the plots as soon as possible, therefore individual bales were not weighed from each plot. Instead, bales were loaded onto semi trailers and weighed with a scale certified by the U.S. Department of Transportation on transport for storage. This weight was divided by the number of bales on the trailer to determine an average bale weight and variation (coefficient of variation = 9%; for further details, see Text S1). We divided the sum of all the trailer weights by the total number of bales to generate an overall average bale weight. The average bale weight was multiplied by the number of bales from each plot to estimate total harvested biomass. The perimeter of the cut area in each plot was measured using a hand-held global positioning system (GPS) (Garmin Ltd., Olathe, Kansas, USA) on an all-terrain vehicle. Biomass yield was determined for each plot as the amount of biomass harvested (Mg) divided by the area cut (ha).

While bales were still in the field, core samples were extracted from bales of harvested biomass for each plot with a hay probe

(Forageurs Corp., Lakeville, MN, USA) attached to an electric drill. One biomass core was collected from every other bale as they were ejected from the baler; therefore the number of core samples was determined by the size of the harvested area within the plot and biomass productivity (mean number of cores in high-intensity plots = 22). Cores were aggregated by plot and weighed wet immediately after collection (mean sample weight = 156 g), dried at 45° C for four days, reweighed and used here to estimate bale yields on a dry matter basis.

Chemical constituents of the biomass were measured from the aggregated core samples for each plot. Biomass samples were dried at 45° C for four days, ground with a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA, USA) to pass a 1 mm screen, and then reground with a cyclone mill. A subsample from each plot was analyzed for N by AgVise Laboratories using methods described on their website (Agvise Inc., Benson MN; http://www.agvise.com).

The concentration of cell wall carbohydrates was determined using near infrared spectroscopy (NIRS) with methods described by Schmer *et al.* [26]. NIRS estimates were from equations built with samples from previous collections, upon which wet chemistry methods were used to directly determine cell wall carbohydrate concentrations (Table S2). The values of xylose, arabinose,

mannose, galactose, and glucose were calculated with methods established by the U.S. Department of Energy to predict theoretical ethanol conversion efficiency (Equation S1, http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html). Calculations used to estimate theoretical ethanol conversion efficiency assume 100% conversion efficiency because realized efficiency rates are not available for production-scale systems.

In the summer of 2009, soil cores were collected to a depth of 20 cm at eight points adjacent to the randomly distributed vegetation quadrats. Soil cores were aggregated by plot and processed and analyzed by AgVise Laboratories for $N–NO_3$, pH, organic matter, and cation exchange capacity.

Plant community composition was visually assessed in 1.0×1.5 m quadrats at 12 random points within each plot in late July and/or early August of 2010 and 2011. A total of 24 quadrats were sampled in the high-intensity treatment plots in 2010 to assess sample power. In 2009, plant community data was collected from quadrats, each 0.75×5 m, in all plots. Quadrat locations were generated with ArcGIS 9.3 (ESRI, Redlands, CA, USA) and loaded to hand-held GPS units. Within each quadrat, surveyors identified all plant species and assigned each a score for relative abundance as a percentage of the canopy cover in the quadrat. Bare ground and litter were also assigned a percentage. Species were aggregated into functional groups for analysis. The average cover value for each functional group was calculated by plot.

Cooperative Farming Agreements, Special Use Permits, and a letter of approval were acquired from the Minnesota Department of Natural Resources, US Fish and Wildlife Service, and the US Department of Agriculture Farm Service Agency for permission to conduct research on state, federal and private land.

Data Analysis

Three response variables related to different components of bioenergy potential were measured in all plots and modeled in this study: biomass yield, theoretical ethanol conversion efficiency, and plant tissue N. Linear mixed effects models were used to test the main effect of location on the three response variables and to determine which covariates were significantly correlated with them. Total variation for each response variable was partitioned into four levels of a temporal/spatial hierarchy that was used as the random structure for the variance components analysis. The largest level of this hierarchy partitioned variance among years, with lower levels partitioning variance between locations, between blocks, and within plots; each level nested within the higher level. A model with only random effects was used to determine the variance at each level of the hierarchical random structure for all three response variables. Equation 1 was modified from West *et al.* [27] to derive variance estimates for each level of the random hierarchy, where ICC_i represents the proportion of variation at level i compared with the total variation.

$$ICC_{Date} = \frac{\sigma^2_{Date}}{\sigma^2_{Date} + \sigma^2_{Location} + \sigma^2_{Block} + \sigma^2}$$

$$ICC_{Location} = \frac{\sigma^2_{Location}}{\sigma^2_{Date} + \sigma^2_{Location} + \sigma^2_{Block} + \sigma^2}$$

$$ICC_{Block} = \frac{\sigma^2_{Block}}{\sigma^2_{Date} + \sigma^2_{Location} + \sigma^2_{Block} + \sigma^2}$$

To quantify the differences in biomass yield, ethanol conversion efficiency, and plant N between locations, a dummy variable was assigned to the south, central, and north locations and was modeled as a categorical main fixed effect. Using location as a fixed effect, various random structures composed of the nested spatial/temporal variables were fit to models and compared using maximum likelihood ratio tests.

Land ethanol yield (l ha^{-1}) was calculated by multiplying ethanol conversion efficiency (l Mg^{-1}) by biomass yield (Mg ha^{-1}) for each plot. A linear regression model was used to estimate the fraction of variation in land ethanol yield due to variation in biomass yield.

For each response variable, we selected a group of candidate covariates *a priori* from a list of measured variables (Table 1). A global model for each response variable included all covariates related to plant community structure and an interaction between each community covariate and the main effect of location. No three-way interactions were tested. Each global model included a best fitting random structure and a first order autocorrelation structure. The global model was reduced by removing the least significant fixed effect determined by t-statistic at $P<0.05$ [28]. This iterative process continued until all fixed effects were removed. The resulting models were compared using Akaike's information criteria adjusted for small sample sizes (AIC$_c$) [29]. The best fitting model was refit using restricted maximum likelihood to generate unbiased parameter estimates. For models without interactions, Tukey's *post hoc* means separation test was used to determine differences between levels of significant main effects.

A mixed effect model was used to test the effect of harvest intensity on the change in biomass yield over time. The difference in biomass yield from the first harvest (2009) to the last (2011) was calculated for plots in the south and central locations to test the hypothesis that trends in biomass yields through time would be the same for plots where all the biomass is removed as plots that include regions of previously unharvested biomass. The change in yield was compared between low- and high-intensity harvest treatments. The model included an interaction between harvest intensity and location while accounting for variation in each plot as a random variable. All statistical analyses were conducted with program R [30].

Results

We analyzed and modeled biomass yield from 109 observations and theoretical ethanol conversion efficiency and plant tissue N from 112 observations from conservation grasslands harvested in autumn of 2009, 2010, and 2011. Weather obstructed biomass harvest at certain plots each year which resulted in an unbalanced data set. No plots were harvested in the north location in 2011 due to expiring land contracts.

The south location received more precipitation during the growing season compared with the north and central locations during all years of the study. Precipitation was lowest in 2009 at the south and central locations, and lowest in 2011 at the north. Over the course of the project, precipitation was the greatest in 2010 and well exceeded the 30-year mean at all locations. In 2011, the north and central locations were below the 30-year mean while precipitation at the central location was higher (Table 2).

Table 1. List and description of all covariates available for analysis.

Effect	Variable	Description
Random	DATE, LOC, BLOCK, PLOT	Nested temporal and spatial variables. Plot nested in block nested in location.
Main	Location	Categorical main effects of location.
Plant Community	C4, C3, Legume, Forb	Continuous measure of mean percent cover of each plant functional group by plot.
Soil Fertility	NO$_3$, OM, pH, CEC	Mean values of N–NO$_3$ (NO$_3$), organic matter (OM), pH, and cation exchange capacity (CEC) by plot.
Plant Composition	PlantN	The concentration of N in harvested biomass tissue.
Precipitation	April, May, June, July, August, September	Total monthly precipitation measured for each year by block.
Interactions	C4×Location, C3×Location, Legume×Location, Forb×Location, Harvest×Location	Interaction between main effects, and between the main effect of location and all plant community covariates

Biomass yield

Without accounting for covariates, mean biomass yield in the south was 55%, 69%, and 55% greater than other locations in 2009, 2010, and 2011 respectively (Figure 2A). Annual plot biomass yield ranged from 0.5 Mg ha^{-1} to 5.7 Mg ha^{-1} and had an overall mean of 2.5 Mg ha^{-1} across all locations and years. Biomass yield increased from 2009 to 2011 in both the south and central locations and in both harvest intensities (Figure 3). The increase in biomass yield through time was the same between harvest intensities (F = 0.48, df = 27, P = 0.49).

Biomass quality

Biomass yield was a significant predictor of the variation in land ethanol yield (F = 5558, df = 1 and 108, P<0.001). The adjusted R-squared was 0.98 for the relationship between biomass yield and land ethanol yield (Figure 4). Mean ethanol conversion efficiency was 450 l Mg^{-1} with a standard deviation of 38 across all locations and years. Mean plant N concentration was 7.1 g kg^{-1} with a standard deviation of 1.5 and was not consistently different among locations and years. Mean plant N was lower and mean ethanol conversion efficiency was greater in the south than the other locations in all three years (Figure 2B and 2C).

Variance components analysis

Results from the intercept-only random effects models suggest that of the total variation in biomass yield, ethanol conversion efficiency, and plant N, the variance between years explained the smallest fraction (Table 3). The largest fraction of the variance in

Table 2. Cumulative precipitation from April through October by location and year, for comparison with other regions.

	2009	2010	2011	30 yr. mean
	(mm)			
North	435	663.46	391.51	442.21
Central	452.64	663.22	538.59	518.92
South	559.09	864.36	577.13	582.93

biomass yield and plant N was partitioned into within-plot variance, while the variation between locations accounted for about one-third for both responses. More than a majority of variation in ethanol conversion efficiency was observed between locations (Table 3).

Bioenergy potential models

Biomass Yield. Measured soil fertility variables did not contribute to explained variation in biomass yield. The effect of forb cover was significant in the best fitting model (Table 4) and influenced biomass yield uniquely in the south compared with the other locations (Table 5, Figure 5B). Specifically, forb cover was negatively correlated with biomass yield in the central and north locations, but positively correlated with biomass yield in the south location. Covariates for May precipitation and legume cover were positively correlated with biomass yield in the best fitting model (Table 5). A model with the random variables plot (identified below as PLOT; see Table 1) nested within block (identified as BLOCK) was superior to a model without random effects (L = 40.77, df = 1, P<0.001). The three best fitting models were similar in their explanatory power determined by AIC$_c$ (Table 4).

Ethanol Conversion Efficiency. The two best fitting models included the effect of location, the cover of C4 grass, and the nitrogen content of harvested biomass as predictors of variation in ethanol conversion efficiency. The best fitting model included the cover of forbs and omitted all interactions between main effect and covariates (Table 4). The cover of C4 grass was positively correlated with ethanol conversion efficiency (Figure 5C), while plant N and forb cover showed negative relationships with ethanol conversion efficiency (Table 5). Ethanol conversion efficiency was significantly greater in the south than the central (P = 0.034) and north (P = 0.020) locations, with a metric ton of biomass producing 12% more ethanol in the south than the average of the central and north locations. There was no significant difference between the central and north (P = 0.947) locations. A model with the random variables BLOCK and DATE was best supported for explaining variation in ethanol conversion efficiency. The random structure was fit to allow unique BLOCK variation around the intercept by DATE. This structure was better supported than the fully nested random structure (L = 13.5, df = 1, P = 0.004) and a model without a random structure (L = 64.7, df = 1, P<0.001). The two best fitting models differed by 0.69 AIC$_c$ points and one parameter (Table 4).
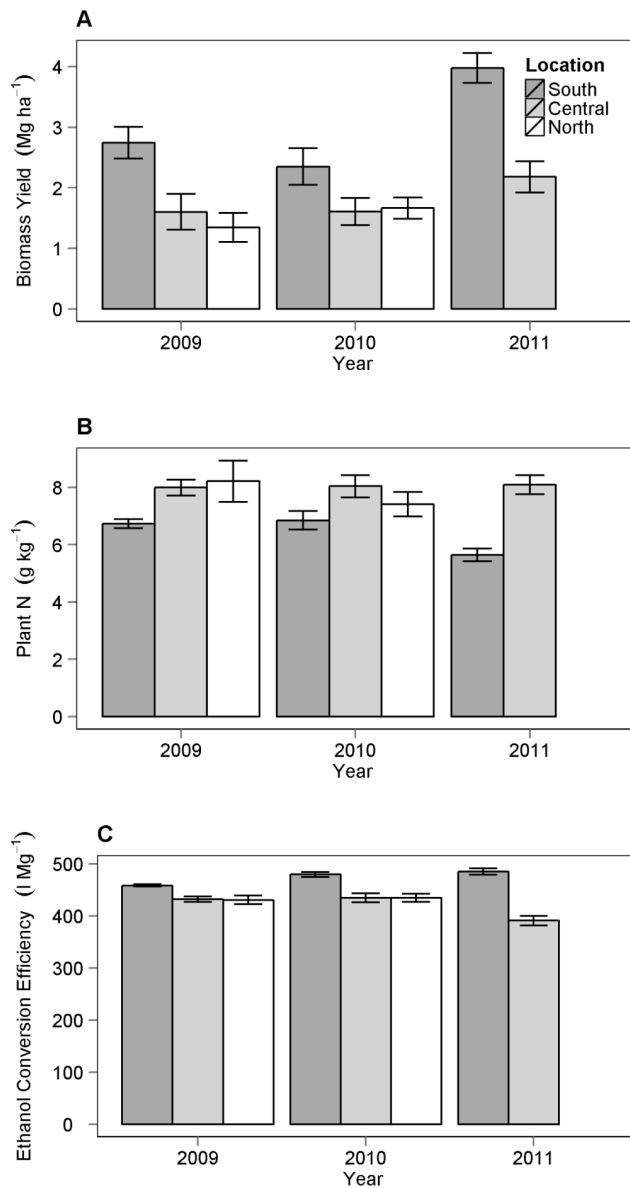
Plant N. The three best fitting models included the main effect of location, C4 cover, and soil N–NO$_3$ concentration

Figure 2. Average values (SE) of response variables by location and year. Mean values of biomass yield (A), plant tissue N (B), and ethanol conversion efficiency (C). Black, gray and white bars are mean values from plots harvested in south, central and north locations respectively.
doi:10.1371/journal.pone.0061209.g002

(Table 4). The best supported model included an interaction term between location and legume cover (Table 5). In the south, legume cover was negatively correlated with plant N as opposed to the positive correlation observed in the central and north locations (Figure 5A). Soil N–NO$_3$ and C4 cover were positively and negatively correlated with plant N respectively (Table 5). The best fitting random structure for modeling the concentration of N in biomass included PLOT nested within BLOCK. This structure was superior to a model without a random component (L = 14.9, df = 1, P<0.001) and to a model with a fully nested hierarchy of random variables (L = 9.2, df = 1, P = 0.003).
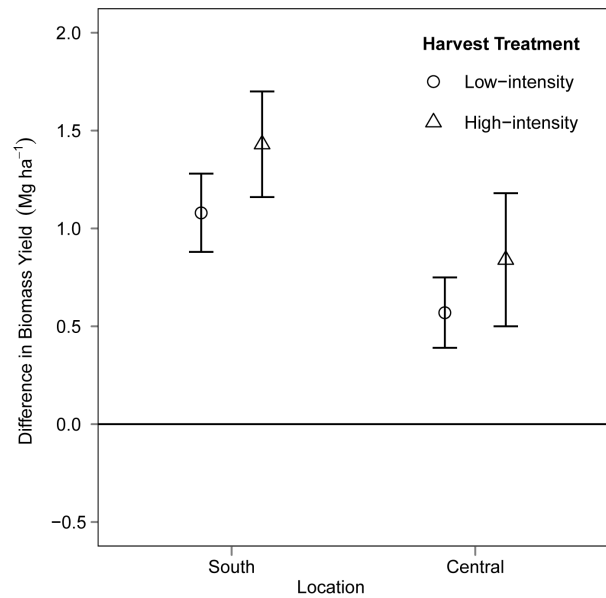
Figure 3. Change in biomass yield from 2009 to 2011 in low- and high-intensity harvest treatments by location. Average change in biomass yield(±90% CI). In low-intensity plots, one third to one half of the annually harvested biomass was from an area not previously harvested. High-intensity harvest plots included biomass from the same area harvested annually.
doi:10.1371/journal.pone.0061209.g003

Discussion

Harvested biomass yields from low-input grasslands managed for conservation was 2.5 Mg ha^{-1} and on average, fluctuated 23% around this mean across the three year study period. Assuming this
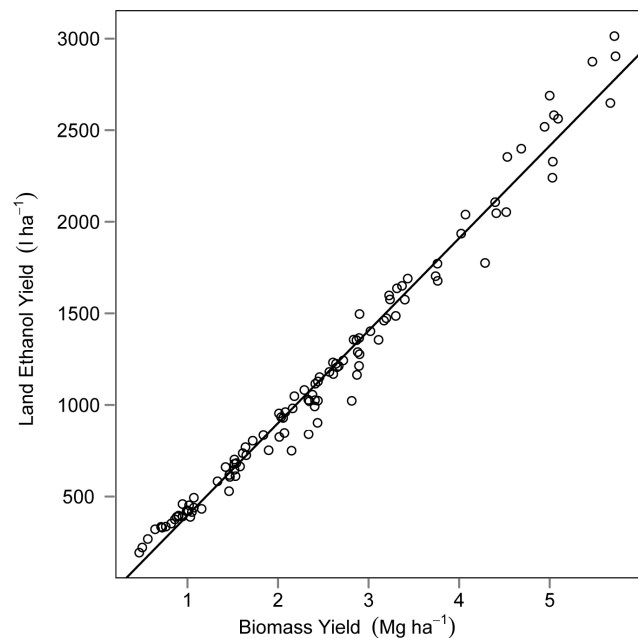
**Figure 4. Correlation between land ethanol yield (l ha^{-1}) and biomass yield (Mg ha^{-1}).** Points represent values from conservation grasslands harvested in the autumn of 2009, 2010, and 2011. Regression line from linear model with R-squared value = 0.98.
doi:10.1371/journal.pone.0061209.g004

Table 3. The contribution of variation from nested random effects for measures of bioenergy quantity and quality.

Nested Sources of Variation	Biomass Yield	Ethanol Conversion Efficiency	Plant N
Between years	0.33 (6%)	$4.6*10^{-3}$ (0%)	$1.0*10^{-4}$ (0%)
Between locations	0.74 (31%)	28.78 (57%)	0.86 (34%)
Between blocks	0.65 (24%)	17.45 (21%)	0.15 (1%)
Within plot (residual)	0.82 (39%)	17.85 (22%)	1.18 (65%)

Variation reported as standard deviation and percent of total variation.
doi:10.1371/journal.pone.0061209.t003
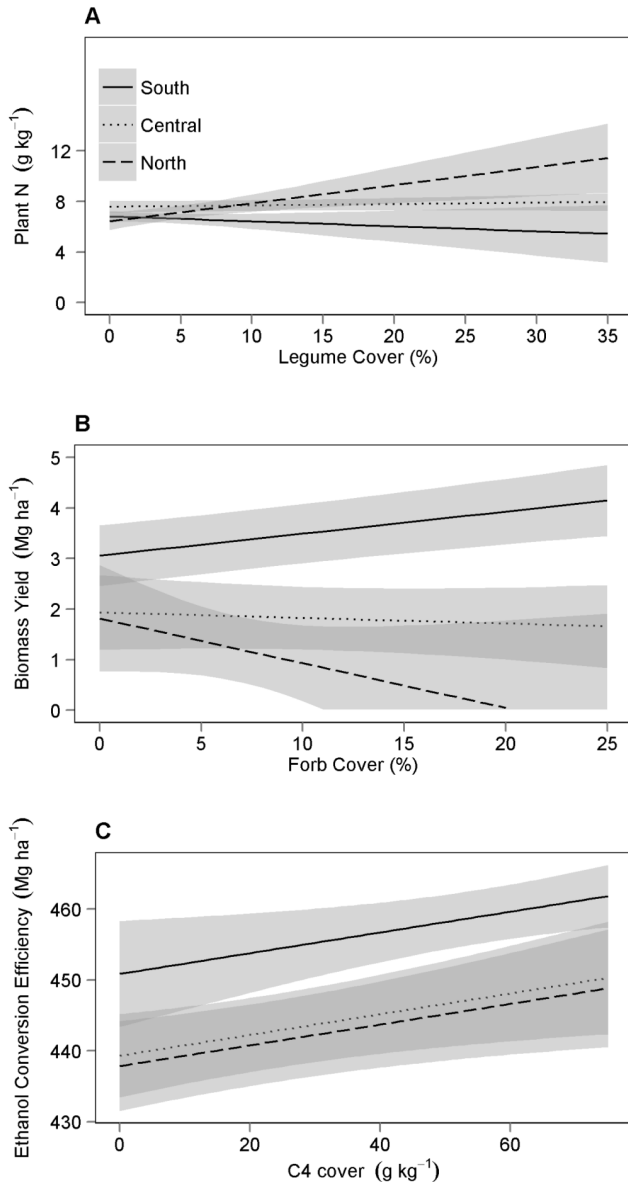
Figure 5. Estimated effect of plant functional group composition on bioenergy potential. Regression line estimates($\pm$90% CI) of the effect of legume cover on the concentration of N in biomass after harvest (A), the effect of forb cover on biomass yield (B), and the effect of C4 cover on ethanol conversion efficiency (C). Estimates are from the best fitting models with all other covariates held constant at their average values.
doi:10.1371/journal.pone.0061209.g005

yield can be achieved from all the conservation grasslands within an 80 km radius of a biorefinery located in the southwest portion of Minnesota (a total of 107,571 ha of conservation grassland or 5.4% of the total area), and that only 75% of the conservation grasslands are harvestable within that area, approximately 1000 Gw*hours of energy is available (Text S2). If divided across the year, this is equivalent to 114 MW of continuous energy from conservation grasslands alone.

Yields were highest in the south location in all years of this experiment, but were 49% lower than first-year hand-cut yield estimates from newly established high diversity mixtures grown in similar regions [31]. Despite similar growing conditions, the high diversity mixtures were grown on fine loam soil with N, P, and K concentrations more than two times higher than concentrations found in our soils. From our southern plots, biomass yield estimates from hand-cut samples collected in late July were 91% and 54% greater than yield values from commercial-scale harvest in 2010 and 2011 respectively (unpublished data), both of which are similar to the harvest efficiency of managed switchgrass plots in Italy [32]. Although leaf loss and reallocation of C to belowground structures can account for 12% to 19% of decreased biomass yields from September to November [33], there is evidence that commercial-scale harvesting techniques can be made more efficient at both cutting more of the material to a desired height and picking up more of the material with a baler to improve yields [32]. It should be noted that stubble and residual litter provides environmental benefits by reducing erosion and providing cover for ground nesting birds, therefore 100% harvest efficiency may not be a desired objective. Observed variation in litter quantities across studies suggests that caution be taken when comparing aboveground productivity estimates and biomass yields between small-scale and large-scale studies that do not use similar cutting and biomass collection methods.

Generally, the concentration of N in herbaceous biomass results in greater NO_X emissions during thermochemical conversion to energy compared with light fuel oil and natural gas [34]. It has been recommended to delay harvesting until after senescence to allow perennial plants to translocate N to belowground tissues for both switchgrass [35] and conservation grassland biomass [16]. Nitrogen content in harvested biomass from this project was similar to conservation grasslands harvested after a killing frost in South Dakota [36]. There is concern that low-input grasslands might not be a long-term viable source of biomass because of N depletion during harvest [37], but those concerns have not yet been tested. There is evidence that long-term annual biomass harvest from low-input grasslands does not decrease yields [38]. Mixed-species grasslands like those used in this project contain legumes that add N annually. N inputs via legumes ranged from 28 to 187 kg ha^{-1} in mowed grass/legume pastures that contained white clover [39], yet studies are needed to determine the net N flux in harvested grassland systems across a range of locations.

Table 4. Top three best-supported models of bioenergy potential measured from conservation grasslands in Minnesota, USA.

Response	Model	Parameters (K)	ΔAIC$_c$
Biomass Yield	Intercept+Location×Forb+May+Legume	12	0.00
	Intercept+Location×Forb+Legume+May+June	13	1.56
	Intercept+Location×Forb+Forb+May	10	2.06
Ethanol conversion efficiency	Intercept+Location+C4+PlantN+Forb	14	0.00
	Intercept+Location+C4+PlantN	13	0.69
	Intercept+Location+C4+Forb+NO3+PlantN	15	1.86
Plant N	Intercept+Location×Legume+C4+NO3	12	0.00
	Intercept+Location×Legume+C4+NO3 +pH	13	0.28
	Intercept+Location+C4+NO3	9	0.42

doi:10.1371/journal.pone.0061209.t004

Variation in biomass yield, ethanol conversion efficiency, and concentration of N in plant tissue was relatively small between years, deviating from each location's average by no more than +/− 27%, 11%, and 7% respectively. This is in contrast to other studies with less mature perennial grasslands (our study sites were all >5years old), where issues with establishment contributed to larger (up to 69%) year-to-year variation in biomass yield [21]. Across the total study area, between-year variability in biomass yield was small despite differences in precipitation. Our results show that precipitation during the month of May measured at the block level is important in determining biomass yield (Figure 6).

Total precipitation may not be a good indicator for predicting biomass yields because high amounts of precipitation during harvesting months may result in lower yields due to leaf losses and other inefficiencies in biomass collection, especially when harvesting with production-scale equipment [32]. Excessive precipitation during autumn months inundated some parts of this experiment and prevented the harvest of certain plots each year. Averaged across all years, 83%, 78%, and 74% of the planned harvested areas were harvested in the south, central and north locations respectively. This percentage increased annually in the south and central locations.

Table 5. Parameter estimates from best-fitted mixed effects models with biomass yield, ethanol conversion efficiency, and plant N as response variables.

Response	Variable	β	SE (β)	DF	t-value	p-value
Biomass Yield	Intercept	2.069	0.381	56	5.432	<0.001
	Location 2	−1.126	0.583	9	−1.932	0.085
	Location 3	−1.243	0.738	9	−1.684	0.126
	May	0.011	0.001	56	9.893	<0.001
	Legume	0.017	0.007	56	2.428	0.018
	Forb	0.044	0.013	56	3.284	0.002
	Location 2×Forb	−0.055	0.026	56	−2.073	0.043
	Location 3×Forb	−0.132	0.076	56	−1.750	0.086
Ethanol Conversion Efficiency	Intercept	529.905	9.680	96	54.743	<0.001
	Location 2	−11.550	4.623	9	−2.498	0.034
	Location 3	−13.005	4.840	9	−2.687	0.025
	C4	0.147	0.070	96	2.081	0.040
	Plant N	−10.812	1.088	96	−9.941	<0.001
	Forb	−0.357	0.203	96	−1.760	0.082
Plant N	Intercept	6.786	0.458	59	14.827	<0.001
	Location 2	0.746	0.400	9	1.862	0.096
	Location 3	−0.384	0.531	9	−0.724	0.488
	C4	−0.017	0.006	59	−2.975	0.004
	Legume	−0.040	0.043	59	−0.925	0.359
	NO3	0.077	0.016	59	4.748	<0.001
	Location2×Legume	0.050	0.044	59	1.137	0.260
	Location3×Legume	0.182	0.071	59	2.579	0.012

doi:10.1371/journal.pone.0061209.t005
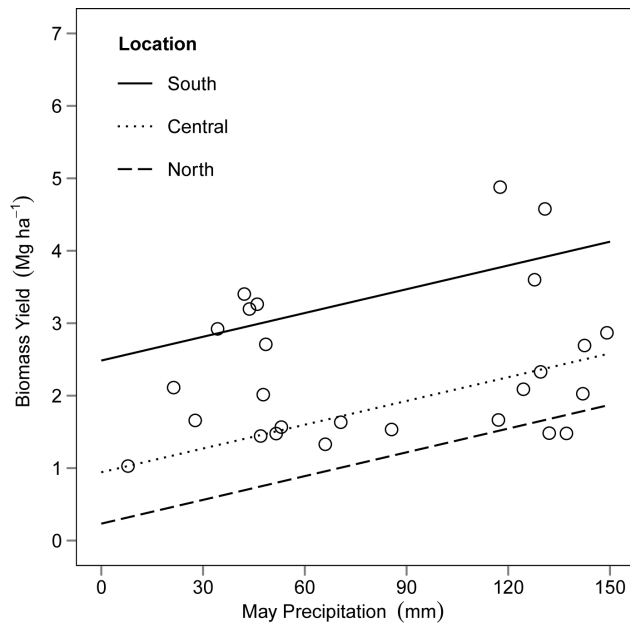
Figure 6. Estimated effect of May precipitation on biomass yield. Dots represent average measured biomass yield and May precipitation values by block. Regression lines are model estimates for bioenergy yield across the precipitation gradient for each location, with all other covariates held constant at their average values.
doi:10.1371/journal.pone.0061209.g006

Table 6. Mean values (SD) of covariates by location across all years from conservation grasslands in Minnesota.

Covariate	South	Central	North
	% cover		
C4	56.86 (18.78)	24.94 (18.37)	20.12 (18.71)
C3	18.15 (16.30)	37.77 (19.58)	45.64 (23.15)
Legume	2.80 (3.22)	8.51 (14.57)	4.81 (5.07)
Forb	6.54 (6.57)	10.35 (5.94)	6.26 (3.22)
NO$_3$	7.84 (3.94)	11.04 (8.35)	13.76 (12.22)
OM	5.27 (1.33)	6.52 (3.04)	5.38 (1.65)
pH	6.67 (0.49)	7.52 (0.37)	7.68 (0.65)
CEC	22.17 (7.55)	25.66 (7.44)	26.19 (8.08)

doi:10.1371/journal.pone.0061209.t006

Consistent values for biomass quality metrics are important for viable biorefinery production. A substantial fraction of the total variation in biomass yield was observed between locations, which is in accordance with studies on the variation of switchgrass yield [21]. About one-quarter of the total variation in biomass yield was measured between blocks, which was similar to the results of yield variation in C3-dominated grasslands analyzed for bioenergy [20]. Florine *et al.* reported smaller total variation in plant N (SD = 0.4 g kg^{-1}) than our results (SD = 1.5 g kg^{-1}) [20]. Total variation in ethanol conversion efficiency was relatively small but greater than reports from switchgrass, yet similar in terms of partitioning between spatial and temporal scales [26].

The variation in land ethanol yield was almost exclusively due to variation in biomass yield (Figure 4). Land managers looking to harvest biomass from conservation grassland for ethanol production would maximize revenues by identify high biomass yielding plots as opposed to harvesting plots based on the theoretical ethanol potential of the plants.

We hypothesized that covariates would explain variation among locations (Table 6). However, for all response variables, location remained a significant variable in the best fitting models (Table 5). Best fitting models for biomass yield and plant N included interactions between location and plant community covariates, which provide limited information to draw conclusions as to why differences in these response variables exist across locations. In terms of ethanol conversion efficiency, location was identified as a main source of variation, therefore suggesting that other factors related to space–factors that were not measured in this study–influenced the response.

Other reports have suggested that plant community characteristics such as C4 grass cover [14] and planted species richness [2] improve biomass yields. In this study, it was the cover of non-legume forbs that explained variation in biomass yield (Table 4 and 5). In the south location, plots with greater average forb cover

had higher biomass yields, while in the central and north locations, increasing forb cover was associated with lower yields. We expected, as Adler *et al.* documented, that the cover of C4 grass would be positively correlated with biomass yield, and our competitive models include that variable (Table 4). It is possible that an increase in forb cover displaces C4 grasses, which would explain the negative correlation between forb cover and biomass yield in the central and north locations. The inverse relationship between forb cover and biomass yield in the south could be driven by a high-yielding forb species that is present or abundant in the south but not in the other locations. We explored this possibility and found that common milkweed (*Asclepias syriaca*) was present in 300 sample points in the south and only 50 and 5 sample points in the central and north locations. Using data from all sample points, a Pearson's correlation test showed that the cover of common milkweed was not correlated to the cover of C4 grass (P = 0.303) but was correlated to biomass yield (P = 0.016). This suggests that common milkweed could increase biomass yield without displacing C4 grass cover (Table 6). Other studies have observed increases in forb abundance without associated decreases in biomass production [40].

Harvested areas in the low-intensity harvest treatments included a fraction of the plot where vegetation was left standing the year before. This did not affect biomass yields compared with completely harvested plots. European mixed-species hay yields did not decrease after decades of annual harvest without nutrient inputs [38], though long term studies are needed to verify if similar patterns exist in North American grasslands. The positive correlation of May precipitation with yield could be because it supplies resources before the peak productivity time of C4 grasses, which contribute to biomass yield when harvested in autumn [36]. Other studies have shown that the variation in June soil moisture was positively correlated with C4 grass productivity [41], but soil moisture measurements were not made in our study.

Maximum theoretical ethanol conversion efficiency values were slightly higher than those reported in switchgrass [26] and similar to mixed prairies [42], and were greater in biomass harvested from the south compared with biomass from the central and north locations (Figure 2C). Studies of switchgrass show that harvesting later after plant senescence results in higher potential ethanol conversion efficiency [43], thus a similar pattern could exist in polyculture grasslands. We harvested plots in sequence from the north to the south so that the plants would be at a similar phenological stage at the time of cutting. A negative correlation between plant tissue N and ethanol conversion efficiency was

apparent in this study (Table 5), and since plant N decreases with senescence, the later harvest date in the south location may have contributed to higher ethanol conversion efficiency found here. Also, our results confirm previous reports of correlations between C4 grass cover and ethanol conversion efficiency [14] (Figure 5C). In general, C4 grasses have higher levels of fermentable sugars than forbs [44]; therefore ethanol conversion efficiency is expected to decrease with increased forb cover relative to C4 dominated stands. As highlighted in this study, Gillitzer *et al.* showed that the relationship between species composition and biomass yield, rather than species composition and ethanol conversion efficiency, is the more dominant driver of land ethanol yield [42,45].

Legumes in mixed-species grasslands fix atmospheric nitrogen, which has several consequences for ecosystem functioning including increased productivity [46]. However, in the case of combustion bioenergy, undesirable consequences of legume biomass come in the form of pollution. Legume biomass has relatively higher levels of tissue N than forbs and grasses, which can lead to greater NO_x emissions during thermochemical energy conversion [34]. The best fitting model identified a relatively strong trend in legume cover and plant N in the north location ($t = 2.579$, $P = 0.012$). Weaker evidence of a relationship was observed in the central ($t = 1.137$, $P = 0.260$) and the south locations ($t = -0.925$, $P = 0.359$), which could be related to the absence or presence of a specific legume species, as observed in other studies [47]. The estimates from this model predict that a four-fold increase in legume cover (from the observed average of 4.8% to 19.2%) in the north location would increase biomass N concentrations approximately 23%, or to a value of 10.2 g N kg^{-1}. Promoting legumes increases functional group diversity, which leads to other ecological benefits including increased soil carbon storage [48]. Also, complementarity among C4 grasses and legumes increases biomass yields [48]. Therefore, we believe that the model-estimated environmental cost of legume abundance in bioenergy grasslands is far outweighed by the ecological and yield benefits they provide.

The three best supported models all suggest that unfertilized soils with naturally higher levels of N–NO$_3$ will produce biomass with greater concentrations of tissue N (Table 4). Elevated levels of soil N–NO$_3$ could come as a result of N fertilizer, which has been considered as a management tool to increase biomass yields in conservation grasslands [8,23]. Fertilization experiments show that higher N fertilizer rates lead to higher concentrations of N in biomass tissue for C3-dominated mixed grasslands [49], for switchgrass [50], and other C4 grasses [51]. Nitrogen fertilization can lead to a loss of species and functional group turnover [52], but when fertilized grasslands are harvested, species diversity has been shown to be maintained [53] or increase [40]. When considering N fertilizers, land managers must weigh the potential benefits for biomass yields against potential detrimental effects including undesirable shifts in species composition and decreased biomass quality.

Conclusions

Biomass quality from mixed-species grasslands not managed for bioenergy is similar to dedicated energy feedstocks, in terms of

theoretical ethanol conversion efficiency and biomass N. Almost all of the variation in land ethanol yield is based on biomass yield, therefore efforts should be focused on maximizing biomass yield rather than biomass quality when managing grasslands for land ethanol yield. A combination of climate, soil fertility, and plant community factors influence overall bioenergy potential. The effect of forbs and legumes on biomass yield and tissue N, respectively, were different in the south compared with the central and north locations. The covariates we measured did not explain why theoretical ethanol conversion efficiency was greater in the south compared with the other locations, but the cover of C4 grass was positively correlated with ethanol conversion efficiency. After three continuous years of harvest, leaving a portion of standing biomass within the harvested area does not influence biomass yield of future harvests. Simply focusing on plant community variables to predict bioenergy potential of conservation grasslands across various locations at the scale we studied will not provide accurate estimates; instead attention should be drawn to local variation in soil fertility, climate, and possibly plant species and interactions between these variables.

Supporting Information

Table S1 Ten most frequently observed species and their average percent cover in sample quadrats.
(DOCX)

Table S2 Calibration statistics for NIRS prediction of forage characteristics and plant cell polysaccharides.
(DOCX)

Equation S1 Equation to estimate theoretical ethanol conversion efficiency from sugar concentrations.
(DOCX)

Text S1 Assessment of bale weight variability for large round bales of biomass harvested from conservation grasslands.
(DOCX)

Text S2 Calculations for estimating residential power production from conservation grasslands in SW Minnesota.
(DOCX)

Author Contributions

Conceived and designed the experiments: JJ CS DW CL. Performed the experiments: JJ. Analyzed the data: JJ. Contributed reagents/materials/analysis tools: JJ JF CS DW CL. Wrote the paper: JJ.

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The Centinel Data Format:
Reliably Communicating through Time and Place

Clarence Lehman[1], Shelby Williams[2], and Adrienne Keen[3]
[1]University of Minnesota, 123 Snyder Hall, 1474 Gortner Avenue, Saint Paul, MN 55108, USA
[2]University of Minnesota, 100 Ecology, 1987 Upper Buford Circle, Saint Paul, MN 55108, USA
[3]London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

> *"A library book lasts as long as a house, for hundreds of years."*
> —Thomas Jefferson, 1821

Abstract—*A common experience among scientists and engineers is storing and sharing data, the capacity for which has advanced immensely since laboratory notebooks were only paper and ink. However, since that time, the sustainability of data has decreased. Even though our digital data should be safer and more secure than ever, a continuing cascade of obsolescence in computer media and software can actually make it less so. Here we outline an ensemble of free tools and techniques that we call "Centinel," designed to manage, communicate, and archive digital datasets. Rather than embedding error-correcting codes as part of the computer media, Centinel exposes them and places them with the data and metadata. Thus even printed copies of the data form reliable storage media that can last indefinitely without intervening attention. Centinel complements standard methods for data sustainability, such as data migration. Unified approaches, as we outline here, benefit reliability and longevity of data.*

Keywords: database, data archive, data longevity, data reliability, error correcting codes

1. Introduction

In 1815 began one of the largest scientific data collection projects ever launched [1]. Legions of surveyors walked regularly spaced transects along 2,500,000,000 meters of the Louisiana Territory, recording the biological species, geographic locations, and diameters of selected trees near periodic sample points—plus other information on soils, vegetation, and boundaries of wetlands. For almost a century the survey continued. Now, another century after the last data were recorded, the results form one of the most visible efforts ever, organizing the rural landscape into square sections along those transects. The results also form one of the best preserved and widely available datasets ever. Think of which present datasets, in your personal experience, are guaranteed to be extant and usable well into the 22nd century.

A large part of the reason the survey data survived was that it was recorded on paper and protected at many different governmental sites. In the meantime, technology changed immensely. Computers emerged and increased in capacity so relentlessly that the Library of Alexandria's ancient charge of organizing and cataloging all human knowledge began to draw within reach. Global access to digital data can make that knowledge available to all. Large-scale private enterprises are aiming at this goal, but individuals in academia and industry are established sources of knowledge and therefore have a special role in achieving this.

Here we are addressing that role—of scientists, engineers, and others who collect empirical data, share it, and want to preserve it for the future. In this report we explain how digital computer techniques of today combine naturally with paper methods of prior centuries to create a form of digital storage that can reliably persist into future centuries and improve electronic processing today.

2. What Centinel is and is not

The general topic that Centinel addresses has been long discussed (e.g., [2] [3] [4] [5] [6] [7] [8] [9]) and a complete solution is not yet available. Centinel combines the words "century" and "sentinel," guarding data for extended periods. One goal for Centinel is to ensure that the digital data it encodes will be accessible in a century or more, without the need for care and intermediate steps by humans. A second goal is to protect data over a shorter term, from the time of initial creation to the time of final processing. Centinel works by (1) keeping all metadata with the data, (2) protecting data with line-by-line error correcting codes, (3) providing a format easily readable by humans as well as computers and scanners, (4) supporting a reliable digital format that works on any media, including paper and verbal communications, to protect data from unintentional alteration, and (5) supplying an extensible, self-defining format with accompanying tools that help computer programmers know that the data entering their programs are correct. Centinel is an approach to data management, but also a set of basic computer utilities for writing, reading, editing, separating, joining, ordering, and aligning data. It avoids structures that are error prone

```
6674844762232577   Keyword SpAbbr: Abbreviations for species names. Abbreviations contain the
0629561874138616     first three letters of the genus name followed by the first three letters
0211050455008008     of the species name. The full species names are recorded with their
5515307245627135     abbreviations in table "species codes" at the end of the chapter.
5915322805104717   Keyword Date: Date species was collected. Format year-month-day.
1453182442695072   Keyword CollID: Unique code assigned to species sample collected.
1382423906566782   Keyword Cover: Estimated canopy cover, in percent. Dashes indicate missing
5953391885352618     data. (See "methods" at the end of the chapter.)
0748783303437946   Keyword HtMax: Maximum height, in meters. Dashes indicate missing data.
0229302812296440     (See "methods" at the end of the chapter.)
0602554115737437   Keyword HtMin: Minimum height, in meters. Dashes indicate missing data.
0229302812296440     (See "methods" at the end of the chapter.)
0000000000000000
1976160343505769   :Site :Code      :SpAbbr :Date       :CollID   :Cover :HtMax :HtMin
4554847814214755   :1600 :P1600D04 :Abibal :1989-08-21 :AMB00555 :   - :   5 :   5
2645745581124348   :1600 :P1600D01 :Abibal :1989-08-21 :AMB00604 :   2 :   1 :   1
1076375677295808   :1600 :R1600EA  :Abibal :1989-08-24 :AMB00666 :   3 :   1 :   1
2000445884315808   :1600 :R1600EA  :Abibal :1989-08-24 :AMB00668 :   5 :   6 :   6
0582355170295008   :1600 :R1600EA  :Abibal :1991-08-05 :AMB01719 :   2 :   2 :   2
1485325476235008   :1600 :R1600EA  :Abibal :1991-08-05 :AMB01722 :   4 :   6 :   6
4100414960104041   :1600 :R1600EA  :Acerub :1991-08-05 :AMB01503 :   2 :   2 :   2
5773084583093978   :1600 :P1600B01 :Agrsca :1989-08-25 :AMB00456 :   3 :   2 :   2
4766066289426272   :1600 :P1600D01 :Amerot :1991-06-17 :AMB01439 :   2 :   2 :   2
```

Figure 1. *Excerpt of a sample Centinel data file from a large ecological database, with metadata above and error correcting codes called "centinels" at left. Here colons separate columns rather than vertical bars. In the Centinel structure, error detection and correction stays with the data rather than with the computer medium.*

and supports good data management practices, for example as outlined in [10] and [11].

Centinel is not intended to substitute for large-scale interactive databases undergoing continual manipulation, such as in PostgreSQL, MySQL, or Access. It is, however, a good format for long and medium-term retention of such databases, as Centinel format can be readily exported from them through simple utility programs, and conversely, imported through conventional means or by scanning. Nor is Centinel intended as a complete solution to the problem of storing all data at national and international scales (e.g. [12] [13]), but rather as a solution for individual research and development groups to help maintain their data.

The Centinel format shown in Figure 1 supports the movement of data through place and time. A dataset documented sufficiently with complete descriptions as its metadata, and protected with error correcting "centinels," can be transmitted to another researcher in a distant place without separate documentation and time spent explaining the data, or equivalently it can be transmitted forward to another researcher in the distant future. In other words, it can be archived. Instead of error detecting and correcting codes being applied to the storage media, as is the common method today, codes in Centinel are applied to the data themselves, and stay with the data through all media changes. That simple but unusual characteristic fills a gap in existing data methods and provides confidence in the data across distant places and times. Multiple printed copies of the data can be stored throughout the world and scanned with optical character recognition in the remote future. The centinels, checked automatically against the scanned results, are the essential link to data reliability.

As in some other databases, Centinel has multiple equivalent formats, which we call "singular," "columnar," and "mixed." Long lines of data in singular format can extend onto new lines, indented as in Figure 1. Here is a simpler file in singular format:

```
Class:   1
ID:      123
Age:     21
Region:  SSA

Class:   1
ID:      47
Age:     7
Region:  UK

Class:   2
ID:      723
Age:     70
Region:  US
```

Below are the same data in columnar format:

```
| Class   | ID      | Age     | Region
| 1       | 123     | 21      | SSA
| 1       | 477     | 7       | UK
| 2       | 723     | 70      | US
```

And below is mixed format:

```
Class: 1
| ID      | Age     | Region
| 123     | 21      | SSA
| 477     | 7       | UK

Class: 2
| 723     | 70      | US
```

These formats are interchangeable. The choice is a matter of space, readability, and ease of processing. All software written to handle Centinel data should process the three formats equally.
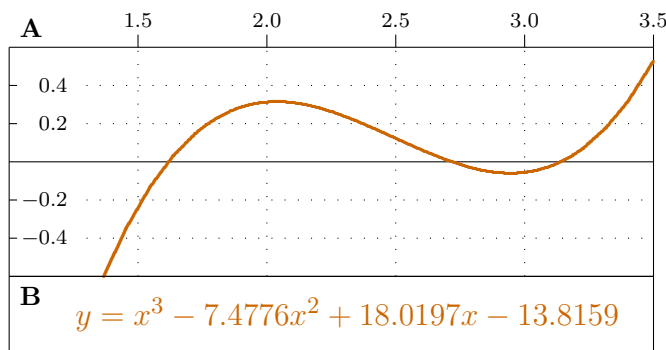
Printed copies of data with error-correcting centinels need not be limited to small data sets. For example, the genome of the fruit fly (*Drosophila melanogaster*), represented with one base-64 symbol for each of its 47 million codons, would require approximately 6000 pages—not absolutely prohibitive to print for an important, expensive dataset. By comparison, the King James Bible is 4.3 million characters, about one-tenth of this genome, and more than one copy of that work has been printed.

3. How Centinel works

Centinel protects data when they are complete and ready to be archived. But it can also be used when the data are first entered, to guard against accidental modifications of datasets undergoing incremental change.

To explain how Centinel works, we must consider what it means for data to be digital. Two properties are essential. First, the data must be represented by "symbols" that have only a finite number of states. Second, the shapes of any two distinct symbols must be separated by a sufficient gap, so that a symbol for one datum does not, except very rarely, degrade into a different symbol for a different datum. Symbols can take various forms—binary 0 and 1 encoded electronically in computer memories are one example of digital data. The Arabic numerals 0–9 printed on paper are another. With these ideas in mind, Figure 2 shows analog versus digital representations of a function, $y = f(x)$.

An analog form on paper could take the form of a graph, Figure 2A. The value on the vertical axis varies smoothly, and can be read to reasonable accuracy with a ruler and a careful eye. However, each time the graph is copied, its accuracy diminishes. The curve becomes successively blurred, the right side may get slightly skewed with respect to the left, and so forth. In contrast, the entire curve in digital form is defined by coefficients, Figure 2B. When this digital version is copied by re-typesetting, it will not degrade, for the individual symbols will be recognized for what they are and reproduced intact. A new font may even change 'x' to 'x', but the meaning of the symbol will remain.

Figure 2. *Non-electronic analog and digital data for the same curve. Printed copies of the digital data (B) will not degrade over time as will the analog version (A) of the same data.*
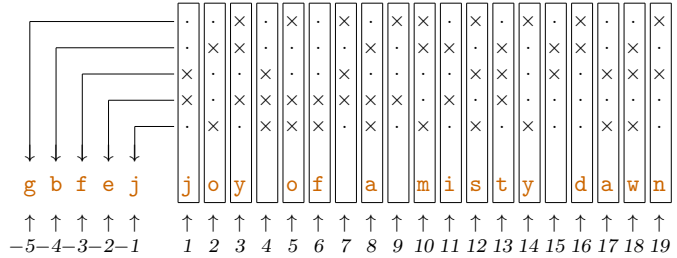
Figure 3. *Error-correcting "centinels" (left) for a 19-character message (right). Each centinel covers a distinct combination of columns, such that any unmatched centinels identify which column is in error and how to correct it. (See code in the appendix for details.)*

Thus digital data are not at all restricted to electronic media, but paper can carry digital data as well, and has done so for millennia. Moreover, some of the most common digital information read by computers today is recorded directly on paper, plastic, metal, and other substrates. The ubiquitous bar code is a case in point, though bar codes are not human-readable as Centinel-protected data are.

A significant separation between symbols in appearance or physical state keeps unavoidable small degradation in information from changing the message, because one symbol does not easily degrade into another. However, separation of symbols is not enough. For highest reliability, error correcting codes must be applied to the digital data to prevent rare alterations of one symbol into another from changing the message, except with negligibly small probability.

Centinel uses a "Hamming code" for arbitrary symbols, a generalization of the original code [14] for binary digits. Such codes we call "centinels," and they appear at the left of each line, at the end of each printed page, and at the end of each file. They can correct any single-symbol error in a line and detect any two-symbol errors. In addition, with high probability they detect multiple-symbol errors, including errors in the centinels themselves.

Each symbol is assigned a small integer and the integers for a given subset of columns are summed. The sum, modulo the number of symbols, is translated back to a symbol, as in columns -1 to -5 of Figure 3. This is repeated for carefully chosen subsets of columns which allow errors to be located and corrected. Then the results are translated to decimal form, as in Figure 1, to mask the actual random combinations of symbols, which by happenstance can spell out any word.

Complete details are in the Centinel algorithms (appendix). These details are part of the metadata and should be included with archived data.

4. Comparison with other approaches

A standard approach to data archiving is a rigorous effort of continually transferring data from old media and old software to new, before the old media and software become completely obsolete—keeping the data "alive" so to speak.

That is called "migration" [12]. It is a practical, well-tested method, though it can be labor intensive and susceptible to catastrophic failure.

Successful migration requires a central discipline maintained over long periods. Any lapse in the chain of migration will result in the complete loss of data. Successful migration will be practical for large, well funded data sets. However, for many small data sets, discipline and funding can easily lapse over long periods of time.

Timing is key, as migration must take place while (1) machines that can read the media still exist, (2) programs encoding the information are still operational, and (3) the media and the information stored on it have not deteriorated.

It follows that the best chance of success in data preservation will be for (1) media that require no advanced or specialized machinery to read them, (2) formats that require no complex computer programs to process them, or at worst require the simplest programs that can be described completely in a few pages of text, as in the Centinel algorithm (appendix), and (3) media and encoding methods that will themselves last a century or more. Centinel allows data preservation with a single migration.

A second method is called "encapsulation." Fully successful migration to new media will be worthless if the software that accesses the data ceases to exist. For example, an organization producing software may go out of existence and no other organization may support the old format. This has happened repeatedly in the history of computing. Encapsulation aims to include with the data all software that accesses the data, in a form that can be translated to future machinery. That is, of course, easiest when the corresponding software is as limited as possible.

Two other methods proposed for data archiving are "emulation" and "technology-preservation." In emulation, the complete hardware and software architectures to retrieve the data are migrated forward with the data and "emulated" on the future system. That practice was widespread and successful among mainframe computers in the 1960s, where one generation of computers would emulate the hardware of the generation before. But as computers become increasingly complex in their architecture and operating software, it becomes difficult to make this practical into the indefinite future.

In technology-preservation, the actual hardware and software is preserved, museum-style, along with the data for future access. This is problematic, however, for today's computers are built for the moment, not built to last, and may not even boot up properly after a decade of disuse.

Therefore, emulation and technology-preservation are not related to Centinel, but migration and encapsulation are. Centinel implements encapsulation in the simplest form—under 100 lines of code (appendix)—and with a single migration, creates digital documents that last as long as possible—up to a century or more.

5. Suggestions

In conclusion, we offer the following: (1) To keep electronic data safe, prepare early for archiving. (2) Archive data in the simplest formats possible. (3) Document data to the highest standards. (4) Associate documentation directly with the data it describes, ideally in the same file. (5) Keep multiple copies in separate locations. (6) Regularly convert working files from proprietary databases to archival format. (7) Keep printed copies of critical data, with Centinel-like guard symbols and documentation for future recovery.

For full details and utility programs supporting this project, see www.cbs.umn.edu/centinel.

6. Acknowledgements

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7. Appendix: The Centinel algorithm

The complete algorithm that encapsulates Centinel files is given here in a subset K&R C [15]. The material below, together with Kernighan and Ritchie's book, should allow the algorithm to be transcribed into future programming languages and the data to be extracted from Centinel files as long as the printed form is extant.

The algorithm adds an error-correcting code to each line of a text-based file, another to each page, and a third to the entire file. Each output line begins with a decimal error correcting code guarding that line, and also guarding the error correcting code itself, then the text of the line. In printed form another decimal code guards the entire page and a third guards the entire file.

In computing the error correcting code, leading and trailing white space is skipped, multiple blanks count as a single blank, and end-of-line codes are not counted. The code at the beginning of the line is not counted either. The assignment between symbols and numbers is specified in array *s* below, where 'a' is number 1, 'b' is number 2, 'A' is number 27, and so forth. Any similar assignment could be substituted.

In the algorithms below, flow control and reserved words are bolded, variables and function names are italicized, and certain operations such as '<=', '>=', '!=', and '==' are displayed in a mathematical form as '$\leq$', '$\geq$', '$\neq$', and '$\equiv$', respectively.

DATA STRUCTURES

#**define** *C*	256	1. Maximum character code plus 1.
#**define** *L*	120	2. Maximum data length, excluding guard symbols.
#**define** *G*	8	3. Number of guard symbols.
#**define** *COL*	9	4. Number of symbols columns displayed on the page.
#**define** *PAGEL*	50	5. Number of lines per page.
#**define** *IDENT*	127	6. Identity symbol.

char *s*[] =   7. Character set available for present application.
```
"_abcdefghijklmnopqrstuvwxyzABCDEFGHIJKLMNOPQRSTUVWXYZ0123456789"
" .,;:!?+-*/\\=\"'()[]{}<>^&%|";
```

int *nchar*;	8. Maximum number of characters in present application.
char *seq*[*C*];	9. Sequence number for each symbol in the set.
char *f*[*C*][*C*];	10. Modulo sum and difference tables.
char *ptn*[*L*][*G* + 1];	11. Pattern of guard symbols for each position.
int *pagef* = *PAGEL*;	12. Number of lines on first page.
int *pages* = *PAGEL*;	13. Number of lines per subsequent page.
int *ipage* = 0;	14. Page index.
int *ifile* = 0;	15. File index.
char *in*[*L* + 1];	16. Input line.
char *line*[*L* + 1], *page*[*L* + 1], *file*[*L* + 1];	17. Current line, page, and file.
char *guard*[*G* + 1];	18. Guard symbols, individual characters.

END OF PAGE

Upon entry to the algorithm, (1) *page* contains a list of symbols representing the current page. **(2)** *ipage* indexes the next entry for the page. **(3)** *a* is set if a blank line should follow the code, indicating end of page. (This is not used on the last page of the file, because the code for the entire file follows immediately.) **At exit, (1)** Guard symbols for the page are displayed. **(2)** *guard* is destroyed. **(3)** *ipage* is set to zero.

```
seqpage(a) int a;
{
   if (ipage ≡ 0) return;
   page[ipage] = 0; ecc(guard, page);
   seqn(guard, "; ", ""); if (a) printf("\n");
   ipage = 0; }
```

MAIN PROGRAM

```
main(argc, argv) int argc; char *argv[ ];
{ char c; int i, j, k;

    if (argc > 1)
    { pagef = atoi(argv[1]);
      if (pagef < 2 || pagef > 100) pagef = PAGEL;
      pages = pagef; }

    if (argc > 2)
    { pagef = atoi(argv[2]);
      if (pagef < 2 || pagef > 100) pagef = PAGEL; }

    s[0] = IDENT;
    for (i = 0; s[i]; i = i + 1) seq[s[i]] = i;
    nchar = i;

    for (i = 0; i < C; i = i + 1)
    for (j = 0; j < C; j = j + 1)
      f[i][j] = IDENT;

    for (i = 0; s[i]; i = i + 1)
    for (j = 0; s[j]; j = j + 1)
    { k = i + j; if (k ≥ nchar) k = k − nchar;
      f[s[i]][s[j]] = s[k]; }

    for (i = 3; i ≤ 7; i = i + 2) colgen(i, G − 1);
    ipage = 0; ifile = 0;

    while (fgets(in, L, stdin))
    { i = strlen(in);
      if (in[i − 1] ≡' \n') in[i − 1] = 0;

      line[0] =' −';
      for (i = j = 0; in[i]; i ++ )
      { c = in[i]; if (seq[c] ≡ 0) c =' ';
        if (line[j] ≡' ' && c ≡' ') continue;
        line[ ++j] = c; }
      line[ ++j] = 0;

      ecc(guard, line + 1); seqn(guard, "", in);

      page[ipage ++] = guard[G − 1];
      if (ipage ≥ pagef) seqpage(1), pagef = pages;

      file[ifile ++] = guard[G − 1];
      if (ifile ≥ L) ifile = ifile − 1; }
    seqpage(0); file[ifile] = 0;
    ecc(guard, file); seqn(guard, ".", "");
    ifile = 0; }
```

1. If an entry parameter has been supplied, take it to be the page length.

2. Determine the number of symbols in the set while developing a list of sequence numbers.

3. Clear the modulo addition table.

4. Construct tables mapping all symbol pairs to corresponding sums.

5. Generate odd guard patterns.

6. Compute the error-correcting code for the line.

7. Compress multiple blanks from the input line.

8. Compute the ECC guard symbols.

9. If this is the end of the page, prepare a code for the entire

10. If this is the end of the page, prepare a code for the entire

11. At the end of the file, prepare a code for the entire file.

COMPUTE CENTINELS

Upon entry to the algorithm, (1) *gs* points to an area of length $G + 1$ to receive the results. **(2)** *line* points to the line. **(3)** G defines the number of guard digits to be computed. **(4)** *ptn* defines which line positions contribute to which guard digits. **(5)** f contains the modulo-addition table for all symbols. **At exit,** *gs* contains the guard symbols for the line.

```
ecc(gs, line) char *gs, *line;
{ int i, j;

    for (i = 0; i < G; i = i + 1) gs[i] = IDENT;

    for (i = 0; i < G; i = i + 1)
    for (j = 0; line[j]; j = j + 1)
      if (ptn[j][i] ≡' X')
        gs[i] = f[gs[i]][line[j]];
    gs[G] = 0; }
```

1. Clear all the guard symbols.

2. Generate each guard symbols.
3. following the table that shows which line positions contribute to which guard symbols.

CONVERT CENTINELS TO INTEGERS

Upon entry to the algorithm, (1) gs contains the guard symbols. **(2)** sep contains a separator character. **(3)** sym contains the string of symbols. **At exit,** gn contains the corresponding integer sequence numbers.

$seqn(gs,\ sep,\ sym)$ **char** $*gs,\ *sep,\ *sym;$
{ **int** $i;$

    for $(i = 0;\ i < G;\ i = i + 1)$ 1. Display the sequence numbers
      $printf("\%02d",\ seq[gs[i]]);$ for the guard symbols.

    $printf("\%s\%s\backslash n",\ sep,\ sym);$ }   2. Display the full line.

GENERATE PERMUTATIONS

Upon entry to the algorithm, (1) n defines the number of guard symbols to be marked. **(2)** k defines the position for the initial mark. **(3)** l defines the column number on the line, starting with 0. **(4)** ptn contains an area to receive the permutations. **(5)** w contains a work area for generating the permutations. **At exit, (1)** All permutations have been generated. **(2)** l is advanced by the number of combinations generated. **(3)** $ptn[0..l]$ contains the permutations generated thus far. **(4)** w contains the most recent permutation generated.

$colgen(n,\ k)$ **int** $n,\ k;$
{ **static char** $w[G + 1] = "";$ **static int** $l = 0;$ **int** $i;$

    if $(w[0] \equiv 0)$ 1. On the first call, establish a
      for $(i = 0;\ i < G;\ i = i + 1)\ w[i] ='\ -';$ null pattern in the array.

    if $(n > 0)$ **for** $(i = k;\ i \geq n - 1;\ i = i - 1)$ 2. Mark the guard symbol for each
    { $w[i] ='\ X';$ possible position and generate
      $colgen(n - 1,\ i - 1);$ all permutations within that
      $w[i] ='\ -';$ }   position.

    else if $(l < L)$ 3. If there are no deeper
    { **for** $(i = 0;\ i < G;\ i = i + 1)$ permutations, save the current
      $ptn[l][i] = w[i];$ permutation and advance the
      $l = l + 1;$ }}   column number.

Using the Centinel Data Format
to Decouple Data Creation from Data Processing
in Scientific Programs

Clarence Lehman[1] **and Adrienne Keen**[2]
[1]University of Minnesota, 123 Snyder Hall, Saint Paul, MN 55108, USA
[2]London School of Hygiene and Tropical Medicine, Keppel St., London WC1E 7HT, UK

"Software is hard. It's harder than anything else I've ever had to do."
—Donald Knuth, 2002

Abstract—*Multi-dimensional numerical arrays are a staple of many scientific computer programs, where processing may be intricate but where data structures can be simple. Data for these arrays may be read into the program from text files assembled in advance, often laboriously from multiple sources or from large-scale databases. Notwithstanding simplicity in the structure of such files, their multi-dimensional nature and the very regularity of their data makes it difficult or impossible to know by inspection that they are assembled exactly as required by the processing programs. Moreover, data errors inadvertently may appear through unintended alteration of some parts of a file while other parts intentionally are being edited. Verifying the correctness of scientific programs is hindered by such difficulties. Here we describe how we have applied the Centinel archival data format to such problems. Centinel (1) provides a format that can be read without difficulty by both people and computers, (2) keeps all metadata locally in the same files as the data themselves, and (3) optionally protects the data with error correcting codes on each row, from the time the data are prepared until they are finally processed. In addition, we show how we have used the Centinel format to produce prototypes of large datasets for initial program testing before the actual data have been prepared. This effort is one step in the uncompromising process of ensuring that complex scientific programs rigorously perform the tasks they are intended to do.*

Keywords: data and metadata, code and metacode, scientific programming, software validation, database, data archives

1. Introduction

Consider the following two files made available to a computer program, each containing an $8{\times}8$ matrix of hypothetical average temperature measurements at points along a latitude line and at times throughout a season. The program expects distances to be represented in successive matrix rows and time in successive columns. In this example, the temperatures increase north to south (top to bottom in the array) and also increase as the season progresses (left to right in the array).

The two files below are identical, just with rows and columns transposed. Suppose one is correct and the other is not. Once read by the program, the data act as parameters, so that verifying the correctness of the program includes knowing the correctness of this data. The question is, how can one verify by inspection which of the two matrices has the correct format and will generate legitimate results in the program?

Input File 1:

18.9	20.7	22.4	24.9	21.7	24.0	23.1	24.9
21.1	24.0	24.2	21.6	25.0	23.0	23.8	24.2
21.7	20.9	24.8	22.7	26.4	25.0	24.8	28.4
23.9	21.7	22.9	24.1	27.0	25.8	28.3	27.6
23.8	22.5	24.8	26.3	25.1	26.9	29.6	29.1
24.3	27.0	25.3	26.2	26.0	25.9	27.0	29.9
25.1	27.2	26.3	28.8	27.4	28.3	29.7	28.6
27.7	27.5	28.2	25.8	29.5	26.5	27.3	30.6

Input File 2:

18.9	21.1	21.7	23.9	23.8	24.3	25.1	27.7
20.7	24.0	20.9	21.7	22.5	27.0	27.2	27.5
22.4	24.2	24.8	22.9	24.8	25.3	26.3	28.2
24.9	21.6	22.7	24.1	26.3	26.2	28.8	25.8
21.7	25.0	26.4	27.0	25.1	26.0	27.4	29.5
24.0	23.0	25.0	25.8	26.9	25.9	28.3	26.5
23.1	23.8	24.8	28.3	29.6	27.0	29.7	27.3
24.9	24.2	28.4	27.6	29.1	29.9	28.6	30.6

The answer is simple. One cannot. Without digging deeper into the processes that created the data files, one cannot know whether rows in the file represent distance and columns represent time, or vice versa. Nor can one be certain of what temperature units are represented. Celsius is plausible if this is a temperate region, but Fahrenheit is equally plausible if this is the subarctic. How the axes are scaled and other basic information about the data are also missing. In the absence

of such information, data development becomes undesirably coupled with software development.

The problems are ameliorated but not solved with "database connectors"—software to access databases from within processing programs. Careful discipline beyond the basic requirements of the database is needed at every step to guarantee that the encoding of the data is known, data transformations are specified, units are clear, and a variety of other items are documented that can otherwise remain underspecified.

Associated mistakes can be spectacular. An unmanned spacecraft vanished in 1999 after a ten-month interplanetary journey, breaking into pieces and burning in the Martian atmosphere in part because some of the units expected by the program did not match those provided in the data (conflicts between English and metric systems) [1]. "Our inability to recognize and correct this simple error has had major implications," according to then-director of JPL, Edward Stone [2]. Results in other scientific programs may be less spectacular but of equal or greater moment. Simulations informing national programs for vaccination and disease control, for instance, or estimating potential climate change from biophysical parameters, can affect millions of people.

In this paper we illustrate the problem and its solution with basic software we developed to connect data that is stored in the Centinal archival format [3]. This software may be used directly in C programs or transcribed to serve other languages. The principles apply to programs that connect to any database.

2. Methods and results

2.1 Problem details

The two sample matrices above are an idealization of an actual situation we confronted in a large-scale scientific simulation developed by one of us (A.K., mathematical model for tuberculosis in the UK [4]). The first version of the simulation program had a standard input specification, represented below in a C-like programming language. The plausible correctness of the program can be verified by inspection.

define N 8
float $a[N][N]$; //Celsius array, $a[g][t]$.
for $(g=0;\ g<N;\ g$++)
  for $(t=0;\ t<N;\ t$++)   (1)
    if $(scanf(\texttt{"\%f"},\ \&a[g][t]) < 1)$
      $ExitMsg(1);$

The input (File 1) can also be inspected—eight lines with eight numbers on each—which matches the program above. The doubly nested loop reads each number on a line into the t dimension, then reads subsequent lines into the g dimension of the array a. Inspection of the code shows that the input file cannot overflow the array, and that missing or non-numeric values will be detected and the subroutine

ExitMsg will be notified to handle them, typically by issuing an error message and terminating the operation.

However, the reason we said *plausible correctness* is that one cannot know by inspection of the data and the code that the order of the loops is correct, nor that the units are indeed Celsius as the program expects. The danger is easy to identify in this basic example, but the dimensionality of arrays in practice commonly grows to five or more and the dangers of undetected errors compound.

2.2 A basic solution

We sought general ways of decoupling the processes of (1) creating the data and verifying the correctness of the created data, and (2) writing the computer program and verifying the correctness of the program's code. Our solution was simple in concept and not difficult to accomplish. We inserted a "decoupling step" between the data and the program, with two components: (1) computer- and human-readable metadata maintained within the file and (2) software that processes not only the data but parts of the metadata as well. Below is an example of File 1 in Centinel format.

Centinel Version of File 1:

2976573	Dataset: Seasonal omega-transformed temperatures.
6519832	Description: This is purely a sample dataset constructed
0823811	for illustration. The data are quite imaginary.
3097624	Label a: Average temperature over time t, location g,
6421009	in degrees Celsius, omega-transformed.
2347567	Label t: Time, two-week intervals from March 21.
2785463	(0=Mar21–Apr03, 7=Jun27–Jul10)
1127554	Label g: Geographic location, half-degree quadrangles
5437743	from the 45th parallel north centered on
8620815	the 100th meridian west.
6584390	(0=45.0–45.5°N, 7=49.0–49.5°N)

		g	a:t=0	a:t=1	a:t=2	a:t=3	a:t=4	a:t=5	a:t=6	a:t=7
9307204										
8217764		7	18.9	20.7	22.4	24.9	21.7	24.0	23.1	24.9
6802135		6	21.1	24.0	24.2	21.6	25.0	23.0	23.8	24.2
1493093		5	21.7	20.9	24.8	22.7	26.4	25.0	24.8	28.4
7564407		4	23.9	21.7	22.9	24.1	27.0	25.8	28.3	27.6
4186572		3	23.8	22.5	24.8	26.3	25.1	26.9	29.6	29.1
3622154		2	24.3	27.0	25.3	26.2	26.0	25.9	27.0	29.9
5894658		1	25.1	27.2	26.3	28.8	27.4	28.3	29.7	28.6
9717717		0	27.7	27.5	28.2	25.8	29.5	26.5	27.3	30.6

Centinel files are ASCII text with three parts: (1) An optional column of numbers at the far left above, which represent error-correcting codes called "centinels." They guard each line against accidental alterations [3]. If the first character of a Centinel file is not a digit '0' to '9', then the column is not included and the file consists only of data and metadata. (2) Metadata, at the top of the example above and to the right of the column of centinels. Metadata describes the data to people and, in certain cases, to computer programs that may process parts of it. Metadata have "keyword–colon–data" format, with indented lines continuing the line above. The last line of metadata contains headings that define

the contents of each column of data. (3) Data, with data elements separated by vertical bars. In this case a column at the left defines the index for each row.

The column of numbers labeled 'g' defines the geographic location of each data element on the line, as described in the metadata above it. Each of the 64 data elements in the array is identified with its geographic location, in column 'g', and with its time, in the column headings marked 'a:t=0' through 'a:t=7'. Each such column heading contains the value of the label to the left of the colon ('a') indexed by the label to the right of the colon ('t') at the index specified to the right of the equal sign. Thus the value in the upper left corner of the data block is $a[g][t] = a[7][0] = 18.9$, the value immediately to its right is $a[7][1] = 20.7$, and so forth until the value in the lower right corner is $a[0][7] = 30.6$. In this way the file is self-defining and the following call to subroutine *Centinel* is sufficient to read it into the array.

define N 8
float $a[N][N]$; //Celsius array, $a[g][t]$.
char $b[\,] = $ "a[g=0~7][t=0~7]";　　　　　　　　(2)
if ($Centinel\,(a,\ b,$ "omega.txt") $\neq 0$) $ExitMsg(1)$;

The second line in the code above specifies the array a and its indexes for the compiler, as before. The third line specifies the array and its indexes for the subroutine *Centinel*, which reads the file. Thus the second line says, "The array a has eight rows indexed by label g in the file and eight columns each indexed by label t in the file." The third line calls the subroutine *Centinel* to read the file. Its first parameter specifies the array to receive the data, in this case a, its second parameter defines the structure of the array and names the index values, and its third parameter is the name of the file to be read. Free source-code copies of the software are available from the authors upon request.

2.3 Equivalent transposed format

We have shown a sample matrix and its transposition, which could not be reliably distinguished, then showed how the first form of the matrix could be reliably represented. For completeness, below is the transposed form of the same matrix, which can also be read with the same call to the subroutine *Centinel*. No changes to the program are needed.

3515117	t	a:g=7	a:g=6	a:g=5	a:g=4	a:g=3	a:g=2	a:g=1	a:g=0
7125262	0	18.9	21.1	21.7	23.9	23.8	24.3	25.1	27.7
0961535	1	20.7	24.0	20.9	21.7	22.5	27.0	27.2	27.5
3303666	2	22.4	24.2	24.8	22.9	24.8	25.3	26.3	28.2
9369193	3	24.9	21.6	22.7	24.1	26.3	26.2	28.8	25.8
8881518	4	21.7	25.0	26.4	27.0	25.1	26.0	27.4	29.5
2627646	5	24.0	23.0	25.0	25.8	26.9	25.9	28.3	26.5
2756293	6	23.1	23.8	24.8	28.3	29.6	27.0	29.7	27.3
9655049	7	24.9	24.2	28.4	27.6	29.1	29.9	28.6	30.6

All lines of metadata but the heading line are identical and therefore not shown again here. Notice that the only differences in the remainder are in the labels on the heading line and in the column for 't', and in the centinels. Those are sufficient to allow the software to load the data into the proper locations of the program's array.

2.4 Equivalent relational format

Any format that properly specifies the data will work. In particular, an ordinary relational database format can be used with the subroutine *Centinel*, as depicted below. We have not used this format in our work nor in this explanation, however, since it is much less compact and therefore harder to examine visually.

2393973	g	t	a
9788239	0	0	27.7
3291521	0	1	27.5
0461845	0	2	28.2
8743319	0	3	25.8
2912616	0	4	29.5
5291316	0	5	26.5
6321936	0	6	27.3
1876497	0	7	30.6
4415933	1	0	25.1
2860027	1	1	27.2
⋮	⋮	⋮	
0270284	7	5	24.0
2154910	7	6	23.1
2712213	7	7	24.9

2.5 Over and under specification

The datafile may contain more data than the array contains. Data corresponding to array indexes that are out of bounds are ignored, as defined in the specifier b. An error indicator will be returned if requested. Also, any labels in the file that are not part of the array are ignored. These are "over-specified" files that contain more information than needed. They allow different parts of a single file to be loaded into different arrays, for example.

Files may also be "underspecified," in that they do not contain enough information to fill the array. For example, any of the three files above could be divided into eight separate files, one for each column of the matrix. When each was read, it would fill in only its column of the array. Multiple files may thus be combined into a single array—convenient for some organizations of data. Of course, in all cases care must be taken not to leave parts of the array undefined.

2.6 Prototyping

The datasets we have shown thus far have single integer indexes in each location. In addition, sequences and ranges of integers can be used in each location, for the purpose of prototyping. Often a program will be ready for partial testing before its data are fully available. We included basic prototyping in the *Centinel* algorithm to allow this.

A set of indexes can be a range of integers separated by a tilde, written 'n_1~n_2', where the n_i are integers, or a

sequence, written '$m_1, m_2, m_3, \ldots, m_k$', where the m_i are integers or ranges of integers. Here are some examples:

Specification	Indexes represented
1	1
0, 1	0 1
0~1	0 1
0, 3~9, 40~38, 2	0 3 4 5 6 7 8 9 40 39 38 2

The example below is related to an actual dataset we used, where a collection of probabilities, p, is indexed in four dimensions by a region $0 \le region \le 2$, a relative year $0 \le year \le 95$, a state $0 \le q \le 8$, and a class $0 \le c \le 3$. This is an array of $3 \cdot 96 \cdot 9 \cdot 4 = 10{,}368$ elements. When the data became available and completely encoded, each array element had its own distinct probability value, but in the meantime program development needed to continue. A file like the following, with appropriate additional descriptive metadata, sufficed for initial testing.

region	year	q	p:c=0	p:c=1	p:c=2	p:c=3	
0~2	0~95	0	0	0	0	0	(line 1)
0	0~95	1~7	0.80	0.60	0.79	0.89	(line 2)
1, 2	0~95	1~7	0.84	0.71	0.88	0.99	(line 3)
0~2	0~95	8	1	1	1	1	(line 4)

When the above file is read, every subarray for $q = 0$ is set to zero (by line 1) and every subarray for $q = 8$ is set to one (by line 4). Of the remaining elements in the array, every subarray for $region = 0$ is set to the vector $0.80, 0.60, 0.79, 0.89$ for $c = 0, 1, 2, 3$ (by line 2), and the remainder is set to the vector $0.84, 0.71, 0.88, 0.99$, for the same values of c (by line 3). Thus the array can be filled initially with appropriate "placeholders." As data are developed, the file can be filled out and the program further tested, until all placeholders are withdrawn and the full 10,368 array entries are individually specified.

2.7 Error correction

The optional error-correcting codes represented by numbers to the left of the lines of data and metadata are "Hamming codes" [5], originally designed for 0–1 bits but redesigned in Centinel for symbols. They allow (1) any single-character error on a line to be corrected, (2) any double-character error to be detected, and (3) the overwhelming majority of multi-character errors also to be detected. The codes are created by the Centinel algorithm [3] or by a text editor that supports the Centinal algorithm.

As mentioned earlier, they guard against accidental modification of one piece of data while editing another. They also make printed copies of the data into reliable long-term storage media for archiving the data. Printed copies of the data can be scanned and verified long into the future, with no intervening migration or maintenance of the data necessary [3].

3. Discussion

3.1 Correctness of scientific programs

Writing software that works is one of the most difficult of human endeavors, and scientific software is at a special disadvantage. Whereas commercial and engineering software can be very complex, its desired behavior can be specified in advance. For example, if a spreadsheet operation is intended to produce the sum of a column of numbers, it is possible to determine whether it is actually doing so. That is, testing is possible. In scientific software, however, testing is often impossible. The program's behavior is often not known because the behavior of the natural system being simulated is not known. Indeed, the whole purpose of the simulation program is to determine how the system behaves.

One aspect among several is "correctness proving." [6] [7] [8] This topic has been well discussed but less well practiced. An essential part is partitioning the software into manageable pieces and documenting each piece so that its correctness can be verified. The ideas discussed in this paper are part of that process—because data read by the program as parameters become part of the program, the program's correctness in turn depends on the data's correctness. Thus the data must also be partitioned into manageable pieces and documented.

The goal is to restrict the range of attention to what can be understood by the human mind in one review session. In software, this can be accomplished by adding "metacode" to the code, describing, among other things, full entry and exit conditions for every module, no matter how small. For data, it can be accomplished by partitioning the data and encapsulating each partition with metadata, as described here.

3.2 Centinel and other forms

This approach can be applied to any database and any programming language that can connect with that database. However, methods such as we have described for partitioning the data into manageable pieces, for documenting it, and optionally for guarding it against unintended alteration, are important with any database. Column names and row names are not required by common spreadsheet software, and spreadsheets for important data are sometimes prepared with little more information than in the sample matrix files shown at the beginning of this paper.

Centinel files may be constructed directly with a text editor. More commonly they are assembled by collections of programs and scripting languages, from databases or from spreadsheets. When created from spreadsheets, column 1 of the spreadsheet can be used solely for metadata, with all actual data beginning in column 2. Then when the files are saved, for example as tab-separated text files, and after the tabs are translated to vertical bars, each actual data line will begin with a vertical bar. Centinel formats can thus be

transferred back and forth to spreadsheet programs without loss of data in either direction.

3.3 Database labels

It is useful to label data elements in the file so that they exactly match corresponding variable names in the program. Doing so means restricting labels to letters and digits, beginning with a letter, and possibly supplemented with optional characters such as underscores. That way no confusion will arise between variables in the program and labels in the database. There is a tendency to try to include metadata in the names of data elements in the database, especially with spreadsheets. For example, a spreadsheet column might be named "%cover-no litter". This is inadvisable for several reasons: (1) even a moderately large amount of metadata in the label is still insufficient to understand what the field really contains; (2) the label will need special characters such as period, hyphen, percent sign, and blank, which have special meanings in most programming languages; and (3) the long label induces a wide column, or alternatively forces part of the label to be hidden.

The approach we use and recommend here is to make a small distinct label, such as in this case "pcover", with metadata like, "Label pcover: Percentage of the area covered by the species in question, when viewed from directly above, relative to the area occupied by living plants (the area not occupied by leaf litter or bare soil)." Not much less than that amount of metadata is necessary for someone familiar with the data to understand what that data element represents, and that amount is too long for a label. Therefore, the better strategy is to use short data labels with ample metadata descriptions carried separately in the file.

3.4 Database metadata

In popular database management systems such as MySQL [9], metadata of the type we advocate can be added, though often not in the same file as the data. At the time of this writing, metadata elements that can be stored as comments in MySQL files are limited to one line of text each and thus are difficult to use for complete metadata. It is always possible to set up special tables to contain the metadata, but that presents other difficulties, for it is harder to maintain metadata when it is in a separate file.

We feel it is important to specify the metadata while creating the data. That is, after all, when the structure and meaning of the data are known. Writing it down then is only incremental time; writing it down later is re-creating a thought process that has already been completed once. Fine details of data and code evaporate from the mind with disappointing ease. Data structures should be defined as carefully as possible beforehand, although achieving good data structures, like good computer programs, can be an iterative process. The best practice is that documentation of the data be maintained at each step of the iteration.

3.5 Database connections versus files

Even when working with a large-scale database that can connect to the program, there is merit in creating files of the Centinel type for communication with the program. Those files fully document the data that will lead to conclusions drawn from the program, and can be used in supplemental material submitted with any publications that result. Recorded in Centinel format, they will be ready for long-term archiving, along with the scientific publication itself. (See example in Appendix.)

Too often, when such files are not created, subsequent changes in the dataset used to draw the conclusions will make it difficult for anyone, including the original authors, to replicate precisely the results. This can make it impossible to precisely compare former conclusions with new conclusions that may arise as conditions change, and may occasionally call into question the original results.

Many other considerations in constructing databases and documenting them lie beyond the scope of our purposes here, but appear in other publications [10] [11] [12].

3.6 Non-relational data

Up to this point we have emphasized ordinary scientific data as stored in relational databases, but any kind of data can be represented in the form we have described. That form allows the data to be written and read directly by simple computer programs and to take forms that adapt to various requirements. As an example, the Centinel format has been applied to large-scale photographic radar images, which can have 10^5 or more levels per spectral band and more than three spectral bands, and exceed the limits of simple image formats such as JPEG and PNG. An image can be represented as a rectangular array of colors, with each color being a set of numeric values. Below is an excerpt from a large array of satellite radar elevation measurements from public NASA databases.

Title:	Earth at maximal ice melt
Contents:	Pixel array, 4320 x 2160

| Spectral bands:	3
Bits per band:	32
Wavelengths:	RGB standard
Resolution:	1/12 degree, latitude and longitude
Data source:	NASA STMR30 database
Produced by:	flood.c
Label Lat:	Latitude band in 1/12 degree resolution. Elevation in meters, Lat0=90N, Lat2160=90S.
Label Lon:	Longitude band in 1/12 degree resolution. Elevation in meters, Lon0=180W, Lon2160=0, Lon4320=180E

Lat	Lon0	Lon1	Lon2	Lon3	Lon4	Lon5	..
0	0,10,880	0,10,880	0,10,880	0,10,880	0,10,880	0,10,880	..
1	0,10,880	0,10,880	0,10,880	0,10,880	0,10,880	0,10,880	..
539	0,10,880	0,10,880	65.66	67.93	85.21	129.23	..
540	0,10,880	0,10,880	76.52	100.53	109.73	129.9	..
:							
2159	2605.37	2605.95	2606.52	2607.23	2607.79	2608.44	..

Ellipsis symbols in the example above (':' and '..') represent material in the file that is not shown here for brevity. Each data element consists of a string of comma-separated numbers following a vertical bar, each number specifying a spectral band. If there are fewer numbers than spectral bands, the last number is taken to be repeated. Thus single numbers represent monochromatic pixels. In this example colors were only used to represent blue water and red coastlines, the remainder representing elevations in meters as monochrome intensities.

The full file is approximately 80 MB uncompressed. Converted to a pixel image, it appears as the following map. As a point of interest, the data represent the results of a flood-fill algorithm estimating coastlines of the planet if all the glacial ice were distributed as water to the oceans.
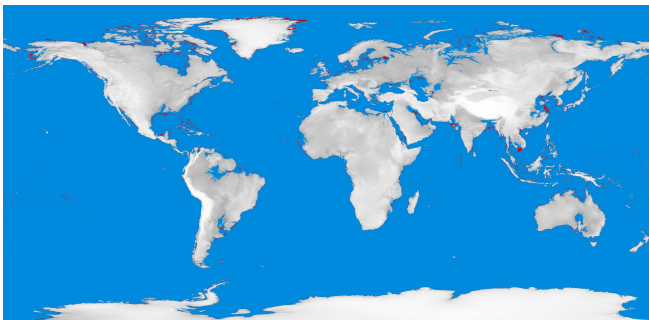
The point of the example above is that data of many kinds can be treated by the methods we describe in this paper, beyond data that are usually considered relational database material.

4. Conclusions

By applying methods of judiciously organizing data and metadata, the processes of data development and software development can be separated. The consequence is data that are better defined, programs that are more often correct, and results that are replicable. Based on the problems and solutions discussed in this paper, we make the following suggestions and recommendations.

A) Use metadata to disentangle data construction from data usage, including data input to scientific programs.

B) Maintain data formats that people can read with ease and computers can access with simple algorithms.

C) Develop metadata concurrently with data collection.

D) Store metadata in the same files as the data themselves.

E) Use data prototyping to test programs before all data are available.

F) Resist the temptation to embed metadata within data labels. Keep labels simple.

G) Maintain archival copies as snapshots of evolving data—especially data used in reaching published scientific conclusions.

H) Include error-correcting codes in archival data to assure integrity independent of changing storage media.

This method has been practical and useful in reducing or eliminating data errors in large-scale simulations [4] and we recommend it for use and extension by others. Code for the functions described here and for related query and maintenance operations on the Centinel format is available free in compilable source files from the authors upon request.

5. Acknowledgements

6. Contributions

A. Keen wrote the simulation programs that inspired the present paper and prepared corresponding simulation data in the format explained here. C. Lehman coded the software for reading Centinel data into scientific programs. Both authors contributed to the development of the Centinel data format and the manuscript.

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7. Appendix

Below is a sample excerpt of a file in Centinel format, used as input to scientific analyses and showing the style of metadata and data specification. At the left of each line are the optional "centinels," error detecting and correcting codes that accompany the file as it is transferred across media, supplementing any such codes that may be part of specific computer media. Thus even printed copies of the file that may be retained indefinitely into the future can be subsequently scanned and the data recovered with the full reliability of any computer medium. Lines beginning with a vertical bar

to the right of the centinel codes are data, in columnar format. Other lines are metadata, describing the data sufficiently well to be understood by a worker in the field who may be accessing the data from a remote place or time. Metadata have "keyword–colon–data" format, with indented lines continuing the line above. Keywords are chosen to fit the data and the needs of processing programs. For example, "Label" is used by query and other database management programs that process the Centinel format. An automatic summary line at the end guards against missing or duplicate lines.

```
3255845646594753   Dataset:    Peatland dates and depths
8969865226586934   By:         Art Dyke, Eville Gorham, Jan Janssens
8286898747137843   Date:       September 15, 2012
0000000000000000
1314776168875326   Contents: Age, depth, and location data for North American
8620213562356287     peatlands. Please consult the publication below for
0901842416681217     details.
0000000000000000
5827730880685764   Publication: This is the archival dataset for "Long-Term
1520774603075243     Carbon Sequestration in North American Peatlands," Gorham,
7259216113317888     Lehman, Dyke, Clymo, and Janssens, Quaternary Science
7505773863167388     Reviews, 2012, doi 10.1016/j.qsciref.2012.09.018.
0000000000000000
1884884179373648   Format: This file is recorded in Centinel format, which is
1043670634366401     for immediate use and long term archiving. The numbers at
6812166714427858     the left are error-correcting and error-detecting codes to
4037101440275922     help ensure that inadvertent alterations of the file will
7313760841625847     not go undetected. See Lehman, Williams, and Keen (2012),
4508626531434354     "The Centinel Data Format: Reliably Communicating through
6206860589148901     Time and Place," International Conference on Information
1864531348064250     and Knowledge Engineering, IKE 12:47-53, Proceedings.
0000000000000000
7770288188098974   Label ID:     Unique identifier for the sample.
0000000000000000
7740581166254016   Label Lat:    Latitude, degrees north of the equator.
6908751974650935                 Negative is south latitude.
0000000000000000
6204302802774740   Label Lon:    Longitude, degrees east of the prime meridian.
4064257255528647                 Negative is west longitude.
0000000000000000
6239741042826543   Label Depth:  Depth of the peatland in centimeters.
0000000000000000
4325213022514926   Label CalBP:  Date of peatland initiation, calendar years
2283760574804679                 before present, reckoned as 1950. Calculated
5705568955445847                 using 2004 international calibration methods.
0000000000000000
4024078842187041   Label Line:   Serial line number.
0000000000000000
5384013588094368   |ID          |Lat        |Lon        |Depth   |CalBP   |Line
8963026933143738   |A-1112      |41.5       |-113.5     | 707.5  |14367   |    1
2355144908347452   |A-2143      |63.33      |-149.     |    .   |14046   |    2
3055234629388956   |A-2147      |63.33      |-149.     |    .   | 6643   |    3
4680358064467548   |A-2163      |63.33      |-152.     |  30.   | 1840   |    4
6020894830527075   |A-219       |42.2       | -88.6    | 175.   |13713   |    5
5124434961658461   |A-9338      |55.15      |-162.95    |    .   |10491   |    6
4450355574122328   |AA-10925    |42.667     | -70.883   | 179.   |13760   |    7
2609664440071317   |AA-20755    |68.02      |-158.73    |    .   |11903   |    8
8704316082120318   |AA-20756    |68.02      |-158.73    | 300.   |10996   |    9
 -2047 LINES OMITTED-| :         | :         | :        |   :    | :      | :
3187374887671188   |Y-2464      |45.08      | -71.08    |    .   |11618   |2056
0513875673303111   |Y-416       |49.62      | -99.43    |    .   | 8878   |2057
4553882162706867   |Y-418       |51.17      |-100.25    |  10.   | 1316   |2058
6580606639747581   |Y-526       |40.025     | -82.975   |    .   |13351   |2059
1002595983626604   |Y-527       |54.8       | -60.82    | 115.   | 4300   |2060
4600813124741886   |Y-762       |46.02      | -61.565   |    .   |12618   |2061
0000000000000000
4314701862316530   Summary: 2061 data lines, 41 metadata lines, Centinel V2.
```

BIOENERGY FROM RESERVE PRAIRIES IN MINNESOTA: MEASURING HARVEST AND MONITORING WILDLIFE

Shelby Williams[1,], Jacob Jungers[1], Kevin Johnson[2], Colleen Satyshur[2], Melissa DonCarlos[2], Robert Dunlap[3], Troy Mielke[2], Joe Schaffer[4], David Tilman[1], Donald Wyse[5], Roger Moon[6], Todd Arnold[3], Clarence Lehman[1]*

Abstract

Conservation of land is valued by society and nurtured by policy that should be informed by science and technology. Over 1.5 million acres are in the Conservation Reserve Program in Minnesota alone. This and similar programs provide ecosystem services, but as prices of commodity crops increase, along with costs of farm operations, many reserve lands may revert to cropland, potentially reducing quality of soil, water, climate, and habitat.

In this study we investigated whether reserve lands could reliably be harvested to yield high quality renewable bioenergy while concurrently preserving resident wildlife populations. Implications can inform policy on earning opportunities from harvested bioenergy while maintaining or expanding conservation lands.

This paper broadly outlines our ongoing, six-year study on production-level harvesting of over 1000 acres of re-established Minnesota prairie. It and its on-line supplementary material (www.cbs.umn.edu/wildlife) focus on protocols, methods, and management practices that have emerged. Results and statistical analyses from this study will be reported in subsequent publications. We describe the logistics of managing a landscape-scale bioenergy research program, with emphasis on harvesting, sampling, and coordination with land managers. In addition, in supplementary material we offer specific protocols to survey small mammals, birds, reptiles, amphibians, and invertebrates. These protocols are intended for researchers to assess whether wildlife populations are affected by various harvesting regimes for bioenergy, and the quality and quantity of bioenergy that can be expected.

The pursuit of principles integrating conservation biology, ecology, agronomy, and energy production, as described here, is an intrinsic part of establishing a viable domestic bio-based economy.

Keywords: bioenergy, wildlife conservation, ecosystem services, multiple use CRP

Introduction

Minnesota grasslands continually produce biomass that largely goes untapped. A properly restored and managed field of mixed grasses, legumes, and other flowering plants offers key ecosystem services including carbon sequestration, enhanced water quality, biodiversity, and wildlife habitat (Foley *et al.* 2005). It also offers flexibility for use as animal feed or forage (Sanderson and Adler 2008). In general, contemporary

[1] Department of Ecology, Evolution, and Behavior, University of Minnesota, MN

[2] Cedar Creek Ecosystem Science Reserve, MN

[3] Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, MN

[4] Minnesota Native Landscapes, MN

[5] Department of Agronomy and Plant Genetics, University of Minnesota, MN

[6] Department of Entomology, University of Minnesota, MN

* Corresponding author

energy crop fields, such as soybean and corn, and some other feedstocks such as miscanthus and switchgrass, support lower levels of wildlife than do diverse grasslands (Robertson e*t al*. 2010; Meehan *et al*. 2010; Gardiner *et al*. 2010). Suitable wildlife habitat in such cases may be reduced by simplification of the landscape, complete harvest of all cover, wetland drainage, chemical application, mechanical injury, and other causes.

But what of restored native grasslands? Can they be harvested sustainably and still provide suitable wildlife habitat? We considered principles of wildlife ecology to design an experiment testing the effects of harvest patterns, edges, and unharvested refuges on production-scale fields within restored native grasslands. We surveyed birds, small mammals, reptiles, amphibians, and invertebrates, including insects and spiders. We also conducted several specialized pilot surveys. This paper outlines the project thus far, five years into a six-year study, to summarize lessons learned. Detailed results and statistical analyses from this study will be reported in subsequent publications.

Site Selection and Logisitics

A common criticism of biomass production is that it uses land that might otherwise be used for food production, leading to increased greenhouse gases, among other outcomes (Fargione *et al.* 2008; Searchinger *et al*. 2008). However, mixed grassland biomass is an exception in that it can be established or restored on marginal land that is either not suitable for typical crop production (Cai *et al.* 2011), or as is more often the case, has been taken out of production because of low yields. For this study, we selected three regions of representative climate, soil, and wildlife composition in western Minnesota spanning the state's latitudinal gradient (Figure 1). These were re-established prairies, restored no less than five years earlier, held in federal, state, or private conservation under fire and weed management appropriate for their region.
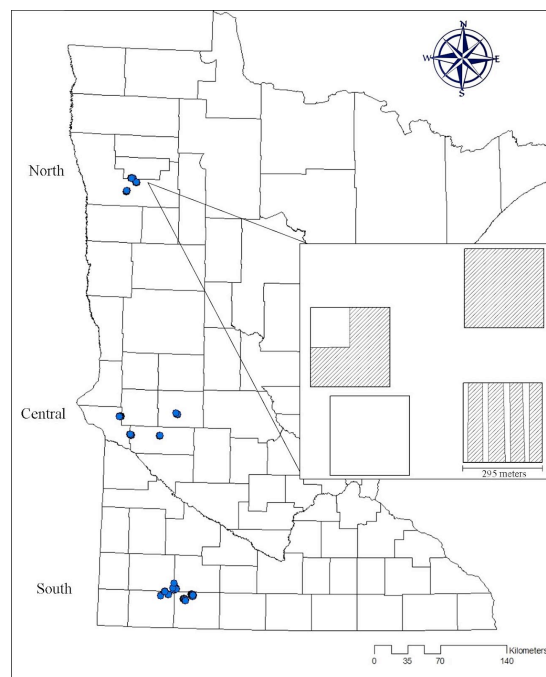
Fig. 1. Three regions in Minnesota where restored grasslands were studied to evaluate effects of biomass harvesting on grassland wildlife. Inset shows one block of four 20-acre plots with one unharvested control and three different harvest plans.

Locating landscapes with enough contiguous, re-established prairie to establish replicated, consistently-sized production scale plots was challenging. We were able to accomplish this goal with plots averaging 20 acres

each, organized into study blocks of an average three-mile radius. The blocks within a region were close enough for harvesting efficiency and delivery to potential biomass consumers. This also kept the soil and climate within each region similar enough to support a randomized block design. We chose plots using detailed maps that included soil and elevation parameters, wetland delineation, and land cover from aerial images, then visited potential locations to determine plot suitability.

Plot distributions within fields required detailed attention. Removing bales would become difficult and expensive if plots were further into fields and away from roads. Wetness and slope were considered as well, especially since the fields used in this study did not have drain tiles. We recognized these as challenges in using marginal agricultural or non-agricultural land for harvesting. Given those constraints, sufficient land for this study was located and partnerships with federal, state, and local agencies and private entities were secured largely within the first year of the project, but required some care and effort.

Wildlife

We evaluated wildlife with biological field crews surveying birds, small mammals, reptiles, amphibians, and invertebrates. Each of these taxa required distinct protocols, detailed in supplementary material (www.cbs.umn.edu/wildlife). Any single survey does not define the response of the landscape to bioenergy harvest, but together these surveys characterize outcomes of harvest management. We identified wildlife to species level where possible, but several taxa were only identified to order, family, or genus (*e.g.* invertebrates, some genera of small mammals).

Invertebrate sampling techniques included sweep nets, pitfalls, and bee bowls. We also developed a new quantitative invertebrate sampling technique (QuIST) to collect all invertebrates within a grassland canopy and calibrate conventional sweep net measurements. Our measurements of invertebrates examine their important roles as food for wildlife and as beneficial predators and pollinators. Small mammals were surveyed because they occupy a central role in grassland ecosystems, consuming invertebrates and plants lower in the food web and in turn becoming food for larger predators. We conducted small mammal surveys in late summer using Sherman live-traps. Reptiles and amphibians are sensitive and susceptible to environmental disturbances and therefore are important indicators. We surveyed them throughout summer using funnel and pitfall live-traps. Grassland birds are of widespread interest, not only for activities such as bird-watching and hunting, but because their populations have declined more precipitously than any other bird guild. We used area-based search methods to survey birds throughout the entire plot, using both auditory and visual cues.

In addition to the wildlife surveys, we conducted vegetation surveys throughout the growing season. These surveys tracked the presence and absence of a variety of plants, percent cover at randomly placed quadrats, and also which plants were blooming and providing resources for pollinators. Other surveys were piloted for special purposes, including winter pellet surveys for deer, artificial nest and predator surveys using trail cameras, snow depth measurements, and nesting waterfowl surveys.

Harvesting

Biomass harvesting was organized in six treatment patterns: 50% harvest in strips, 50% harvest in blocks, 75% harvest in strips, 75% harvest in blocks, 100% harvest, and 0% harvest. Patterns were designed to test for the importance of unharvested areas in providing wildlife refuges, connectivity, edges, and landscape complexity. Harvesting was guided by semi-permanent bamboo poles placed in the plots. Following harvest, we traced the edges on all-terrain vehicles using global positioning systems (GPS) to record actual harvested areas, which occasionally differed from the plan due to temporary wetlands or other obstructions. We collected sample cores from biomass bales and analyzed them for minerals and other factors (Jungers *et al*. 2011).

We did not employ custom equipment for harvesting. A discbine with multiple small spinning heads was used for all cutting. After the biomass was cut, it was roller-conditioned and dispensed to form windrows. The discbine head worked well for cutting the various plant types in the project and cut both wet and dry material. It also allowed greater ground speed. However, it can be expensive to repair if damaged by rocks or other debris, which can occur on marginal lands targeted for this study.

During the first harvest season (2009), the discbine head was mounted on a two-wheel-drive, self-propelled, swathe-type cutter, but this was suboptimal because the unit was difficult to transport between plots, and it got stuck in wet ground. Accordingly, in 2010 and later, we mounted the discbine on a four-wheel drive tractor, which solved the transportation problem and also provided the versatility of another tractor on site. A high capacity wheel V-rake worked well to combine two windrows of cut biomass into one windrow and also to flip the material to speed drying. If conditions were dry, the biomass did not have to be raked.

We tested both round and square balers. Both produced large bales of similar size. In 2009, the large square baler produced 4' x 4' x 8' twine-tied bales weighing around 1,000 pounds at 15% moisture. They stacked, hauled, and transported well—better than round bales—and had no tendency to roll on slopes. However, they were not as resistant to rain. The square baler was effective but heavy for its tire size and difficult to load for transport. In 2010, we switched to a round baler, which produced a 4' wide by 6' high bale wrapped with plastic net. That size allowed easy hauling by truck to final destinations. Round, net-wrapped bales can be left outdoors for up to three years or more without cover, allowing storage in the field, where costs are lower.

Available time windows for harvesting were relatively short, due in part to regulations of land managers, but also to weather conditions. For example, many wildlife management areas by regulation cannot be harvested before November 1, sometimes leaving little available time before snowfall. Occasionally, wet conditions or snow prevented a complete fall harvest. Where possible, harvesting was then completed the following spring. The best method for transporting bales from the field was tractors with front and rear-mounted bale spikes. With these, bales can be placed a safe distance from the roadside for future transport or loaded directly onto trucks.

A practical consideration for geographically broad studies like this is preventing the spread of weeds, so for this project transportation equipment carried on-board air compressors to clean machinery before departing any plot.

Public Involvement

Ultimately policy flows from the public, and with that in mind we dedicated part of the project to meetings and demonstrations for agencies, landowners, news media, and the general public. During multiple fall harvest seasons, we advertised in local media and moved a representative set of harvesting equipment to one of our 20-acre plots that was close to a roadway with safe parking nearby. We presented the ideas of ecosystem services from multiple concurrent uses of land and conducted discussion and feedback sessions. Conditions permitting, we demonstrated the harvesting process to those not familiar with issues of harvesting non-agricultural land. Attendance was good and responses were enthusiastic, though more people interested in land management and wildlife attended than those interested purely in bioenergy. These sessions were then distributed more broadly through news reports and photos in local newspapers.

Discussion

With proper planning, diverse, re-established grasslands can provide multiple benefits to conservation lands and to agricultural lands used for bioenergy (Tilman *et al.* 2006). Harvesting can provide an easier and less expensive management alternative to prescribed burning on conservation lands. As riparian buffers, prairies can be planted and subsequently harvested alongside waterways, with bioenergy revenues potentially making such buffers profitable and allowing them to be wider than they otherwise could be. Wet and mesic prairies

could be established to catch drain-tile runoff of food-crop fields and remove nitrogen and other nutrients before they reach natural watersheds, increasing bioenergy yields in the process. Integrating animal production for food onto grassland bioenergy fields may offer further opportunities for managing a multifunctional system. For example, if grazers use mixed-species grasslands in the spring and consume cool-season grasses, that could maximize the growth of warm-season grasses and bring higher bioenergy yields. Lower potential yields than heavily managed monoculture grasses are offset by putting to use land that is not suitable for heavy management, and providing broad conservation services to society across the landscape.

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

JACOB M. JUNGERS[1], CRAIG C. SHEAFFER[1], JOSEPH FARGIONE[2] and CLARENCE LEHMAN[3]

[1]Department of Agronomy and Plant Genetics, University of Minnesota, Saint Paul, MN, USA, [2]North America Region, The Nature Conservancy, Minneapolis, MN, USA, [3]College of Biological Sciences, University of Minnesota, Saint Paul, MN, USA

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

Correspondence: Jacob M. Jungers, tel. 612-625-0745, fax 612-625-1268, e-mail: junge037@umn.edu

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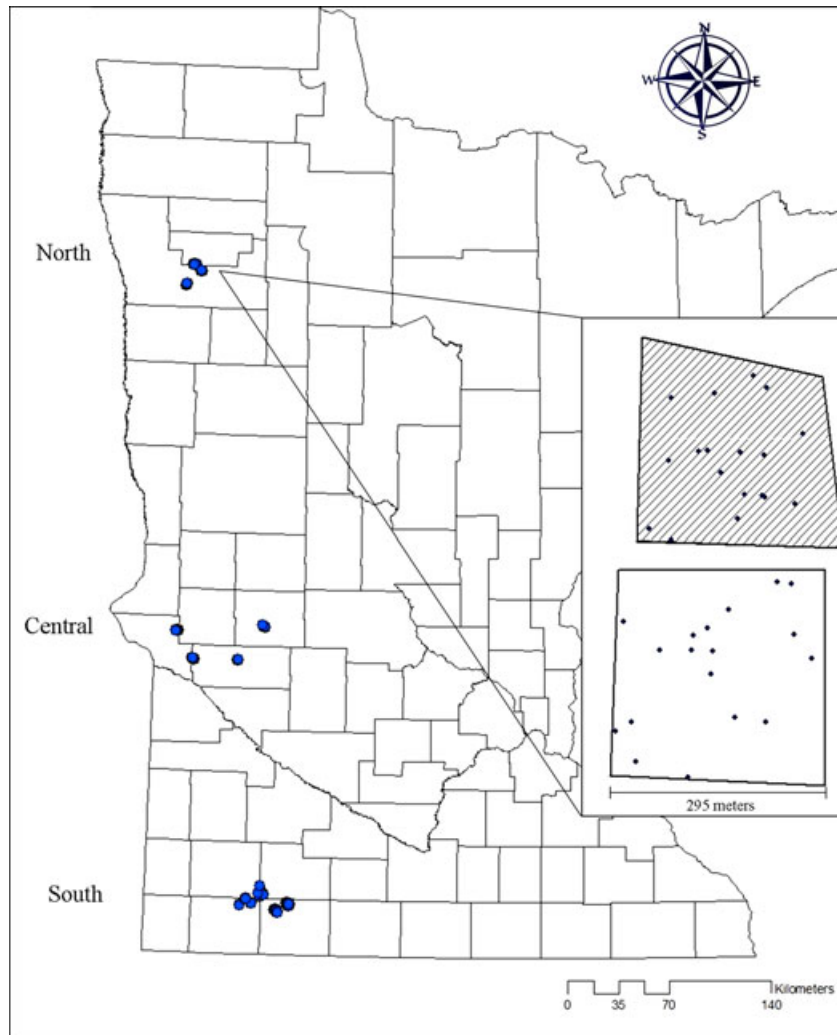
Fig. 1 Map of the study area in Minnesota, USA with each point representing a block of plots. Inset shows one block containing a 100% harvest plot and an unharvested control plot with randomly distributed sample quadrats where plant community composition was measured in 2011.

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&statef-ips=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 × 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 m^2 per plot, which was equivalent to the area of five 1.0 × 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 m^2.

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 two percent 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 m^2 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 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 ($R^2 = 0.34$, $P = 0.007$).

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	*Andropogon gerardii*	1	37
	Panicum vigratum	2	14
	Schizachyrium scoparium	3	16
	Sorghastrum nutans	4	14
	Bouteloua curtipendula	5	3
C3 grass	*Poa pratensis*	1	20
	Bromus inermis	2	21
	Phalaris arundinacea	3	31
	Agropyron repens	4	11
	Elymus canadensis	5	8
Nonlegume forb	*Solidago* spp.	1	8
	Cirsium arvense	2	3
	Asclepias syriaca	3	3
	Taraxacum officinale	4	1
	Lactuca scariola	5	1
Legume	*Melilotus* spp.	1	8
	Dalea purpurea	2	4
	Medicago lupulina	3	3
	Dalea candida	4	4
	Astragalus canadensis	5	5
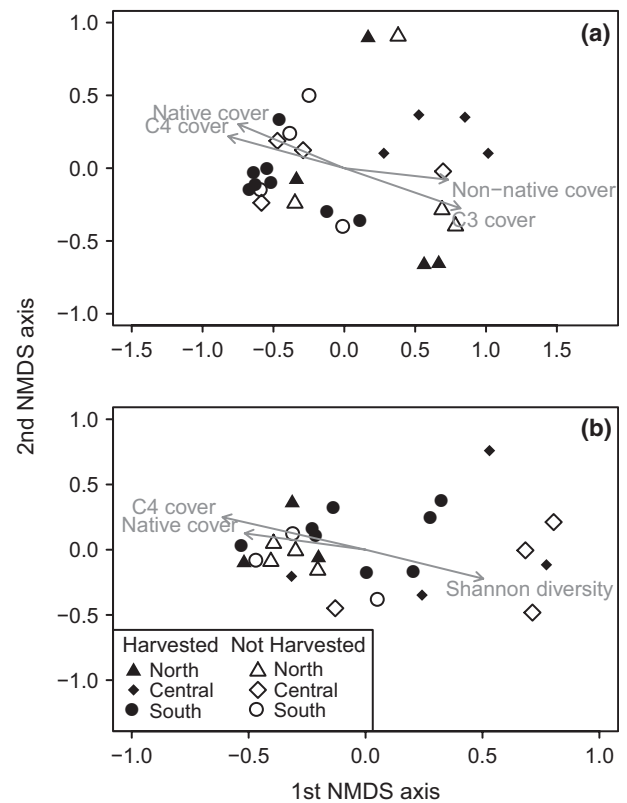
Fig. 2 Nonmetric multidimensional scaling ordination of plant communities in grasslands prior to biomass harvest (a) and following two (North) and three (Central and South) years of biomass harvest (b). Lines represent gradients for metrics of plant community composition, with the length of the line 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 × Year and Location × 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 × 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 × 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 × Treatment: $R^2 = 0.08$, $P = 0.189$) and after 2 or 3 years of biomass harvest (Location × 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	t	P
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
	Harvested × North	−0.568	0.727	−0.782	0.443
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 overlap 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
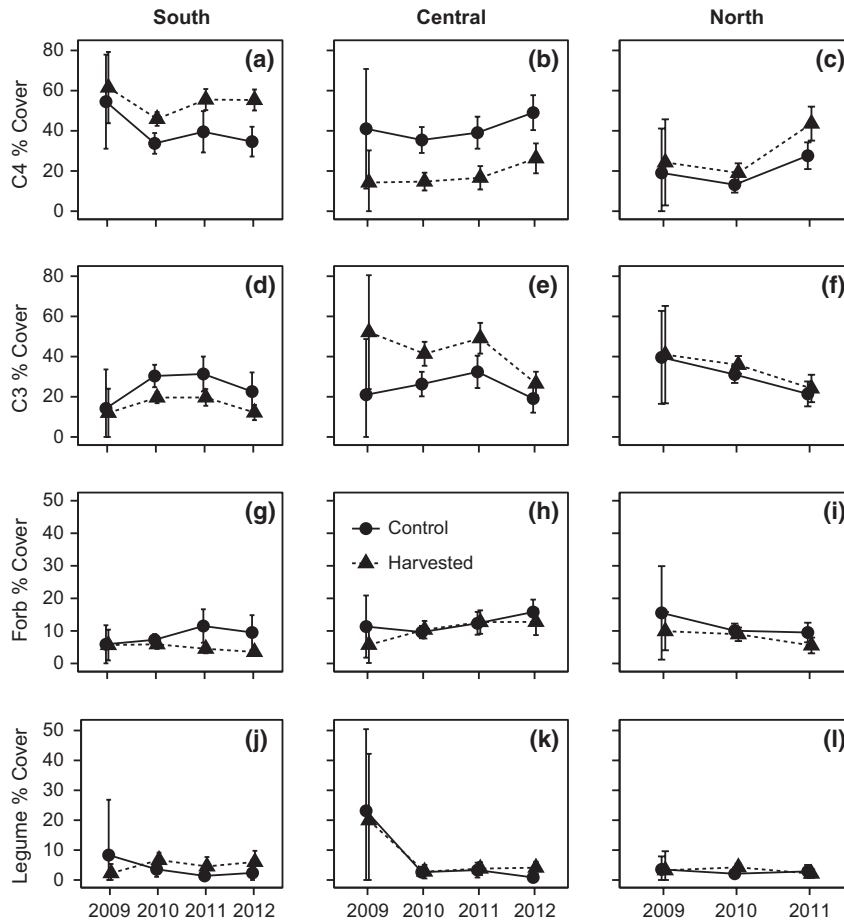
Fig. 3 Average percent cover of the four major plant functional groups in harvested and unharvested plots located in the south, central and north locations from 2009 (pretreatment) to 2012.

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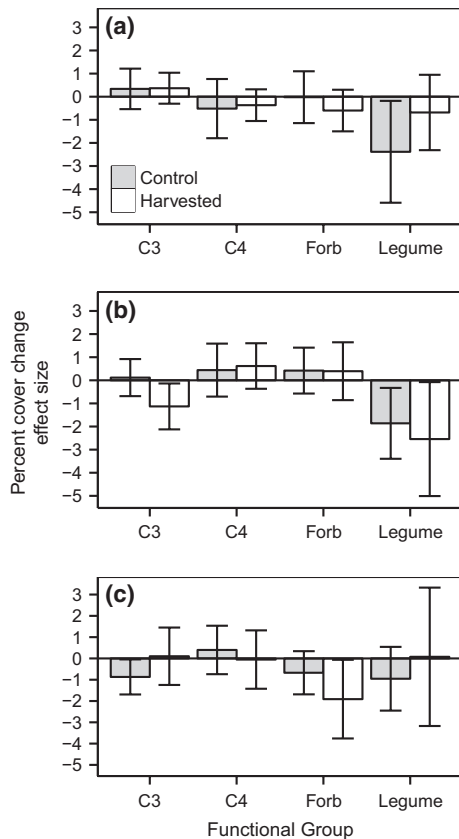
Fig. 5 Effect sizes (Hedges' *g*) and associated 95% confidence intervals for the change in functional group cover from pretreatment to final year posttreatment conditions in the south (a), central (b), and north (c) locations in Minnesota, USA.

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.

Acknowledgements

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Bioenergy and Wildlife: Threats and Opportunities for Grassland Conservation

Author(s): Joseph E. Fargione, Thomas R. Cooper, David J. Flaspohler, Jason Hill, Clarence Lehman, Tim McCoy, Scott McLeod, Erik J. Nelson, Karen S. Oberhauser, and David Tilman

Bioenergy and Wildlife: Threats and Opportunities for Grassland Conservation

JOSEPH E. FARGIONE, THOMAS R. COOPER, DAVID J. FLASPOHLER, JASON HILL, CLARENCE LEHMAN, TIM McCOY, SCOTT McLEOD, ERIK J. NELSON, KAREN S. OBERHAUSER, AND DAVID TILMAN

Demand for land to grow corn for ethanol increased in the United States by 4.9 million hectares between 2005 and 2008, with wide-ranging effects on wildlife, including habitat loss. Depending on how biofuels are made, additional production could have similar impacts. We present a framework for assessing the impacts of biofuels on wildlife, and we use this framework to evaluate the impacts of existing and emerging biofuels feedstocks on grassland wildlife. Meeting the growing demand for biofuels while avoiding negative impacts on wildlife will require either biomass sources that do not require additional land (e.g., wastes, residues, cover crops, algae) or crop production practices that are compatible with wildlife. Diverse native prairie offers a potential approach to bioenergy production (including fuel, electricity, and heat) that is compatible with wildlife. Additional research is required to assess the compatibility of wildlife with different composition, inputs, and harvest management approaches, and to address concerns over prairie yields versus the yields of other biofuel crops.

Keywords: corn, biofuel, grassland, wildlife, cellulosic ethanol

Agriculture has a major effect on the status and integrity of natural ecosystems. Improvements in agricultural practices over the last century have increased productivity and thus the footprint for land and resource use is smaller than it otherwise would have been. However, modern agriculture still adversely affects habitat conservation, water and air quality, carbon sequestration in the soil, and soil fertility (e.g., Foley et al. 2005).

To mitigate the environmental impacts caused by agriculture, the US federal government has developed and implemented various land conservation programs, the most prominent of which is the Conservation Reserve Program (CRP; see, e.g., *www.ncga.com/files/pdf/ConservingLand FutureGenerations.pdf*). The original purpose of the CRP, a voluntary program that pays rent annually to landowners who enroll their agricultural land and convert it to perennial grasslands, was to support commodity prices, reduce soil erosion, and improve water quality on highly erodible croplands (FAPRI 2007). The CRP has also benefited wildlife (e.g., Reynolds 2005, Herkert 2007, Niemuth et al. 2007, Riffell et al. 2008), and the program has evolved over time to more explicitly target benefits beyond soil erosion, including the enhancement of wildlife habitat.

Biofuel production offers the potential to bolster energy security, support rural economies, and reduce greenhouse gas (GHG) emissions. However, biofuel production also has potentially large land-use impacts. Greater demand for biofuels has caused—and may continue to cause—retired croplands to be put back into crop production (Secchi and Babcock 2007, Searchinger et al. 2008). Current US law mandates production of 136 billion liters of biofuel by 2022, which is 740% more than was produced in 2006. High gas prices also contribute to the demand for biofuel production, but given current subsidies and mandates, expansion of biofuel production is assured even if gas prices drop. That expansion may threaten some of the gains the CRP and other land conservation programs have made over the last two decades in the conservation of wildlife, ecosystem services, and biodiversity.

This article provides a framework for assessing the potential environmental impacts of existing and prospective methods of bioenergy production, with a focus on impacts on wildlife. We focus on the effects of biofuel feedstock production on wildlife, although we recognize that wildlife conservation is only one of the benefits that society derives from its lands. We believe that ecosystem services, including wildlife

production, require special consideration because these services are typically external from market considerations and incentives, making them vulnerable to loss from unintended consequences of policy or shifts in market forces.

Although biofuel in the form of ethanol is the current focus of bioenergy production in the United States, we use the more inclusive term "bioenergy" to include all useful forms of energy that can be extracted from biological crops, residues, or wastes (i.e., liquid fuel, electricity, heating, cooling). Bioenergy includes biodiesel made from fats and oils (e.g., soy oil and canola oil), ethanol made from sugars and starches (e.g., corn grain and sugarcane), cellulosic ethanol (ethanol made from plant biomass either through fermentation or thermochemical processes), and bioelectricity and bioheat (e.g., from biomass burners or gasifiers). We consider the effects of biomass production on both terrestrial and aquatic systems. We define wildlife broadly to include all nondomesticated animals, although we focus primarily on birds because (a) they are the primary species of management concern in grasslands at risk of conversion to bioenergy crops, and (b) there are limitations in the primary literature on potential impacts on other species.

A continuum of effects on wildlife

Bioenergy can be produced using a variety of feedstocks and methods. If nonurban land use is classified along a continuum of intensity of use ranging from intensive agriculture to nature preserves, bioenergy can be produced across almost the entire continuum. At one end of the spectrum, bioenergy can be produced with intensively managed monocultures of annual food crops. This method of production can have large environmental consequences, including habitat loss and the off-field impacts of fertilizer and pesticide runoff (e.g., Foley et al. 2005). Toward the other end of the spectrum, bioenergy can be produced by sustainably harvesting biomass from systems with high plant diversity and low agriculture input (Tilman et al. 2006).

The quality of habitat and the production of ecosystem services on a landscape are affected by several aspects of agricultural production (figure 1). The value of an area as wildlife habitat is influenced by the vegetation type, including plant diversity and whether these plants are invasive; the timing and frequency of harvest; stubble height; refugia; and landscape context. Whether the bioenergy crop represents a net gain or loss of habitat depends on the type of land that it is replacing. Agriculture production in one area can affect habitat in another through fertilizer runoff, pesticide drift, and sedimentation of aquatic habitat. The value of the ecosystem services produced on and around a bioenergy crop field is influenced by the field's productivity, the interannual variability of productivity, the nutrient uptake of crops, rates of carbon seques-

tration, and hunting leases, among other factors. Many of the environmental impacts of bioenergy production on agricultural fields can be minimized by low-input systems with diverse native species. However, the major drawback of less-intensive systems is that more land is generally required to generate a given amount of energy than would be required by more-intensive systems that use fertilizer, pesticide, and monocultures of high-yield cultivars to maximize productivity. Here we consider a range of methods for producing bioenergy, starting with corn and moving on to less-intensive methods, and evaluate their observed and potential impacts on wildlife.

Current and projected ethanol production and land requirements

In the United States, growing demand for corn ethanol, largely fueled by production subsidies and gasoline blending mandates, has led to an increase in the amount of land used to produce corn (figure 2b, 2c). Most of the recent expansion in corn area has come at the expense of land previously used for other crops, especially soybeans (figure 2c). Some land that
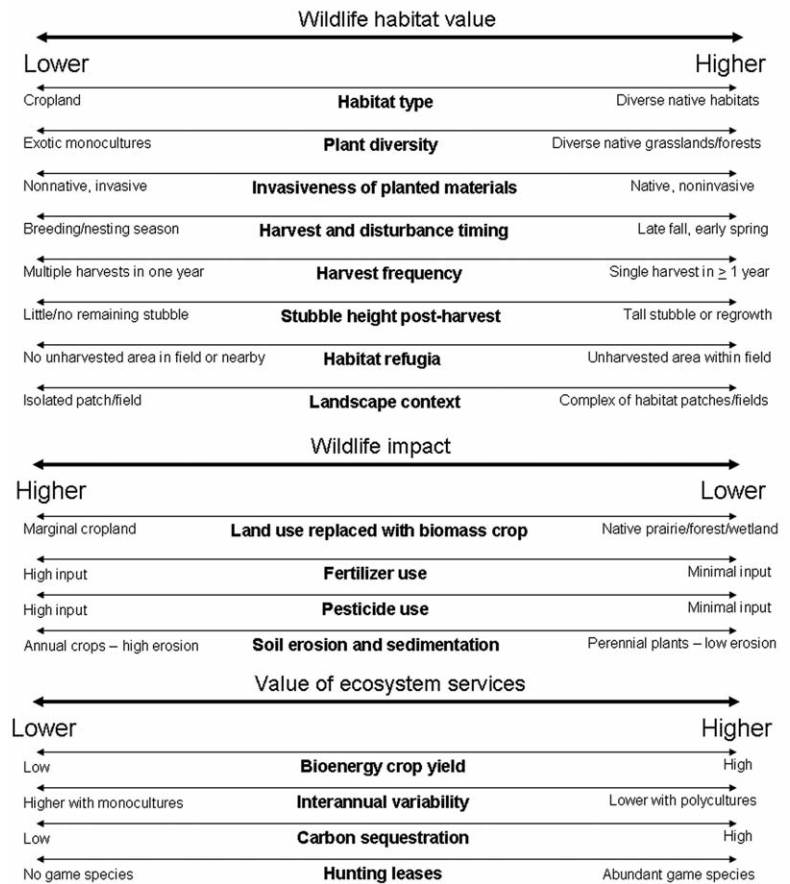
Figure 1. Factors influencing wildlife habitat value, wildlife impacts, and ecosystem services of bioenergy crops. For each factor, the qualities associated with greater wildlife or ecosystem service benefit (or less impact) are listed on the right side of the figure, and the qualities that are associated with less wildlife or ecosystem service benefit (or greater impact) are listed on the left side of the figure.
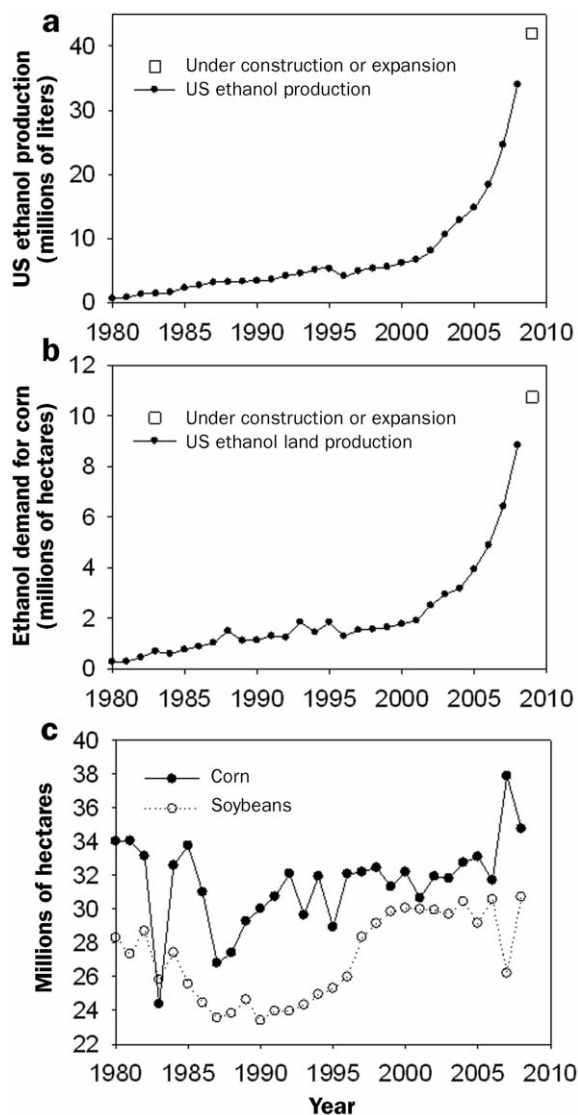
Figure 2. US ethanol production (RFA 2008), land demand for ethanol production, and area planted for corn and soybeans (USDA 2009). Land demand for ethanol production is based on each year's actual yields, area planted, and area harvested (USDA 2009).

had previously been planted alternately with corn and soybeans now is planted continuously with corn. This practice lowers yields and increases nutrient additions and emissions, as discussed below. Also, some land that is now used to produce corn was under perennial vegetation, primarily grasses, just several years ago.

Data on exactly how much grassland has been converted to corn production are not available. However, several lines of evidence indicate that grassland has been and will be converted to crop production as a result of the higher demand for corn.

First, the amount of land enrolled in the CRP peaked at 14.9 million hectares (ha) in September 2007. In October 2007, CRP lands had declined by 931,000 ha (USDA 2007). Of those lands no longer in the program, 850,000 ha were grass-

lands, and the remainder had been enrolled to promote tree or wetlands conservation practices. Second, the Food, Conservation, and Energy Act of 2008 reduced the total area that may be enrolled in the CRP to 12.9 million ha by 2010, which ensures that the trend of expiring CRP acres and declining enrollments will continue. This mandate reduces the ceiling of allowable area, but it does not provide a floor of required area, so it is unclear how deep the loss of CRP-enrolled lands will ultimately be. The US Department of Agriculture has projected that CRP area will bottom out at 12.2 million ha in 2013 before rebounding to 12.9 million ha in 2017 (USDA 2009). Economic analyses, however, suggest the potential for deeper losses. Secchi and Babcock (2007) estimated that 49% to 61% of the land enrolled in the CRP in Iowa would eventually be converted back to cropland if corn prices were fixed at $3 or $4 per bushel, respectively, for an extended period. Given that corn prices ranged from $3 to $7 per bushel in 2008 and are projected to remain greater than $3.65 until 2018 (USDA 2009), a significant drop in CRP area in Iowa is likely to occur. As a final piece of evidence of CRP losses, the Farm Service Agency indicates that more than 345,000 ha of the 3.2 million ha of CRP land in the prairie pothole region of the Northern Great Plains expired in 2007. Another 1.4 million ha will expire from 2008 to 2012 unless new opportunities to reenroll in CRP become available (figure 3).

Not all of the grassland being converted to cropland has been cropped in the past. Some of the land currently being converted to cropland is native prairie that has been pastured but never plowed. This land is vulnerable to conversion as a result of both higher crop prices and profits, and challenging grazing economics. For example, cropland conversion totaled more than 203,000 ha of native prairie in North Dakota, South Dakota, and Montana between 2002 and 2007 (Scott Stephens, Director of Conservation Planning and Programs, Ducks Unlimited, Bismarck, North Dakota, personal communication, 30 March 2009), and 5.2% (36,540 ha) of remaining native grassland in the Missouri Coteau of North Dakota and South Dakota was lost from 1984 to 2003 (Stephens et al. 2008).

Significant investment in the ethanol industry over the past few years, buoyed by renewable fuel mandates and industry subsidies, means that corn-ethanol production capacity in this country will continue to grow strongly. As of late 2008, the United States had a 42-billion-liter annual capacity (RFA 2008). Ongoing construction (including new plants and expansion of existing plants) will result in a production capacity of about 50 billion liters. Capacity would have to go even higher to meet goals in the renewable fuels standard of the Energy Independence and Security Act of 2007 (EISA; Sissine 2007), which mandates production of 57 billion liters of biofuel by 2015, all of which are expected to be made from corn grain. Assuming an industrywide conversion rate of 10.6 liters of ethanol per bushel of corn (current conversion rates are about 10.4 liters per bushel; FAPRI 2008), an average annual corn yield of 417 bushels per ha (current yields are 380 bushels per ha; USDA 2009), and that 98% of
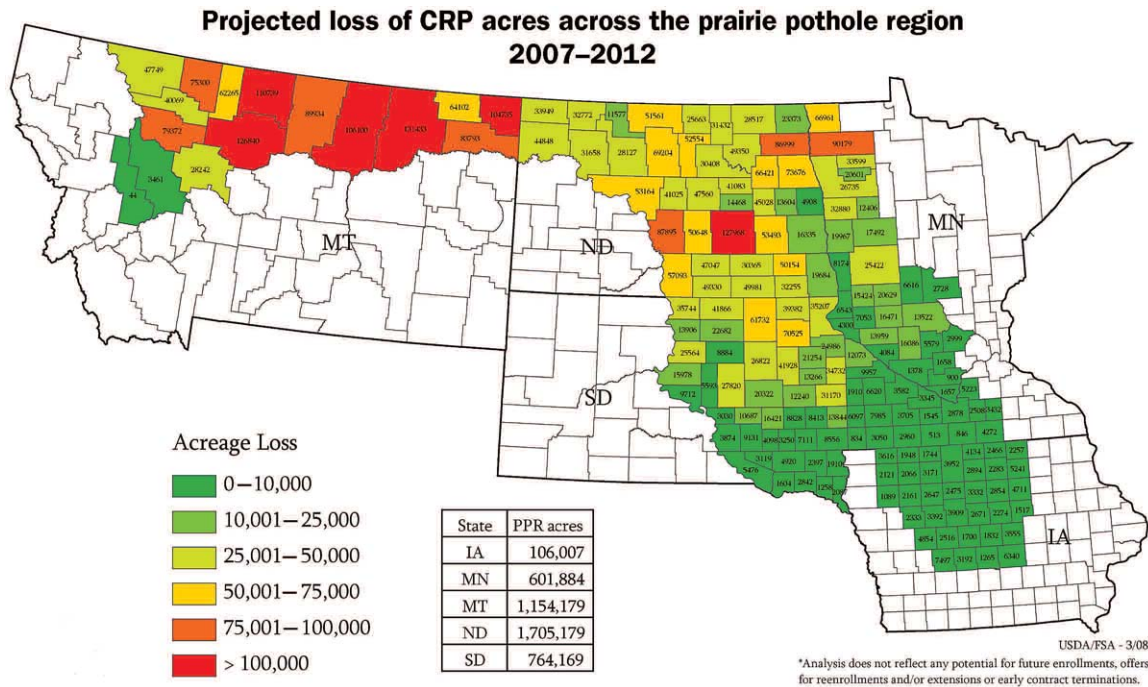
Figure 3. Projected loss of Conservation Reserve Program (CRP) acres across the prairie pothole region from 2007 to 2012. This is calculated as the sum of the acres whose contracts expire in these years and whose owners have declined or were not offered the chance to reenroll in CRP.

planted hectares are harvested (historic rate), meeting the 57-billion-liter mandate with corn ethanol would require about 13.1 million ha of planted corn, or about 6.7 million more ha of corn than was planted for ethanol production in 2006. The net increase in demand for cropland will be less than this, because corn ethanol production also yields the coproduct "distiller's grain," which is used as animal feed and displaces corn and soybean meal (e.g., Klopfenstein et al. 2008), but the land-use impact of this displacement is poorly quantified. We expect that some of the expansion of corn ethanol production will come at the expense of perennial grassland conversion, judging from the analyses and ob-served losses discussed above.

Potential impacts of corn ethanol on wildlife and fisheries

The conversion of CRP land to cropland has potentially significant impacts on grassland wildlife such as nesting birds and mammals (Reynolds 2005, Herkert 2007, Niemuth et al. 2007). For example, Herkert (2007) showed that population trends for the Henslow's sparrow (*Ammodramus henslowii*) in Illinois counties were related to the amount of CRP land, and attributed the recovery of this species primarily to the increase in perennial grasslands created by the CRP. Results from a study on the value of CRP to grassland birds in North and South Dakota indicated that almost two million birds of five grassland nesting species would be lost without the CRP in those two states (Niemuth et al. 2007). Reynolds (2005) estimated that CRP habitat in the prairie pothole region adds an additional 2.1 million ducks annually to the fall flight.

To meet the greater demand for corn, many farmers have planted corn in the same field continuously from year to year. Compared with the more common corn and soybean rotation, continuous corn planting requires more fertilizer inputs (Katsvairo and Cox 2000), results in greater nitrogen leaching, is more susceptible to buildups of soil pathogens, and lowers annual yields by about 14% (Pikul et al. 2005). Lower yields from continuous corn mean that, in a given year, more land is required to meet the same demand, thus increasing competition with wildlife for land. Corn also requires more fertilizer than soybeans do, especially when it does not follow soybeans in a crop rotation (soybeans increase soil fertility because they fix atmospheric nitrogen). Moreover, it is more difficult to use conservation tillage on continuous corn because the buildup of residue leads to lower yields in subsequent years (Wilhelm and Wortmann 2004). Thus, continuous corn planting may reduce the amount of land in conservation tillage and intensify soil erosion.

Conversion of grassland to corn has significant impacts on freshwater ecosystems. Intact grasslands retain soil and nitrogen—for example, the amount of nitrate leaving tile-drained CRP grasslands was 98% lower than the amount leaving continuous corn (Randall et al. 1997). Sediment in-creases turbidity, raising temperatures and degrading habi-tat for coldwater fish such as trout. Nitrates are carried through freshwater systems, leading to algal blooms and hypoxia, creating "dead zones" such as the one in the Gulf of Mexico. In 2007, the dead zone in the Gulf of Mexico was 65% larger than average (1990–2006), and in 2008 it reached its second-largest size ever at 20,689 square kilometers (km^2)

(NOAA 2007, 2008). Producing the mandated 57 billion liters of corn ethanol will make it practically impossible to meet the federal goal of reducing the dead zone to less than 5000 km^2, according to Donner and Kucharik (2008).

Ethanol production requires substantial water use. Ethanol factories use 3 to 5 liters of water to produce 1 liter of ethanol (Keeney and Muller 2006). However, water usage in ethanol production is dwarfed by the amount of water needed to grow corn. Irrigated corn requires about 785 liters of irrigation water for every liter of ethanol produced (Aden 2007). About 19% of US corn comes from irrigated land (figures on area irrigated are from USDA [2004]; irrigated yields data are from Aden [2007]). This means that ethanol, on average, requires about 147 liters of irrigation water for every liter of ethanol produced. About 70% of this water is lost in crop production (primarily through transpiration and evaporation), and about 30% is returned to the surface and groundwater through runoff and infiltration (Mubako and Lant 2008). Although water may be used sustainably, in some places it is being removed at unsustainable rates from aquifers or it competes with other uses of surface waters, including the maintenance of aquatic biodiversity (Roberts et al. 2007).

Potential bioenergy sources

There are other possible options for future bioenergy sources, many of which would quite likely replace wildlife habitat with bioenergy crops and negatively affect wildlife. However, at least two ways of producing bioenergy may be compatible with wildlife. The first is to use biomass sources that do not require additional land, and thus do not increase the footprint of agriculture. The second is to produce biomass with land-use practices that are compatible with wildlife. Biomass sources that do not require additional land include wastes such as agricultural residues, cover crops, and, potentially, algae. Practices that are compatible with wildlife may include a variety of perennial biomass crops. Whether a particular project has effects that are negative, neutral, or positive for wildlife will depend on explicit consideration of wildlife impacts in the project-planning stages, and on actions taken to avoid incompatible land uses and management practices.

Wastes can be used to create bioenergy (fuel, heat, electricity) without requiring additional land. Potential sources include wastes from agricultural, municipal, animal, food industry, and forestry sources. Depending on how much cellulosic ethanol efficiencies can be improved, it would require 199 million to 282 million metric tons of biomass to meet the current renewable fuels standard of 79 billion liters of advanced biofuel by 2022 (mandated in addition to the 57 billion liters that can be supplied by corn ethanol). The US Department of Energy and the Department of Agriculture (Perlack et al. 2005) estimated that with 25% increases in yield, annual supplies of crop residues could provide 244 million metric tons (however, maintaining soil organic carbon may limit potential residue removal; Wilhelm et al. 2007), process residues could provide 36 million metric tons, and manure could provide 40 million metric tons (Perlack et al. 2005) of

material suitable for bioenergy production. Animal waste from concentrated feeding operations can produce methane that can be burned to produce electricity. Forestry waste is available from logging and sawmills, forest thinning (e.g., for fuel-load reduction), packaging and durable good wastes, and from storm- or pest-damaged trees. However, the retention of fine and coarse woody debris after logging is essential to maintain the wildlife value of forests (Pedlar et al. 2002). To avoid unintended consequences, plans to increase the removal of woody biomass from logged sites need to be carefully evaluated for their potential impacts on wildlife. Although the use of mill waste does not carry such risks, the potential to expand that use is relatively small since most mill waste is already used for energy or other coproducts. The unexploited capacity of forestry waste residues for bioenergy production is estimated at 70 million metric tons annually in the United States, with an additional potential of 54 million metric tons annually from fuel-load reductions (Perlack et al. 2005).

The most commonly discussed agricultural by-product is corn stover (leaves and stalks remaining in a field after harvest). Corn stover is produced in large quantities, may be relatively inexpensive, and is a uniform feedstock. However, the use of corn stover raises environmental concerns because of increased soil erosion (Graham et al. 2007) and further depletion of soil organic carbon stocks (Wilhelm et al. 2007). If concerns about wind and soil erosion are addressed, some 54 million metric tons of stover could be collected annually (Graham et al. 2007). However, this does not take into account concerns about depleting organic soil carbon stocks, which not only would increase carbon dioxide emissions, and thus contribute to climate change, but also may reduce yields (Wilhelm et al. 2007). Promisingly, long-term research suggests stover removal may be sustainable in terms of yields, soil quality, and soil carbon if practiced in combination with no-till farming (Moebius-Clune et al. 2008). The use of stover or other agricultural residues or cover crops could reduce the amount of habitat converted to bioenergy production because it can be supplied from land currently planted in corn. Conversely, if corn stover boosts the profits associated with corn production, this could lead to increased corn production and greater conversion of habitat to corn. The use of corncobs in cellulosic ethanol production would increase the amount of ethanol produced per ha by about 25% over the use of corn alone, without raising concerns over reductions in soil carbon.

Algae, which do not require soil for growth, have also been proposed as a source of bioenergy (Sheehan et al. 1998). Algae can be grown in freshwater or saltwater, and thus conflicts with wildlife can be avoided more readily than is the case with other bioenergy crops. Algae can also have extremely high yields (45 metric tons per ha per year). From an aquatic wildlife perspective, however, there could be unintended impacts on habitat quality (e.g., the release of modified algae could invade natural ecosystems).

Several energy crops have been proposed, including native species such as switchgrass (*Panicum virgatum*) and big bluestem (*Andropogon gerardii*), and exotic species such as *Mis-*

canthus (*Miscanthus giganteus*), common reed (*Phragmites australis*), reed canary (*Phalaris arundinacea*), hybrid poplar (*Populus* spp.), and camelina (*Camelina sativa*). Often, the introduction of exotic plant species produces undesirable consequences for native habitats and native wildlife species. Native wildlife species have not evolved with monocultures of exotic plants, and they may not be able to use such monocultures as habitat. For example, *Miscanthus* produces nine-foot-tall thickets (similar to bamboo) that are unlike the plant communities with which native North American species have evolved. Proposals to plant woody crops in areas typically dominated by grasslands raise similar concerns about wildlife impacts.

In general, the net effect of crops on wildlife will depend on the land use that they are replacing. Perennial energy crops are likely to provide better habitat than annual crops. For example, compared with corn, monocultures of switchgrass benefit some bird species of management concern, while other bird species have shown no benefit (Murray et al. 2003). Similarly, *Miscanthus* may provide better habitat than annual crops, although this may be a transient response associated with greater weed abundance in recently established *Miscanthus* fields (Bellamy et al. 2009). However, perennial crops can be grown in places not suited to existing crops, such as some existing grassland, thus potentially posing a broader threat of conversion to wildlife habitat than existing biofuel crops.

Assessing potential impacts on wildlife

Biomass crops may provide habitat if they are similar to native ecosystems, depending on the harvest management of the crops. In addition, biomass crops may pose a risk of off-field negative impacts if they become invasive and spread beyond field borders. Similarity to native ecosystems, harvest management, and invasive potential are reviewed below.

Risk of invasiveness. Biomass crops may pose a risk of becoming invasive if exotic crop species are used, if exotic or native species are modified through breeding or genetic engineering, or if species native to the United States are used outside their home range (Raghu et al. 2006, Barney and Ditomaso 2008). If native species are bred to increase yield, they may differ significantly from unmodified cultivars. Native or exotic species may be genetically modified to promote cultivation, yield, or other characteristics affecting bioenergy usage. Breeding and genetic modification of species may make species more likely to become invasive, as desirable agronomic traits such as a fast growth rate and high establishment success are also associated with successful invasive species (CAST 2007). Because biomass crops are typically harvested after they have set seed, there is opportunity for propagule spread before harvest or during transport. This increases the risk of invasion, which rises with greater propagule pressure (the number of seeds that are released to the environment). *Miscanthus giganteus* is a naturally occurring hybrid with sterile seed, which reduces its risk of becoming

invasive. However, *M. giganteus* still poses a risk of invasion through rhizomes; further, continued sterility is not guaranteed, and any variety with viable seed could spread rapidly (Raghu et al. 2006).

Similarity to native ecosystems. The diverse prairie ecosystem has been proposed as a bioenergy source with unique benefits for wildlife and carbon sequestration (Tilman et al. 2006). Diverse prairie is dominated by perennials, obviating the soil erosion, energetic, and financial costs associated with annual planting. When cropland is planted to perennial plants, soil carbon increases (FAPRI 2007). Diverse prairie communities have higher rates of carbon sequestration than do monocultures or low-diversity prairie (Tilman et al. 2006). In particular, seed mixes that include legumes, which fix nitrogen, result in dramatically increased rates of carbon storage compared with the mixes of several warm-season grasses commonly used in conservation practices (Fornara and Tilman 2008). The risk of invasion is greatly reduced when using native species of local ecotype. Because these communities are relatively self-sustaining, few fertilizers or herbicides are needed (at least after initial establishment), reducing the environmental and energetic costs associated with these inputs. Thus, even though perennial monocultures tend to require lower inputs than do annual crops, diverse prairie grasses require even fewer inputs.

Diverse communities also benefit wildlife. Experimental manipulations of biodiversity show that insect diversity is positively correlated with plant diversity (Haddad et al. 2001). The nectar produced by forbs in grasslands supports insects that can benefit insect-pollinated crops in nearby fields (Ockinger and Smith 2007). The benefit of plant diversity to wildlife also appears to hold higher up the food chain—for example, a survey of Wisconsin grasslands found that the diversity of birds was positively correlated with plant diversity (Sample 1989). Thus, although perennial monocultures and perennial polycultures both provide more wildlife benefits than corn does, diverse mixtures provide the most.

Harvest management. Without periodic management to reduce the litter layer and encourage new growth, grasslands produce less biomass (Knapp and Seastedt 1986) and lose their habitat value for many wildlife species (e.g., Roth et al. 2005). This highlights the potential for biomass harvests to increase the wildlife value of grasslands, but that potential will be realized only if wildlife values and landscape context are taken into consideration in harvest planning.

Harvest management of biomass fields will play a large role in determining vegetation structure, and thus the fields' value for wildlife habitat. Harvest management considerations include the seasonal timing of harvest, the height at which vegetation is harvested, and the proportion of available grassland that is harvested. Grassland bird species are adapted to particular ranges of habitat conditions (e.g., Sample and Mossman 1997). For example, some species prefer short stubble, which allows them to detect predators, and other species prefer long stubble, which allows them to avoid detection by

predators (Whittingham et al. 2006). Extensive harvest of vegetation will very likely favor grassland birds requiring short, sparse vegetation (e.g., grasshopper sparrow [*Ammodramus savannarum*] and Savannah sparrow [*Passerculus sandwichensis*]) and negatively affect those requiring tall, dense vegetation (e.g., sedge wren [*Cistothorus platensis*] and Henslow's sparrow). The best harvest scenario is likely to be one that produces a mosaic of harvested and unharvested patches, but further research is needed to determine the appropriate scale of these patches. Small habitat patches may suffer higher predation rates, making these patches population sinks rather than sources.

The proper time to harvest depends on the species of management concern, whether those species are migratory or resident, and the timing of the life-cycle events that have the greatest impact on populations (nesting, brood rearing, winter, etc.). Harvest should not occur during the established primary nesting season (PNS) (figure 4). Biomass could be harvested either before or after PNS. From a wildlife perspective, having multiple harvest times (early fall, postfrost, early spring) could provide a mosaic of habitat conditions suiting a wider range of species, as well as provide feedstocks to a biomass facility at different times of the year. However, depending on the species of management concern, either fall or spring harvests may be preferred. Harvesting in early spring would collect less biomass because of lodging (i.e., plants falling over) during the winter, but may be beneficial if biomass storage space is limited, and would benefit wildlife that require winter or residual cover, such as harriers (*Circus cyaneus*), pheasants (*Phasianus colchicus*), sedge wren, and Henslow's sparrow (George et al. 1979, Evrard and Bacon 1998, Roth et al. 2005). Early spring harvests must occur before the established PNS for each state to minimize impacts on grassland birds. Fall harvests typically occur after the first killing frost, well after the PNS for grassland birds. Earlier harvests, timed to coincide with the end of the nesting season, may benefit wildlife by allowing sufficient regrowth to provide winter cover and spring nesting. However, the effects of earlier harvest on the productivity and composition of the biomass crop are not well known and should be monitored to avoid unintended shifts in composition.

Residual cover (i.e., stubble) is of paramount importance to nesting ducks and other birds, particularly early nesting species such as mallard (*Anas platyrhynchos*) and northern pintail (*Anas acuta*) that arrive on northerly breeding grounds before the onset of the growing season (e.g., Jarvis and Harris 1971). Nest success for grassland nesting ducks increases with the height, structure, and amount of residual cover on the landscape. However, because it is unclear what stubble height would allow both sufficient nesting habitat for ducks and reasonable biomass yield, research is needed to understand the trade-offs between leaving stubble for ground-nesting birds and other wildlife and harvesting stubble for increased biomass yields.

Stubble may also benefit soils and yields. Stubble may reduce soil erosion caused by wind, particularly in northern climates that experience snowfall. The presence of stubble will help catch and maintain snow cover, which can improve spring soil moisture and may boost yields of desired perennial grasses. Research is needed to determine whether there is a relationship between stubble height and subsequent yields, and if so, what minimum and maximum stubble heights will produce the desired benefits.

The ideal proportion or configuration of unharvested to harvested land to maximize the wildlife benefit is not yet known. For example, would it be better to leave 20% of each field unharvested, or to let one out of five fields go unharvested, to serve as refuges? Research on nesting waterfowl in the prairie pothole region clearly indicates that nesting success
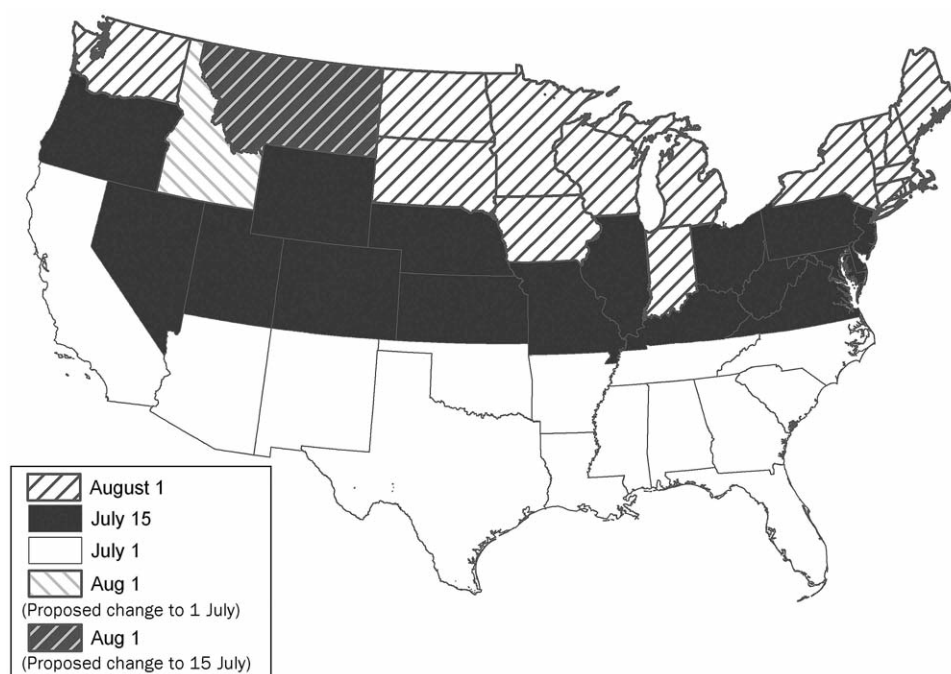
Figure 4. Established ending dates for primary nesting season for the purposes of management on Conservation Reserve Program (CRP) lands (USFSA 2008). These dates are established by National Resources Conservation Service rulemaking under federal law, and any management that occurs on CRP land, such as emergency haying or mid-contract management, must occur outside of primary nesting season.

increases with the amount of perennial cover, as measured across a range of scales. Species abundance of grassland birds is highly dependent on landscape context (e.g., Cunningham and Johnson 2006). Larger blocks of grassland are more likely to provide nesting and winter cover for a wide range of bird species (e.g., Winter et al. 2006). Wildlife-friendly bioenergy crops are most likely to achieve the most wildlife benefit if they are components of landscapes that already contain a large portion of grasslands, rather than isolated fragments among cropland. This could be especially beneficial for area-sensitive, grassland wildlife species such as prairie chickens and Henslow's sparrows. Bioenergy facilities will also benefit from being located in landscapes with high perennial biomass production. Thus, if bioenergy demand is met with biomass production that is compatible with wildlife, the location of bioenergy facilities near grassland habitat could benefit both wildlife and the bioenergy industry.

Even grasslands primarily managed for wildlife could provide biomass for bioenergy. Biomass on these lands could periodically be harvested as part of normal establishment and management practices aimed at, for example, controlling invasive weeds such as Canada thistle (*Cirsium arvense*) and leafy spurge (*Euphorbia esula*); providing an alternative to burning to control woody encroachment or litter buildup; and supplying short, sparse vegetation for species requiring this structure.

Assessing the feasibility of wildlife-friendly bioenergy crops

Although it is possible to produce biomass in ways that are compatible with wildlife, there are several open questions about its feasibility. Specifically, native perennial crops need to be feasible from the standpoints of economic, agronomic, and technological considerations, and of land and seed availability, if they are to become a significant portion of the energy portfolio.

One concern associated with the use of low-input, native prairie grasses is their yield relative to that of other proposed bioenergy crops (Schmer et al. 2008). Because there are no direct comparisons of native prairie grasses with other potential biomass crops using the same site, soils, and climate, it is premature to draw firm conclusions about yield differences. Comparisons of yields from different biomass crops at different sites, often with different rates of fertilization or other management practices, are problematic because farm trials generally occur on high-yielding cropland, whereas prairie yields are often measured on low-yielding lands that are unsuitable for farming. In farm trials, yields of fertilized switchgrass in North Dakota, South Dakota, and Nebraska averaged between 5.2 and 11.1 metric tons per ha (Schmer et al. 2008). In those same states, unfertilized prairie yields ranged from 3.4 to 5.7 metric tons per ha (Risser et al. 1981). *Miscanthus* is among the highest-yielding biomass crops, with fertilized yields in Europe averaging 22 metric tons per ha (Heaton et al. 2004). *Miscanthus* has high water demands, and this yield average included irrigated field trials (Heaton

et al. 2004). Yields of unfertilized native prairie grasses of up to 13.7 metric tons per ha have been reported from Illinois (Oesterheld et al. 1999). These examples illustrate high-yield potential from prairie grasses but also reveal a gap between reported yields for prairies and fertilized bioenergy crops. Direct comparisons of different potential biomass crops and native prairie on similar soils and under similar fertilization and irrigation regimes are needed to accurately quantify yield differences on a given site. It may also be possible to fertilize native prairie in a way that increases its yields while maintaining its wildlife value. Although fertilization typically reduces the diversity of plant communities, it may be possible to maintain plant diversity in communities that are both fertilized and harvested (Collins et al. 1998).

Establishing diverse mixtures of native perennial vegetation is expensive at present, in part because of high seed costs, which may initially hinder the large-scale establishment of diverse prairie grasses for bioenergy production. To encourage the use of diverse mixtures and their associated wildlife benefits, the government, bioenergy industry, and conservation community would need to work together to increase supply and lower seed prices or otherwise offset the higher cost of seeds. Cost-share programs could share establishment costs for projects resulting in quantifiable benefits for targeted wildlife populations or for projects allowing public access for recreation. Federal, state, and nongovernmental wildlife organizations could help provide the technical expertise needed for successfully establishing native grasslands, reducing costs associated with poor establishment.

Production costs of native grasses are estimated at $39 to $61 per metric ton, including land rental rates (Tiffany et al. 2006). This would be reduced to $22 to $33 per metric ton if land rental rates were excluded (cost estimates do not include any capital or hired labor costs). Transportation costs vary greatly depending on the size of the source area (Tiffany et al. 2006), and average costs increase from about $3.48 to $12.08 per metric ton as the source radius increases from 16 to 80 km. Community-scale projects with modest biomass requirements or higher-yielding crops would allow smaller source areas for biomass production, significantly reducing transportation costs.

It is unclear whether biomass fermentation processes currently under development will call for uniform feedstocks, which could limit the use of diverse prairie in ethanol production. However, diverse plantings can be burned to produce heat and electricity, or gasified to produce heat and electricity, or gasified to produce syngas, which is converted through the Fischer-Tropsch or other catalytic processes to gasoline, diesel, or ethanol (McKendry 2002). Cogeneration, the production of both heat and electricity, can be an extremely efficient way to extract energy from biomass through either burning or gasification (McKendry 2002).

Recent global analyses suggest that approximately 385 million to 472 million ha of abandoned farmland could be used to produce approximately 1.4 billion to 2.1 billion metric tons of biomass annually (Campbell et al. 2008). In the

United States, CRP contracts allow haying and grazing management, if that is written into the CRP contract with the landowner. However, harvest should follow the management guidelines in the contract, and some lands should not be eligible for harvest because of their slope; the presence of wetlands; or their importance to wildlife species of local, state, or national concern. Additional research is needed to identify where suitable lands occur in sufficient densities to support bioenergy facilities.

Landscape and adaptive management considerations

Whether bioenergy production is beneficial to wildlife or not will depend on many factors in addition to the composition of the crops. Most important, it will depend on the landscape context in which the bioenergy crops are planted. To deal with these external factors, managers should have explicit objectives, defined at the correct scale, and use adaptive management to tailor practices to local and changing conditions.

Management for wildlife could focus on overall biodiversity, on particular species groups, or on specific species. Managing for specific species is often the easiest task, especially when the ecological needs of the species are well understood. The US Fish and Wildlife Service has developed a series of habitat evaluation tools that help land managers evaluate impacts on fish and wildlife habitat resulting from changes in water or land use, as well as assess the suitability of habitat for fish and wildlife species (USFWS 1980). Species-specific habitat suitability index (HSI) models (USFWS 1981) use quantitative relationships between environmental variables and habitat suitability to arrive at a numerical index of habitat suitability (USFWS 1981). There are currently HSI models for 157 species, many of which use prairie or grassland habitats (USGS 2008).

When managing to maximize overall biodiversity, it may be possible to choose umbrella species to represent the habitat needs of other groups (e.g., Mac Nally and Fleishman 2004). Generally, a diverse array of native plants provides food for a diversity of native herbivores and nectivores, particularly insects, which in turn can provide food for a diversity of birds (Sample 1989, Haddad et al. 2001).

It is not possible to know a priori the exact management practices and species combinations that will simultaneously optimize bioenergy production and benefit wildlife under all conditions over time. Therefore, in parallel with an emerging bioenergy industry, both ongoing monitoring and experiments are important to provide site-specific information and allow the industry to adapt as learning occurs, technologies emerge, and conditions change. Adaptive management includes clearly defined and measurable management objectives, monitoring or experiments to assess progress toward objectives, and adjustment in response to measured outcomes (e.g., Wilhere 2002). Inclusion of such adaptive management experiments in major bioenergy projects offers the best chance of creating projects that provide both bioenergy and wildlife benefits.

Policy and carbon emissions

US policy promoting biofuels has been driven primarily by interest in energy independence, rural economic development, and reducing GHG emissions. Given the large land-use implications of biofuels policy, the wildlife conservation implications of policy also merit consideration. Production of biofuel crops that leads to direct or indirect clearing of natural habitats will harm wildlife and, when the full costs of production and use are considered, are likely to increase carbon emissions (Fargione et al. 2008, Searchinger et al. 2008). Thus, policies requiring biofuels to meet carbon emission standards (now being discussed in the implementation and interpretation of EISA and various state policies) are likely to benefit wildlife by discouraging some types of conversion of natural habitat resulting from biofuel production. The establishment of carbon markets that provide economic incentives to reduce carbon emissions from natural ecosystems will also benefit wildlife. Maintaining or increasing terrestrially stored carbon, however, is not enough to guarantee wildlife benefits. For example, growing *Miscanthus* for bioenergy and converting native grassland to do so would most likely have negative impacts on wildlife even though it would probably reduce carbon emissions from petroleum use. This indicates a need for policy that goes beyond carbon considerations to explicitly address sustainability standards for biomass production, including the impacts on wildlife.

Summary and conclusions

The area in the United States devoted to corn crops is increasing, partially at the expense of perennial grasslands, with negative effects on wildlife and water quality. The recent corn ethanol boom has already been associated with the loss of more than 850,000 ha of set-aside grassland in the United States and with a 4.9-million-ha increase in corn cropland used for ethanol between 2005 and 2008. Evidence for current and future impacts on grasslands includes data on declining CRP enrollment, increasing corn area, conversion of virgin prairies, and economic analyses of future CRP enrollment. The increase in land area in grasslands from CRP starting in 1986 has had clear wildlife benefits for birds, fish, and other taxa, and for freshwater stream ecosystems in general. These benefits will erode, and wildlife populations and water quality will decline, as CRP land is lost. Thus, increased corn production for ethanol threatens wildlife and ecosystem services.

New conservation strategies are needed to protect grassland wildlife habitat. Increases in conservation payments, while needed, may reduce only a relatively small portion of expected habitat loss. Using new markets for biomass offers the tantalizing prospect of maximizing the amount of perennial grassland, land that could benefit wildlife, provide income to farmers, and contribute to domestic renewable energy production. By incorporating wildlife, water quality, carbon sequestration, and other ecosystem services in the up-front planning and consideration of biomass feedstocks, incentives could be used to encourage farmers to grow and harvest biomass for bioenergy using practices that simultaneously

provide society with multiple benefits. Opportunities to harvest native perennial plants may provide incentives to keep land in current conservation programs. However, additional incentives or regulations would be required to ensure that planting and management decisions made on the basis of short-term biomass yield, or yield and carbon sequestration, also benefit wildlife.

We have suggested several important research directions that would help bioenergy fulfill its promise of sustainable energy production. These include bettering our understanding of the effects of conversion of natural habitat to bioenergy production; researching the effects of crop or plant community composition, annual harvests, refugia, stubble height, and minimal fertilization on sustainable yield and wildlife and plant diversity; and investigating the possibility of using biomass sources that do not require a bigger agricultural footprint, such as from agricultural and other wastes. Natural resource managers and environmental scientists are well positioned to inform the policies and practices of bioenergy production. Providing society with the multiple benefits of sustainable energy and a sustainable environment will require increased partnership between natural resource managers and the bioenergy industry.

Acknowledgments

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Joseph E. Fargione (jfargione@tnc.org) is with the North American region of The Nature Conservancy in Minneapolis. Thomas R. Cooper works for the Division of Migratory Birds, US Fish and Wildlife Service, Ft. Snelling, Minnesota. David J. Flaspohler is with the School of Forest Resources and Environmental Science, Michigan Technical University, Houghton, Michigan. Jason Hill is with the Department of Applied Economics at the University of Minnesota in St. Paul. Clarence Lehman and David Tilman are ecologists in the Department of Ecology, Evolution, and Behavior at the University of Minnesota in St. Paul. Tim McCoy works for the Nebraska Game and Parks Commission in Lincoln, Nebraska. Scott McLeod is with the Great Plains Regional Office of Ducks Unlimited in Bismarck, North Dakota. Erik J. Nelson works for the Natural Capital Project, Woods Institute for the Environment, at Stanford University in California. Karen S. Oberhauser works for the Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, St Paul.

Managing Conservation Grasslands for Bioenergy and Wildlife

A Dissertation
SUBMITTED TO THE FACULTY OF
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DOCTOR OF PHILOSOPHY

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Abstract

Greenhouse gas emissions continue to rise while native grassland habitat continues to decline. A potential solution to both of these conservation priorities may exist in bioenergy. Various state and federal agencies maintain tracts of conservation grasslands, usually native perennial plants, for recreation and habitat. If biomass from conservation grasslands can be harvested without harming habitat and wildlife, then sales of grassland biomass to bioenergy producers may be the economic catalyst to expand conservation grassland acreage. This dissertation reports the bioenergy potential of conservation grasslands, how that potential can be improved, and possible effects of biomass harvest on grassland plants, ducks, and pheasants. Chapter one quantifies the bioenergy potential of biomass from conservation grasslands and identifies environmental characteristics that influence that potential. Chapter two reports an agronomically optimum nitrogen fertilization rate to increase bioenergy yields from switchgrass (*Panicum virgatum*) and mixed-species grasslands. Chapter three summarizes the effects of biomass harvest on plant diversity and species composition. Chapter four relates plant diversity and composition to duck and pheasant nest density and survival, and measures the effect of biomass harvest on both metrics of reproduction. Some major conclusion include: (1) Estimates of bioenergy potential suggest that 50% of the conservation grassland acreage within an 80 km radius of southwestern Minnesota could produce 75,700,000 liters of ethanol annually. (2) On average, bioenergy yields are predicted to increase by 52% when fertilized with agronomically optimum nitrogen rates ranging from 61 to 87 kg N ha^{-1}. (3) Biomass harvest did not affect plant species richness, species or functional group

diversity, nor change the relative abundance of the main plant functional groups in conservation grasslands. (4) Pheasant and duck nest success rates were similar in harvested and unharvested regions of conservation grasslands, but nest density was greater in unharvested regions. Overall, a substantial amount of renewable energy can be produced from harvested conservation grassland biomass without detrimental effects on plant communities or nesting pheasants and ducks.

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Preface

The big picture

In effort to learn how we can manage our planet for perpetual habitability, my dissertation research focused on addressing two major environmental problems: the increasing concentration of atmospheric CO_2 and loss of natural ecosystems. The prospects of restoring and harvesting biomass from naturalized grasslands to produce bioenergy may offer solutions to both of these problems. If a bioenergy market could provide the economic incentives to restore and manage grasslands in agriculturally dominated regions, the grassland bioenergy scenario becomes even more intriguing. This was the inspiration for my research.

I was first captivated by the complex interconnections among environmental problems after studying the Millennium Ecosystem Assessment. In the Upper Midwest, the conversion of remnant prairie to farmland destroys habitat for native species, but also transfers carbon from the soil to the atmosphere. However, converting prairie to monoculture row crops allows the US to fulfill food demands with less land. The solutions to these environmental problems may also be connected. The concept of managing grasslands to produce bioenergy and support native species seems like a possible "win-win" scenario, but research is needed to determine if a management plan can achieve both objectives while being economically viable. With this dissertation, my objective is to fill some of these and other knowledge gaps related to the use of grassland for bioenergy.

Grassland bioenergy offers new opportunities to diversify agriculture at multiple scales. At the field scale, grasslands grown for bioenergy can be composed of multiple species, which makes them more resilient to extreme environmental events like droughts or insect outbreaks. At the farm scale, a bioenergy industry provides a market for producers to grow biomass in fields that are not suitable for row crops. Diversifying market opportunities for producers also reduces economic risks compared to farms that rely on revenues from one crop. Grassland bioenergy may be an option to expand agricultural diversity. I hope that results from this research can help guide the development of more diverse and sustainable agricultural systems that limit carbon emission, support native flora and fauna, and enhance rural economies.

Technical notes

Here, I define conservation grasslands as areas that have been restored to mixtures of perennial species by state and federal programs. Not all programs have similar guidelines for what species are planted. Some programs allow non-native species like smooth brome (*Bromus inermis* Leyss.), while others require a certain proportion of sown seeds to be grasses or forbs. The number of sown species also varies by program. This research was conducted on conservation grasslands managed under three different programs; Wildlife management areas (WMAs; state managed), waterfowl production areas (WPAs; federally managed), and the conservation reserve program (CRP; privately managed and federally supported). The WMAs and WPAs are similar in that the primary objective of the managers is to provide habitat for wildlife.

At the time of this printing, chapter one has been published in the journal *PLoS One* with coauthors Joe Fargione, Craig Sheaffer, Don Wyse, and Clarence Lehman (Jungers *et al.* 2013). Chapter two has been submitted and is in review for *Biomass and Bioenergy* with coauthors Craig Sheaffer and John Lamb. Chapter three is being formatted for *Biological Conservation*, and chapter four has been submitted, reviewed, revised as requested, and resubmitted to *American Midland Naturalist* with coauthors Todd Arnold and Clarence Lehman. Throughout this dissertation I refer to "we" or "our" rather than "I" or "my" in reference to co-authorship.

Chapter 1

Title: Energy potential of biomass from conservation grasslands in Minnesota, USA

Perennial biomass from grasslands managed for conservation of soil and biodiversity can be harvested for bioenergy. Until now, the quantity and quality of harvestable biomass from conservation grasslands in Minnesota, USA was not known, and the factors that affect bioenergy potential from these systems have not been identified. We measured biomass yield, theoretical ethanol conversion efficiency, and plant tissue nitrogen (N) as metrics of bioenergy potential from mixed-species conservation grasslands harvested with commercial-scale equipment. With three years of data, we used mixed effects models to determine factors that influence bioenergy potential. Sixty conservation grassland plots, each about 8 ha in size, were distributed among three locations in Minnesota. Harvest treatments were applied annually in autumn as a completely randomized block design. Biomass yield ranged from 0.5 to 5.7 Mg ha^{-1}. May precipitation increased biomass yield while precipitation in all other growing season months showed no affect. Averaged across all locations and years, theoretical ethanol conversion efficiency was 450 l Mg^{-1} and the concentration of plant N was 7.1 g kg^{-1}, both similar to dedicated herbaceous bioenergy crops such as switchgrass. Biomass yield did not decline in the second or third year of harvest. Across years, biomass yields fluctuated 23% around the average. Surprisingly, forb cover was a better predictor of biomass yield than warm-season grass with a positive correlation with biomass yield in the south and a negative correlation at other locations. Variation in land ethanol yield was

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almost exclusively due to variation in biomass yield rather than biomass quality,

therefore efforts to increase biomass yield might be more economical than altering

biomass composition when managing conservation grasslands for ethanol production.

Our measurements of bioenergy potential, and the factors that control it, can serve as

parameters for assessing the economic viability of harvesting conservation grasslands for

bioenergy.

1.1 Introduction

Perennial biomass is an alternative to conventional starch-based biofuel feedstocks such

as corn. It may improve land-use efficiency, reduce greenhouse gas emissions, promote

biodiversity, and support other components of sustainability (Tilman *et al*. 2006,

Fargione *et al*. 2008, Robertson *et al*. 2011a). Research comparing ecosystem services of

various native and non-native perennial bioenergy crops in the Upper Midwest indicates

that bioenergy systems with more plant species support greater avian diversity (Meehan

et al. 2010), abundance and diversity of beneficial arthropods (Gardiner *et al*. 2010),

carbon storage and complexity of belowground food webs (Glover *et al*. 2010). In many

regions of North America, diverse grasslands have not produced as much gross biomass

as dedicated energy crops grown in monoculture such as switchgrass (*Panicum virgatum*

L.; Johnson *et al*. 2010). This has initiated questions regarding the economic viability of

diverse grassland bioenergy, yet few studies have quantified bioenergy yields from

diverse perennial plantings over multiple years. Only recently have studies compared the

bioenergy potential of mixed-species grasslands harvested with production-scale

techniques in various regions of the Upper Midwest (Lee *et al*. 2013).

Growing biomass on land unsuitable for commodity crops transforms the economic outlook for bioenergy systems. Bioenergy production from feedstocks grown on marginal or underutilized land, such as land enrolled in the Conservation Reserve Program (CRP), can provide immediate greenhouse gas benefits (Gelfand *et al*. 2011) while avoiding competition for land between food and energy crops (Hill *et al*. 2006). One idea is to harvest biomass from CRP land as revenue to supplement government subsidies, potentially incentivizing renewal of CRP contracts and offsetting recent trends in expiring CRP acreage (Olson 2007). Current CRP regulations do not allow biomass harvest from land enrolled in the program. If economic opportunities from bioenergy initiate new regulations that allow biomass harvest, these regulations should be designed to support the original intentions of the CRP, including improved wildlife abundance (Wiens *et al*. 2011), an important component of biodiversity.

Other conservation lands managed for wildlife by state, federal, and non-profit agencies have been planted with mixtures of perennial grassland species. These may serve as biomass sources for energy production. Studies are underway to determine the effects of biomass harvest on resident wildlife in various types of conservation grasslands (Jungers *et al*. 2011). If research concludes that conservation grasslands can be managed for bioenergy and biodiversity simultaneously, then the quality and quantity of harvested biomass from conservation lands should be considered before bioenergy management is implemented.

The amount of bioenergy from conservation grasslands depends on both biomass quantity and quality. One means of measuring biomass quantity is to multiply yields from CRP fields in different regions of North America by estimates of available acreage (Adler *et al*. 2009, Venuto and Daniel 2010, Cai *et al*. 2011, Lee *et al*. 2013). These yields can then be extrapolated to estimate biomass from land not currently enrolled in, but eligible for conservation programs. Another important component of predicting bioenergy potential is biomass quality, often defined by the mineral and sugar concentrations of the biomass. Mineral concentrations are used to predict conversion efficiency for thermochemical energy production. High concentrations of alkali metals in post-combustion ash lead to slagging and fouling in thermochemical systems (Baxter *et al*. 1998), while high concentrations of N, S, and other elements pose issues of oxide emissions and possibly nutrient removal from soils in long-term harvested systems (Robertson *et al*. 2011b). Predicting the efficiency of biofuel production with biochemical technologies requires measuring the plant sugar and carbohydrate concentrations. High values of cellulose and hemicellulose relative to lignin results in greater liquid biofuel potential (David and Ragauskas 2010).

Variation in the quantity and quality of grassland biomass with respect to energy production – hereafter called bioenergy potential – can occur due to variation in plant species composition, geographic location, and management activities. Plant composition influences bioenergy potential with studies indicating positive relationships between (i) biomass yield and planted species richness (Tilman *et al*. 2006) and (ii) relative cover of warm-season grasses (C4) and lignocellulose ratios that favor ethanol production (Adler

et al. 2009). In southern Iowa, spatial variation in biomass yield and elemental composition was greater within fields than between fields and was correlated to individual species within cool-season (C3) grasslands (Florine *et al.* 2006). A broad-scale analysis of switchgrass yields across the Great Plains indicated that within-field variation is small enough to consider the mean biomass yield of a field for modeling purposes (Schmer *et al.* 2009). Di Virgilio *et al.* found correlations between switchgrass yields and both soil fertility and moisture, which were interpreted as sources of within-field variation (2007).

Management activities, including harvest, also affect bioenergy potential. Harvesting biomass after senescence allows for plants to translocate nutrients to belowground tissues, but harvesting post-senescence means that vegetation is removed after peak biomass and lodging have occurred. In Oklahoma and South Dakota, delaying harvest until October increased yields and decreased N and ash concentrations in CRP biomass compared to pre-peak biomass harvests (Mulkey *et al.* 2006, Venuto and Daniel 2010). Harvesting switchgrass-dominated CRP lands every year compared with alternate years increased yields (Lee *et al.* 2007a), while deferring harvest to more than two year intervals lowered bioenergy potential in Canadian conservation grasslands managed for wildlife (Jefferson *et al.* 1999).

In the present study, we modeled bioenergy potential of conservation grasslands based on three response variables related to quantity and quality: biomass yield, theoretical ethanol conversion efficiency, and plant tissue N. We used data collected from large-scale plots

distributed across three locations of western Minnesota and harvested with commercial-scale tools and techniques. Our objectives were (i) to determine biomass yields, theoretical ethanol conversion efficiency, and plant tissue N content from conservation grasslands, (ii) to measure the variability of bioenergy potential along a latitudinal gradient in western Minnesota, and (iii) to understand what factors affect bioenergy potential by modeling the three response variables with data on plant communities, soil fertility, precipitation, and management activities while accounting for space and time. Two harvest treatments were used to determine if yields from completely harvested plots followed similar trends through time as yields from plots that included previously unharvested regions of biomass. Our results are intended to aid policy and land-management decisions regarding the use of conservation grasslands for bioenergy production in the Upper Midwest, USA.

1.2 Methods

1.2.1 Experimental design

In 2008, we located and delineated 60 plots within existing grasslands enrolled in a conservation program. Plots were distributed among three locations (hereafter north, central, and south locations) spanning a latitudinal gradient in western Minnesota, USA (Figure 1.1). Soils of the south are glacial till, the north are laucustrine, and the central has regions containing both. Forty plots were located on conservation grasslands managed by the Minnesota Department of Natural Resources (DNR), eight plots managed by the US Fish and Wildlife Service, and 12 plots managed by private landowners as part of the CRP. Each plot was about 8 ha (20 acres; mean = 8.1 ha, SD =

0.5 ha) in size and contained a mixture of grasses and forbs. All plots were established more than five years prior to the project start date. Three of 12 CRP plots were planted with perennial introduced grasses and legumes (CP1) and the rest with perennial native grasses (CP2). The DNR plots were established with different species, but all were categorized as "restored/planted tall grass prairie". A list of the most frequently observed species is in Table A.1. Plots were managed periodically for woody species with prescribed fire and/or mechanical harvest prior to the project start date. Fire was not implemented on our plots during the duration of the study. Occasional spot-spraying of herbicides was done in the south location to control invasive species.

Within each location, treatments were replicated in four blocks (Figure 1.1). Each block contained a control (no harvest) and three harvested plots. Since the control plots were not harvested, this analysis does not include data from those plots. Plots were randomly assigned a harvest treatment, and, for this analysis, were considered either a high- or low-intensity harvest. High-intensity treatments involved a complete harvest of the assigned plot while low-intensity treatments involved a partial harvest so that the plot contained a refuge of standing vegetation of 2 or 4 ha. The harvest treatments were designed to maintain other uses of the grassland, such as habitat for wildlife. In low-intensity harvest treatments, the refuge moved annually within the fixed plot area so that each year, a portion of the harvested area contained biomass that was not harvested the previous year. At all three locations, each block included one control plot, one high-intensity treatment, and two low-intensity treatments with refuges of 2 ha. A separate sub-study allowed the establishment of extra plots in the south location. Blocks in the south location included

one extra high-intensity treatment plot and two extra low-intensity treatment plots (totaling seven plots per block). The extra low-intensity treatment plots had refuges of 4 ha. Twenty-four plots were scheduled to be harvested in the south and twelve in each the central and north locations. Weather prevented the harvest of certain plots each year. No plots were harvested in the north in 2011 due to expiring land contracts.

1.2.2 Field and laboratory methods

A single operator harvested the plots between late October and mid December in 2009, 2010, and 2011. No plots were harvested after the first significant snowfall. Vegetation was harvested to a target height of 15 cm with a self-propelled windrower with a mounted disc cutter. When conditions were deemed dry enough by the operator, the cut biomass was immediately baled using a large round baler. If the cut biomass required drying, it was raked into larger windrows and left to dry before being baled. Due to time constraints and landowner regulations, bales were removed from the plots as soon as possible, therefore individual bales were not weighed from each plot. Instead, bales were loaded onto semi trailers and weighed with a scale certified by the U.S. Department of Transportation on transport for storage. This weight was divided by the number of bales on the trailer to determine an average bale weight and variation (coefficient of variation = 9%; for further details, see Text A.1). We divided the sum of all the trailer weights by the total number of bales to generate an overall average bale weight. The average bale weight was multiplied by the number of bales from each plot to estimate total harvested biomass. The perimeter of the cut area in each plot was measured using a hand-held global positioning system (GPS) (Garmin Ltd., Olathe, Kansas, USA) on an all-terrain vehicle.

Biomass yield was determined for each plot as the amount of biomass harvested (Mg) divided by the area cut (ha).

While bales were still in the field, core samples were extracted from bales of harvested biomass for each plot with a hay probe (Forageurs Corp., Lakeville, MN, USA) attached to an electric drill. One biomass core was collected from every other bale as they were ejected from the baler; therefore the number of core samples was determined by the size of the harvested area within the plot and biomass productivity (mean number of cores in high-intensity plots = 22). Cores were aggregated by plot and weighed wet immediately after collection (mean sample weight = 156 g), dried at 45º C for four days, reweighed and used here to estimate bale yields on a dry matter basis.

Chemical constituents of the biomass were measured from the aggregated core samples for each plot. Biomass samples were dried at 45º C for four days, ground with a Wiley mill (Thomas-Wiley Mill Co., Philadelphia, PA, USA) to pass a 1 mm screen, and then reground with a cyclone mill. A subsample from each plot was analyzed for N by AgVise Laboratories using methods described on their website (Agvise Inc., Benson MN; http://www.agvise.com).

The concentration of cell wall carbohydrates was determined using near infrared spectroscopy (NIRS) with methods described by Schmer *et al*. (2012). NIRS estimates were from equations built with samples from previous collections, upon which wet chemistry methods were used to directly determine cell wall carbohydrate concentrations

(Table A.2). The values of xylose, arabinose, mannose, galactose, and glucose were calculated with methods established by the U.S. Department of Energy to predict theoretical ethanol conversion efficiency (Equation A.1, http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html). Calculations used to estimate theoretical ethanol conversion efficiency assume 100% conversion efficiency because realized efficiency rates are not available for production-scale systems.

In the summer of 2009, soil cores were collected to a depth of 20 cm at eight points adjacent to the randomly distributed vegetation quadrats. Soil cores were aggregated by plot and processed and analyzed by AgVise Laboratories for $N-NO_3$, pH, organic matter, and cation exchange capacity.

Plant community composition was visually assessed in 1.0 x 1.5 m quadrats at 12 random points within each plot in late July and/or early August of 2010 and 2011. A total of 24 quadrats were sampled in the high-intensity treatment plots in 2010 to assess sample power. In 2009, plant community data was collected from quadrats, each 0.75 x 5 m, in all plots. Quadrat locations were generated with ArcGIS 9.3 (ESRI, Redlands, CA, USA) and loaded to hand-held GPS units. Within each quadrat, surveyors identified all plant species and assigned each a score for relative abundance as a percentage of the canopy cover in the quadrat. Bare ground and litter were also assigned a percentage. Species were aggregated into functional groups for analysis. The average cover value for each functional group was calculated by plot.

Cooperative Farming Agreements, Special Use Permits, and a letter of approval were acquired from the Minnesota Department of Natural Resources, US Fish and Wildlife Service, and the US Department of Agriculture Farm Service Agency for permission to conduct research on state, federal and private land.

1.2.3 Data Analysis

Three response variables related to different components of bioenergy potential were measured in all plots and modeled in this study: biomass yield, theoretical ethanol conversion efficiency, and plant tissue N. Linear mixed effects models were used to test the main effect of location on the three response variables and to determine which covariates were significantly correlated with them. Total variation for each response variable was partitioned into four levels of a temporal/spatial hierarchy that was used as the random structure for the variance components analysis. The largest level of this hierarchy partitioned variance among years, with lower levels partitioning variance between locations, between blocks, and within plots; each level nested within the higher level. A model with only random effects was used to determine the variance at each level of the hierarchical random structure for all three response variables. Equation 1 was modified from West *et al.* (2007) to derive variance estimates for each level of the random hierarchy, where ICC_i represents the proportion of variation at level *i* compared with the total variation.

Equation 1.1

$$ICC_{Date} = \frac{\sigma^2_{Date}}{\sigma^2_{Date} + \sigma^2_{Location} + \sigma^2_{Block} + \sigma^2}$$

$$ICC_{Location} = \frac{\sigma^2_{Location}}{\sigma^2_{Date} + \sigma^2_{Location} + \sigma^2_{Block} + \sigma^2}$$

$$ICC_{Block} = \frac{\sigma^2_{Block}}{\sigma^2_{Date} + \sigma^2_{Location} + \sigma^2_{Block} + \sigma^2}$$

To quantify the differences in biomass yield, ethanol conversion efficiency, and plant N between locations, a dummy variable was assigned to the south, central, and north locations and was modeled as a categorical main fixed effect. Using location as a fixed effect, various random structures composed of the nested spatial/temporal variables were fit to models and compared using maximum likelihood ratio tests.

Land ethanol yield (l ha^{-1}) was calculated by multiplying ethanol conversion efficiency (l Mg^{-1}) by biomass yield (Mg ha^{-1}) for each plot. A linear regression model was used to estimate the fraction of variation in land ethanol yield due to variation in biomass yield.

For each response variable, we selected a group of candidate covariates *a priori* from a list of measured variables (Table 1.1). A global model for each response variable included all covariates related to plant community structure and an interaction between each community covariate and the main effect of location. No three-way interactions were tested. Each global model included a best fitting random structure and a first order autocorrelation structure. The global model was reduced by removing the least significant fixed effect determined by t-statistic at $P < 0.05$ (Zuur *et al*. 2010). This iterative process

continued until all fixed effects were removed. The resulting models were compared using Akaike's information criteria adjusted for small sample sizes (AIC_c) (Burnham and Anderson 2002). The best fitting model was refit using restricted maximum likelihood to generate unbiased parameter estimates. For models without interactions, Tukey's *post hoc* means separation test was used to determine differences between levels of significant main effects.

A mixed effect model was used to test the effect of harvest intensity on the change in biomass yield over time. The difference in biomass yield from the first harvest (2009) to the last (2011) was calculated for plots in the south and central locations to test the hypothesis that trends in biomass yields through time would be the same for plots where all the biomass is removed as plots that include regions of previously unharvested biomass. The change in yield was compared between low- and high-intensity harvest treatments. The model included an interaction between harvest intensity and location while accounting for variation in each plot as a random variable. All statistical analyses were conducted with program R (R Development Core Team 2010).

1.3 Results

We analyzed and modeled biomass yield from 109 observations and theoretical ethanol conversion efficiency and plant tissue N from 112 observations from conservation grasslands harvested in autumn of 2009, 2010, and 2011. Weather obstructed biomass harvest at certain plots each year, which resulted in an unbalanced data set. No plots were harvested in the north location in 2011 due to expiring land contracts.

The south location received more precipitation during the growing season compared with the north and central locations during all years of the study. Precipitation was lowest in 2009 at the south and central locations, and lowest in 2011 at the north. Over the course of the project, precipitation was the greatest in 2010 and well exceeded the 30-year mean at all locations. In 2011, the north and central locations were below the 30-year mean while precipitation at the central location was higher (Table 1.2).

1.3.1 Biomass yield

Without accounting for covariates, mean biomass yield in the south was 55%, 69%, and 55% greater than other locations in 2009, 2010, and 2011 respectively (Figure 1.2A). Annual plot biomass yield ranged from 0.5 Mg ha^{-1} to 5.7 Mg ha^{-1} and had an overall mean of 2.5 Mg ha^{-1} across all locations and years. Biomass yield increased from 2009 to 2011 in both the south and central locations and in both harvest intensities (Figure 1.3). The increase in biomass yield through time was the same between harvest intensities ($F = 0.48$, df $= 27$, $P = 0.49$).

1.3.2 Biomass quality

Biomass yield was a significant predictor of the variation in land ethanol yield ($F = 5558$, df $= 1$ and 108, $P < 0.001$). The adjusted R^2 was 0.98 for the relationship between biomass yield and land ethanol yield (Figure 1.4). Mean ethanol conversion efficiency was 450 l Mg^{-1} with a standard deviation of 38 across all locations and years. Mean plant N concentration was 7.1 g kg^{-1} with a standard deviation of 1.5 and was not consistently

different among locations and years. Mean plant N was lower and mean ethanol

conversion efficiency was greater in the south than the other locations in all three years

(Figure 1.2B and 1.2C).

1.3.3 Variance components analysis

Results from the intercept-only random effects models suggest that of the total variation

in biomass yield, ethanol conversion efficiency, and plant N, the variance between years

explained the smallest fraction (Table 1.3). The largest fraction of the variance in

biomass yield and plant N was partitioned into within-plot variance, while the variation

between locations accounted for about one-third for both responses. More than a majority

of variation in ethanol conversion efficiency was observed between locations (Table 1.3).

1.3.4 Bioenergy potential models

Biomass Yield: Measured soil fertility variables did not contribute to explained variation

in biomass yield. The effect of forb cover was significant in the best fitting model (Table

1.4) and influenced biomass yield uniquely in the south compared with the other

locations (Table 1.5, Figure 1.5B). Specifically, forb cover was negatively correlated with

biomass yield in the central and north locations, but positively correlated with biomass

yield in the south location. Covariates for May precipitation and legume cover were

positively correlated with biomass yield in the best fitting model (Table 1.5). A model

with the random variables plot (identified below as PLOT; see Table 1.1) nested within

block (identified as BLOCK) was superior to a model without random effects ($L = 40.77$,

df = 1, $P < 0.001$). The three best fitting models were similar in their explanatory power determined by AIC_c (Table 1.4).

Ethanol Conversion Efficiency*: The two best fitting models included the effect of location, the cover of C4 grass, and the nitrogen content of harvested biomass as predictors of variation in ethanol conversion efficiency. The best fitting model included the cover of forbs and omitted all interactions between main effect and covariates (Table 1.4). The cover of C4 grass was positively correlated with ethanol conversion efficiency (Figure 1.5C), while plant N and forb cover showed negative relationships with ethanol conversion efficiency (Table 1.5). Ethanol conversion efficiency was significantly greater in the south than the central ($P = 0.034$) and north ($P = 0.020$) locations, with a metric ton of biomass producing 12% more ethanol in the south than the average of the central and north locations. There was no significant difference between the central and north ($P = 0.947$) locations. A model with the random variables BLOCK and DATE was best supported for explaining variation in ethanol conversion efficiency. The random structure was fit to allow unique BLOCK variation around the intercept by DATE. This structure was better supported than the fully nested random structure ($L = 13.5$, df $= 1$, $P = 0.004$) and a model without a random structure ($L = 64.7$, df $= 1$, $P < 0.001$). The two best fitting models differed by 0.69 AIC_c points and one parameter (Table 1.4).

Plant N: The three best fitting models included the main effect of location, C4 cover, and soil N-NO_3 concentration (Table 1.4). The best-supported model included an interaction term between location and legume cover (Table 1.5). In the south, legume cover was

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negatively correlated with plant N as opposed to the positive correlation observed in the central and north locations (Figure 1.5A). Soil N-NO$_3$ and C4 cover were positively and negatively correlated with plant N respectively (Table 1.5). The best fitting random structure for modeling the concentration of N in biomass included PLOT nested within BLOCK. This structure was superior to a model without a random component (L = 14.9, df = 1, P < 0.001) and to a model with a fully nested hierarchy of random variables (L = 9.2, df = 1, P = 0.003).

1.4 Discussion

Harvested biomass yields from low-input grasslands managed for conservation was 2.5 Mg ha^{-1} and on average, fluctuated 23% around this mean across the three-year study period. Assuming this yield can be achieved from all the conservation grasslands within an 80 km radius of a biorefinery located in the southwest portion of Minnesota (a total of 107,571 ha of conservation grassland or 5.4% of the total area), and that only 75% of the conservation grasslands are harvestable within that area, approximately 1000 Gw*hours of energy is available (Text A.2). If divided across the year, this is equivalent to 114 MW of continuous energy from conservation grasslands alone.

Yields were highest in the south location in all years of this experiment, but were 49% lower than first-year hand-cut yield estimates from newly established high diversity mixtures grown in similar regions (Mangan *et al*. 2011). Despite similar growing conditions, the high diversity mixtures were grown on fine loam soil with N, P, and K concentrations more than two times higher than concentrations found in our soils. From

our southern plots, biomass yield estimates from hand-cut samples collected in late July were 91% and 54% greater than yield values from commercial-scale harvest in 2010 and 2011 respectively (unpublished data), both of which are similar to the harvest efficiency of managed switchgrass plots in Italy (Monti *et al*. 2009). Although leaf loss and reallocation of C to belowground structures can account for 12% to 19% of decreased biomass yields from September to November (Sanderson *et al*. 1999), there is evidence that commercial-scale harvesting techniques can be made more efficient at both cutting more of the material to a desired height and picking up more of the material with a baler to improve yields (Monti *et al*. 2009). It should be noted that stubble and residual litter provides environmental benefits by reducing erosion and providing cover for ground nesting birds, therefore 100% harvest efficiency may not be a desired objective. Observed variation in litter quantities across studies suggests that caution be taken when comparing aboveground productivity estimates and biomass yields between small-scale and large-scale studies that do not use similar cutting and biomass collection methods.

Generally, the concentration of N in herbaceous biomass results in greater NO_X emissions during thermochemical conversion to energy compared with light fuel oil and natural gas (Nussbaumer 2003). It has been recommended to delay harvesting until after senescence to allow perennial plants to translocate N to belowground tissues for both switchgrass (Ogden *et al*. 2010) and conservation grassland biomass (Venuto and Daniel 2010). Nitrogen content in harvested biomass from this project was similar to conservation grasslands harvested after a killing frost in South Dakota (Mulkey *et al*. 2008). There is concern that low-input grasslands might not be a long-term viable source of biomass

because of N depletion during harvest (Russelle *et al*. 2007), but those concerns have not yet been tested. There is evidence that long-term annual biomass harvest from low-input grasslands does not decrease yields (Jenkinson *et al*. 1994). Mixed-species grasslands like those used in this project contain legumes that add N annually. N inputs via legumes ranged from 28 to 187 kg ha^{-1} in mowed grass/legume pastures that contained white clover (Ledgard 2001), yet studies are needed to determine the net N flux in harvested grassland systems across a range of locations.

Variation in biomass yield, ethanol conversion efficiency, and concentration of N in plant tissue was relatively small between years, deviating from each location's average by no more than +/- 27%, 11%, and 7% respectively. This is in contrast to other studies with less mature perennial grasslands (our study sites were all > 5 years old), where issues with establishment contributed to larger (up to 69%) year-to-year variation in biomass yield (Schmer *et al*. 2009). Across the total study area, between-year variability in biomass yield was small despite differences in precipitation. Our results show that precipitation during the month of May measured at the block level is important in determining biomass yield (Figure 1.6). Total precipitation may not be a good indicator for predicting biomass yields because high amounts of precipitation during harvesting months may result in lower yields due to leaf losses and other inefficiencies in biomass collection, especially when harvesting with production-scale equipment (Monti *et al*. 2009). Excessive precipitation during autumn months inundated some parts of this experiment and prevented the harvest of certain plots each year. Averaged across all years, 83%, 78%, and 74% of the planned harvested areas were harvested in the south,

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central and north locations respectively. This percentage increased annually in the south and central locations.

Consistent values for biomass quality metrics are important for viable biorefinery production. A substantial fraction of the total variation in biomass yield was observed between locations, which is in accordance with studies on the variation of switchgrass yield (Schmer *et al*. 2009). About one-quarter of the total variation in biomass yield was measured between blocks, which was similar to the results of yield variation in C3-dominated grasslands analyzed for bioenergy (Florine *et al*. 2006). Florine *et al*. (2006) reported smaller total variation in plant N (SD = 0.4 g kg^{-1}) than our results (SD = 1.5 g kg^{-1}). Total variation in ethanol conversion efficiency was relatively small but greater than reports from switchgrass, yet similar in terms of partitioning between spatial and temporal scales (Schmer *et al*. 2012).

The variation in land ethanol yield was almost exclusively due to variation in biomass yield (Figure 1.4). Land managers looking to harvest biomass from conservation grassland for ethanol production would maximize revenues by identify high biomass yielding plots as opposed to harvesting plots based on the theoretical ethanol potential of the plants.

We hypothesized that covariates would explain variation among locations (Table 1.6). However, for all response variables, location remained a significant variable in the best fitting models (Table 1.5). Best fitting models for biomass yield and plant N included

interactions between location and plant community covariates, which provide limited

information to draw conclusions as to why differences in these response variables exist

across locations. In terms of ethanol conversion efficiency, location was identified as a

main source of variation, therefore suggesting that other factors related to space – factors

that were not measured in this study – influenced the response.

Other reports have suggested that plant community characteristics such as C4 grass cover

(Adler *et al.* 2009) and planted species richness (Tilman *et al.* 2006) improve biomass

yields. In this study, it was the cover of non-legume forbs that explained variation in

biomass yield (Table 1.4 and 1.5). In the south location, plots with greater average forb

cover had higher biomass yields, while in the central and north locations, increasing forb

cover was associated with lower yields. We expected, as Adler *et al.* (2009) documented,

that the cover of C4 grass would be positively correlated with biomass yield, and our

competitive models include that variable (Table 1.4). It is possible that an increase in forb

cover displaces C4 grasses, which would explain the negative correlation between forb

cover and biomass yield in the central and north locations. The inverse relationship

between forb cover and biomass yield in the south could be driven by a high-yielding

forb species that is present or abundant in the south but not in the other locations. We

explored this possibility and found that common milkweed (*Asclepias syriaca*) was

present in 300 sample points in the south and only 50 and 5 sample points in the central

and north locations. Using data from all sample points, a Pearson's correlation test

showed that the cover of common milkweed was not correlated to the cover of C4 grass

($P = 0.303$) but was correlated to biomass yield ($P = 0.016$). This suggests that common

milkweed could increase biomass yield without displacing C4 grass cover (Table 1.6).
Other studies have observed increases in forb abundance without associated decreases in
biomass production (Jarchow and Liebman 2012).

Harvested areas in the low-intensity harvest treatments included a fraction of the plot
where vegetation was left standing the year before. This did not affect biomass yields
compared with completely harvested plots. European mixed-species hay yields did not
decrease after decades of annual harvest without nutrient inputs (Jenkinson *et al*. 1994),
though long term studies are needed to verify if similar patterns exist in North American
grasslands. The positive correlation of May precipitation with yield could be because it
supplies resources before the peak productivity time of C4 grasses, which contribute to
biomass yield when harvested in autumn (Mulkey *et al*. 2008). Other studies have shown
that the variation in June soil moisture was positively correlated with C4 grass
productivity (Nippert *et al*. 2005), but soil moisture measurements were not made in our
study.

Maximum theoretical ethanol conversion efficiency values were slightly higher than
those reported in switchgrass (Schmer *et al*. 2012) and similar to mixed prairies (Jarchow
et al. 2012), and were greater in biomass harvested from the south compared with
biomass from the central and north locations (Figure 1.2C). Studies of switchgrass show
that harvesting later after plant senescence results in higher potential ethanol conversion
efficiency (Adler *et al*. 2006), thus a similar pattern could exist in polyculture grasslands.
We harvested plots in sequence from the north to the south so that the plants would be at

a similar phenological stage at the time of cutting. A negative correlation between plant tissue N and ethanol conversion efficiency was apparent in this study (Table 1.5), and since plant N decreases with senescence, the later harvest date in the south location may have contributed to higher ethanol conversion efficiency found here. Also, our results confirm previous reports of correlations between C4 grass cover and ethanol conversion efficiency (Adler *et al.* 2009) (Figure 1.5C). In general, C4 grasses have higher levels of fermentable sugars than forbs (Lee *et al.* 2007b); therefore ethanol conversion efficiency is expected to decrease with increased forb cover relative to C4 dominated stands. As highlighted in this study, Gillitzer *et al.* (2012) showed that the relationship between species composition and biomass yield, rather than species composition and ethanol conversion efficiency, is the more dominant driver of land ethanol yield (Jarchow *et al.* 2012).

Legumes in mixed-species grasslands fix atmospheric nitrogen, which has several consequences for ecosystem functioning including increased productivity (Tilman *et al.* 1997). However, in the case of combustion bioenergy, undesirable consequences of legume biomass come in the form of pollution. Legume biomass has relatively higher levels of tissue N than forbs and grasses, which can lead to greater NO_x emissions during thermochemical energy conversion (Nussbaumer 2003). The best fitting model identified a relatively strong trend in legume cover and plant N in the north location ($t = 2.579$, $P = 0.012$). Weaker evidence of a relationship was observed in the central ($t = 1.137$, $P = 0.260$) and the south locations ($t = -0.925$, $P = 0.359$), which could be related to the absence or presence of a specific legume species, as observed in other studies (Spehn *et*

al. 2002). The estimates from this model predict that a four-fold increase in legume cover (from the observed average of 4.8% to 19.2%) in the north location would increase biomass N concentrations approximately 23%, or to a value of 10.2 g N kg^{-1}. Promoting legumes increases functional group diversity, which leads to other ecological benefits including increased soil carbon storage (Fornara and Tilman 2008). Also, complementarity among C4 grasses and legumes increases biomass yields (Fornara and Tilman 2008). Therefore, we believe that the model-estimated environmental cost of legume abundance in bioenergy grasslands is far outweighed by the ecological and yield benefits they provide.

The three best-supported models all suggest that unfertilized soils with naturally higher levels of $N-NO_3$ will produce biomass with greater concentrations of tissue N (Table 1.4). Elevated levels of soil $N-NO_3$ could come as a result of N fertilizer, which has been considered as a management tool to increase biomass yields in conservation grasslands (Mulkey *et al*. 2006, Lee *et al*. 2013). Fertilization experiments show that higher N fertilizer rates lead to higher concentrations of N in biomass tissue for C3-dominated mixed grasslands (Malhi *et al*. 2010), for switchgrass (Guretzky *et al*. 2010), and other C4 grasses (Waramit *et al*. 2011). Nitrogen fertilization can lead to a loss of species and functional group turnover (Suding *et al*. 2005), but when fertilized grasslands are harvested, species diversity has been shown to be maintained (Collins *et al*. 1998) or increase (Jarchow and Liebman 2012). When considering N fertilizers, land managers must weigh the potential benefits for biomass yields against potential detrimental effects including undesirable shifts in species composition and decreased biomass quality.

1.5 Conclusions

Biomass quality from mixed-species grasslands not managed for bioenergy is similar to dedicated energy feedstocks, in terms of theoretical ethanol conversion efficiency and biomass N. Almost all of the variation in land ethanol yield is based on biomass yield, therefore efforts should be focused on maximizing biomass yield rather than biomass quality when managing grasslands for land ethanol yield. A combination of climate, soil fertility, and plant community factors influence overall bioenergy potential. The effect of forbs and legumes on biomass yield and tissue N, respectively, were different in the south compared with the central and north locations. The covariates we measured did not explain why theoretical ethanol conversion efficiency was greater in the south compared with the other locations, but the cover of C4 grass was positively correlated with ethanol conversion efficiency. After three continuous years of harvest, leaving a portion of standing biomass within the harvested area does not influence biomass yield of future harvests. Simply focusing on plant community variables to predict bioenergy potential of conservation grasslands across various locations at the scale we studied will not provide accurate estimates; instead attention should be drawn to local variation in soil fertility, climate, and possibly plant species and interactions between these variables.

Figure 1.1. Study areas in Minnesota, located in the Upper Midwest, USA. Research blocks are indicated by circles within the outline of Minnesota in north, central, and south locations. Inset outlines treatments within blocks.
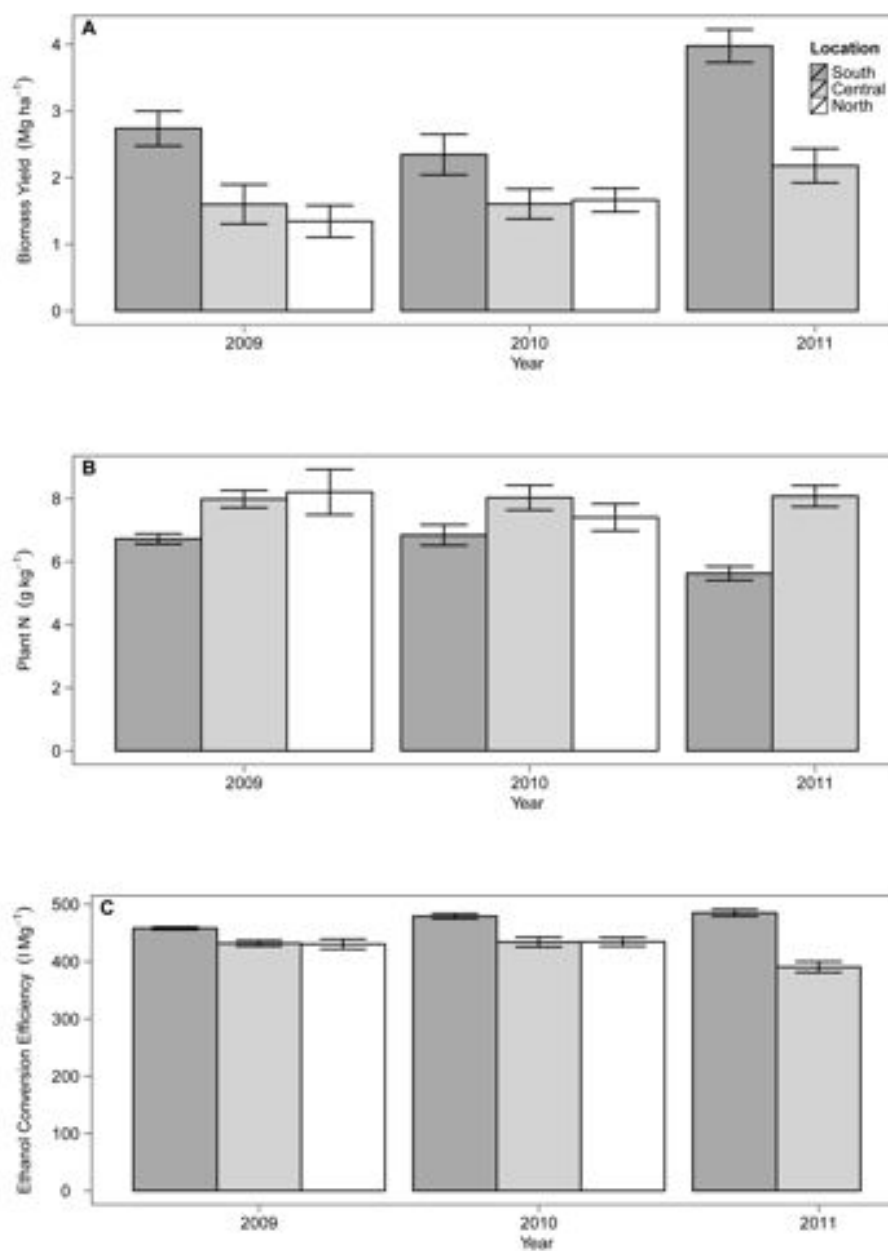
Figure 1.2. Average values (SE) of response variables by location and year. Mean values of biomass yield (A), plant tissue N (B), and ethanol conversion efficiency (C). Black, gray and white bars are mean values from plots harvested in south, central and north locations respectively.
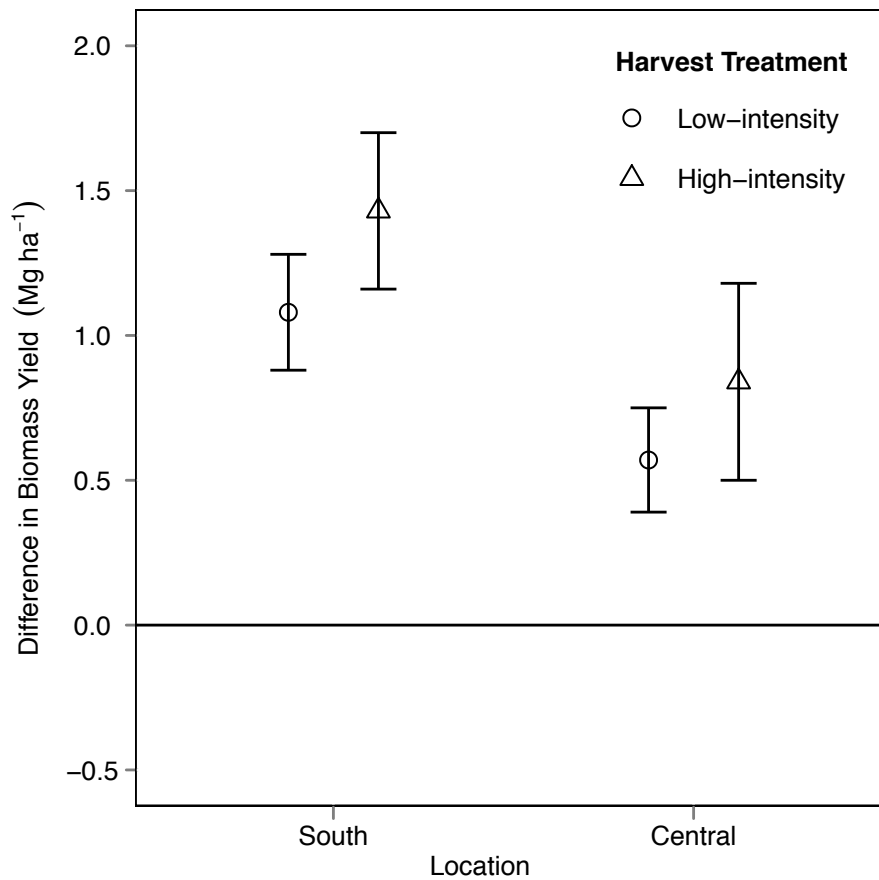
Figure 1.3. Change in biomass yield from 2009 to 2011 in low- and high-intensity harvest treatments by location. Average change in biomass yield (± 90% CI). In low-intensity plots, one third to one half of the annually harvested biomass was from an area not previously harvested. High-intensity harvest plots included biomass from the same area harvested annually.
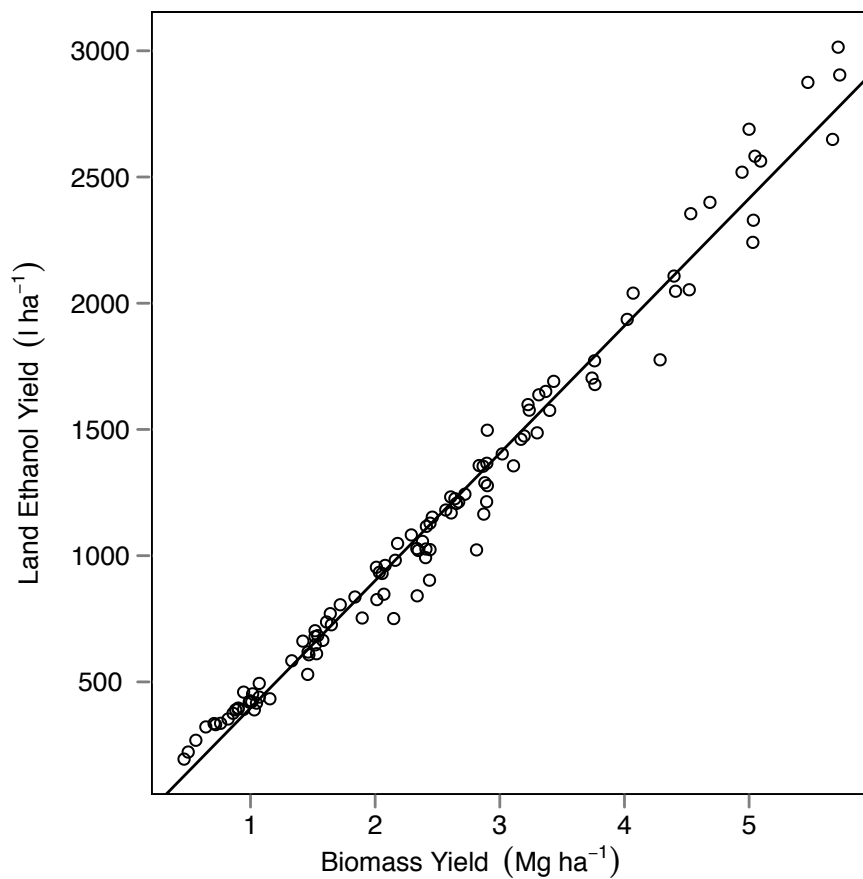
Figure 1.4. Correlation between land ethanol yield (l ha^{-1}) and biomass yield (Mg ha^{-1}). Points represent values from conservation grasslands harvested in the autumn of 2009, 2010, and 2011. Regression line from linear model with R^2 value = 0.98.
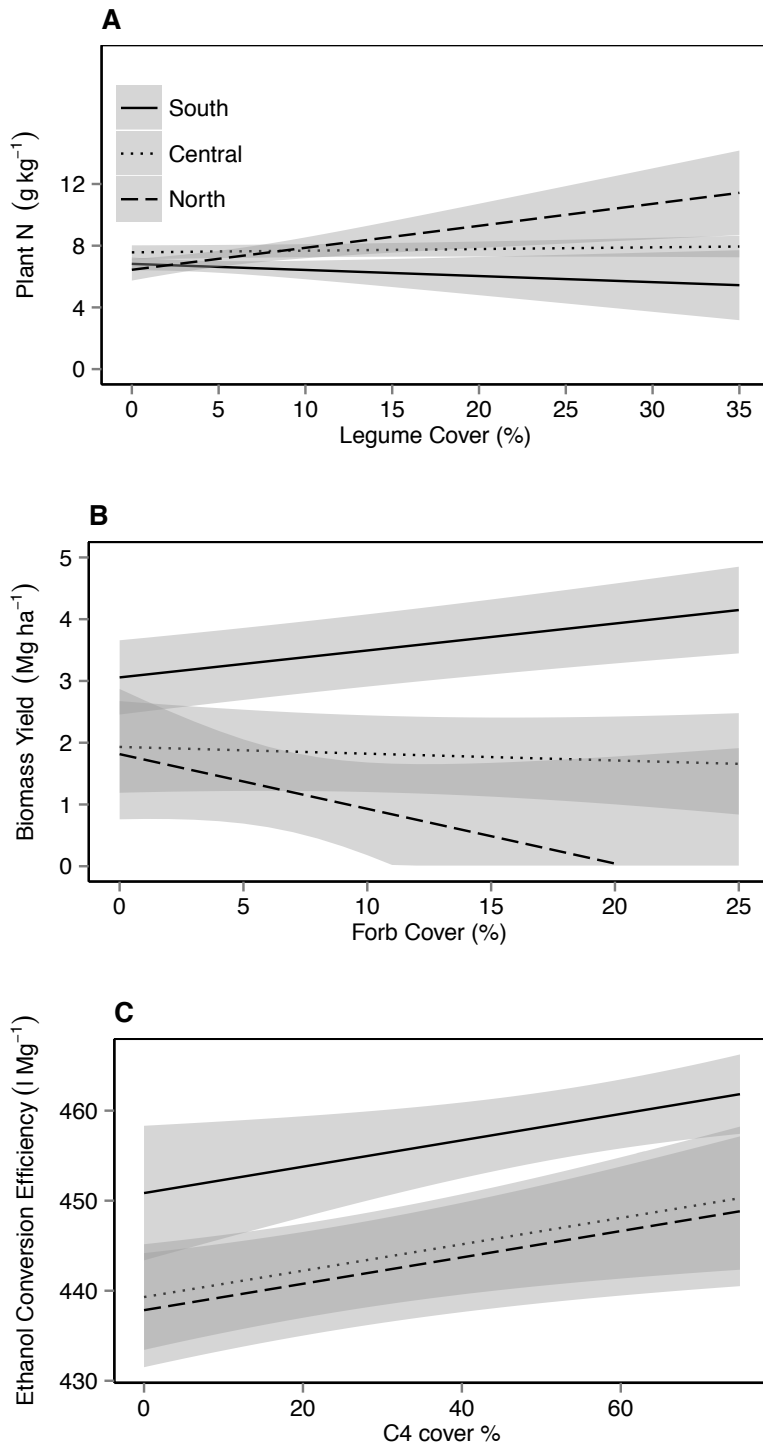
Figure 1.5. Estimated effect of plant functional group composition on bioenergy potential. Regression line estimates (± 90% CI) of the effect of legume cover on the concentration of N in biomass after harvest (A), the effect of forb cover on biomass yield (B), and the effect of C4 cover on ethanol conversion efficiency (C). Estimates are from the best fitting models with all other covariates held constant at their average values.
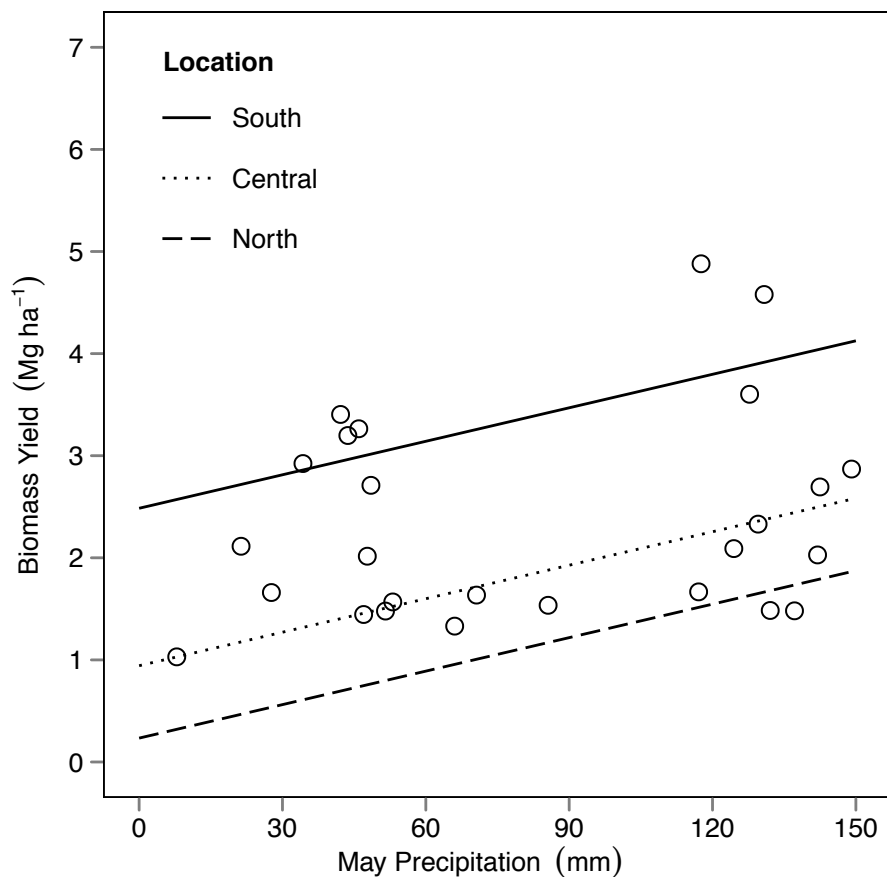
Figure 1.6. Estimated effect of May precipitation on biomass yield. Dots represent average measured biomass yield and May precipitation values by block. Regression lines are model estimates for bioenergy yield across the precipitation gradient for each location, with all other covariates held constant at their average values.

Table 1.1. List and description of all covariates available for analysis.

Effect	Variable	Description
Random	DATE, LOC, BLOCK, PLOT	Nested temporal and spatial variables. Plot nested in block nested in location.
Main	Location	Categorical main effects of location.
Plant Community	C4, C3, Legume, Forb	Continuous measure of mean percent cover of each plant functional group by plot.
Soil Fertility	NO$_3$, OM, pH, CEC	Mean values of N-NO$_3$ (NO$_3$), organic matter (OM), pH, and cation exchange capacity (CEC) by plot.
Plant Composition	PlantN	The concentration of N in harvested biomass tissue.
Precipitation	April, May, June, July, August, September	Total monthly precipitation measured for each year by block.
Interactions	C4 x Location, C3 x Location, Legume x Location, Forb x Location, Harvest x Location	Interaction between main effects, and between the main effect of location and all plant community covariates

Table 1.2. Cumulative precipitation from April through October by location and year, for comparison with other regions.

	2009	2010	2011	30 yr. mean[1]
			(mm)	
North	435	663.46	391.51	442.21
Central	452.64	663.22	538.59	518.92
South	559.09	864.36	577.13	582.93

[1]30 yr mean: http://hurricane.ncdc.noaa.gov/climatenormals/clim81/MNnorm.pdf
Minnesota Climatology Working Group:
http://climate.umn.edu/hidradius/HIDENbrowse_PHP.asp

Table 1.3. The contribution of variation from nested random effects for measures of bioenergy quantity and quality.

Nested Sources of Variation[1]	Biomass Yield	Ethanol Conversion Efficiency	Plant N
Between years	0.33 (6%)	$4.6*10^{-3}$ (0%)	$1.0*10^{-4}$ (0%)
Between locations	0.74 (31%)	28.78 (57%)	0.86 (34%)
Between blocks	0.65 (24%)	17.45 (21%)	0.15 (1%)
Within plot (residual)	0.82 (39%)	17.85 (22%)	1.18 (65%)

[1]Variation reported as standard deviation and percent of total variation.

Table 1.4. Top three best-supported models of bioenergy potential measured from conservation grasslands in Minnesota, USA.

Response	Model	Parameters (K)	ΔAIC_c
Biomass Yield	Intercept + Location x Forb + May + Legume	12	0.00
	Intercept + Location x Forb + Legume + May + June	13	1.56
	Intercept + Location x Forb + Forb + May	10	2.06
Ethanol conversion efficiency	Intercept + Location + C4 + PlantN + Forb	14	0.00
	Intercept + Location + C4 + PlantN	13	0.69
	Intercept + Location + C4 + Forb + NO3 + PlantN	15	1.86
Plant N	Intercept + Location x Legume + C4 + NO3	12	0.00
	Intercept + Location x Legume + C4 + NO3 +pH	13	0.28
	Intercept + Location + C4 + NO3	9	0.42

Table 1.5. Parameter estimates from best-fitted mixed effects models with biomass yield, ethanol conversion efficiency, and plant N as response variables.

Response	Variable	β	SE (β)	df	t	P
Biomass Yield	Intercept	2.069	0.381	56	5.432	< 0.001
	Location 2	-1.126	0.583	9	-1.932	0.085
	Location 3	-1.243	0.738	9	-1.684	0.126
	May	0.011	0.001	56	9.893	< 0.001
	Legume	0.017	0.007	56	2.428	0.018
	Forb	0.044	0.013	56	3.284	0.002
	Location 2 x Forb	-0.055	0.026	56	-2.073	0.043
	Location 3 x Forb	-0.132	0.076	56	-1.750	0.086
Ethanol Conversion Efficiency	Intercept	529.905	9.680	96	54.743	< 0.001
	Location 2	-11.550	4.623	9	-2.498	0.034
	Location 3	-13.005	4.840	9	-2.687	0.025
	C4	0.147	0.070	96	2.081	0.040
	Plant N	-10.812	1.088	96	-9.941	< 0.001
	Forb	-0.357	0.203	96	-1.760	0.082
Plant N	Intercept	6.786	0.458	59	14.827	< 0.001
	Location 2	0.746	0.400	9	1.862	0.096
	Location 3	-0.384	0.531	9	-0.724	0.488
	C4	-0.017	0.006	59	-2.975	0.004
	Legume	-0.040	0.043	59	-0.925	0.359
	NO3	0.077	0.016	59	4.748	< 0.001
	Location2 x Legume	0.050	0.044	59	1.137	0.260
	Location3 x Legume	0.182	0.071	59	2.579	0.012

Table 1.6. Mean values (SD) of covariates by location across all years from conservation grasslands in Minnesota.

Covariate	South	Central	North
		—% cover—	
C4	56.86 (18.78)	24.94 (18.37)	20.12 (18.71)
C3	18.15 (16.30)	37.77 (19.58)	45.64 (23.15)
Legume	2.80 (3.22)	8.51 (14.57)	4.81 (5.07)
Forb	6.54 (6.57)	10.35 (5.94)	6.26 (3.22)
NO_3	7.84 (3.94)	11.04 (8.35)	13.76 (12.22)
OM	5.27 (1.33)	6.52 (3.04)	5.38 (1.65)
pH	6.67 (0.49)	7.52 (0.37)	7.68 (0.65)
CEC	22.17 (7.55)	25.66 (7.44)	26.19 (8.08)

Chapter 2

Title: The effect of nitrogen, phosphorus, and potassium fertilizers on prairie biomass yield, ethanol yield, and nutrient harvest.

Native prairie plants can be managed to provide biomass for cellulosic ethanol production, however, there is inadequate information in northern latitudes regarding the effects of fertilizers on biomass and ethanol yields. We evaluated biomass yield, land ethanol yield (theoretical ethanol production per unit area), and nutrient harvest in grasslands managed across a gradient of nitrogen (N), phosphorus (P), and potassium (K) fertilizers at three locations in Minnesota, USA from 2008 to 2009. The Austin and Lamberton locations were planted with a mixture of prairie plants; while the Rosemount location was solely switchgrass (*Panicum virgatum* L.). Model-based estimations of agronomically optimum nitrogen rates (AONRs) for land ethanol yield were determined for five of six site-year environments. Five response functions were modeled for land ethanol yield, each predicting a unique AONR with varying degrees of confidence. The linear plateau function was best-supported for four of six environments. Agronomically optimum nitrogen rates ranged from 61 to 87 kg N ha^{-1}, and on average, yielded 3161, 2090, 3182 L ethanol ha^{-1} at Austin, Lamberton, and Rosemount, respectively. Phosphorus and K fertilizers did not affect land ethanol yield. Nitrogen, P, and K removed during biomass harvest increased with N fertilization, and averaged 30.9, 5.7, and 20.3 kg ha^{-1} at the AONRs. Nitrogen use efficiency declined with N fertilization during drier years. We recommend fertilizing with between 61 and 87 kg N ha^{-1} to

maximize cellulosic ethanol production from grasslands. Soil P and K should be

monitored as nutrients are removed during repeated biomass harvests.

2.1 Introduction

The United States Department of Agriculture estimates that more than 50 billion liters of

advanced biofuels will be produced from dedicated energy crops by 2022 to meet the

larger national target of 80 billion liters (USDA 2010). One advanced biofuel is cellulosic

ethanol, which is an alternative transportation fuel that can be derived from perennial,

non-food crops to limit greenhouse gas emissions and promote energy security (Tilman *et*

al. 2009). Perennial grasses such as switchgrass (*Panicum virgatum* L.), Miscanthus

(*Miscanthus* X *giganteus*), and big bluestem (*Andropogon gerardii* Vitman) have been

identified as potential dedicated energy crops for cellulosic ethanol based on their

relatively high yields and their adaptability to a broad range of growing conditions

(Sanderson and Adler 2008). Much of the research on dedicated energy crops has focused

on maximizing yields by growing them in monoculture (Heaton *et al*. 2004, Wang *et al*.

2010). However, mixtures of native perennial plants that include species from multiple

plant functional groups – such as warm-season (C4) grasses, cool-season (C3) grasses,

legumes, and non-legume forbs – can increase biomass yields (Marquard *et al*. 2009,

Jarchow *et al*. 2012) and provide additional ecosystem services compared to

monocultures (Tilman *et al*. 1997, Pokorny *et al*. 2005, Fornara and Tilman 2008).

Grasslands with a mixture of grasses and legumes produced more biomass when

harvested in autumn than most monocultures across eight study sites in Minnesota, USA

(Mangan *et al*. 2011). In other studies, C4 grass/legume bicultures had greater

harvestable biomass and belowground carbon accumulation than monocultures (Fornara

and Tilman 2008).

Although cellulosic biofuel feedstocks may be harvested from fields sown with dedicated

energy crops, mixed-species biomass from marginal land has direct greenhouse gas

mitigation potential that rivals dedicated energy crops (Gelfand *et al*. 2013). For example,

there are more than 1.4 million ha of perennial grassland seeded in the Conservation

Reserve Program (CRP) in Minnesota, North Dakota, and South Dakota. Perennial

grassland biomass yields from marginal land enrolled in the CRP were as high as 7.9 Mg

ha^{-1} without fertilization (Zamora *et al*. 2013), but the bioenergy production potential of

these lands managed with fertilization is uncertain.

The effect of fertilization on biomass yield has been studied for various bioenergy

feedstocks to identify optimal fertilization rates (Heaton *et al*. 2004, Waramit *et al*. 2011,

Garten Jr. *et al*. 2011, Sindelar *et al*. 2012). In most studies, linear regression was used to

fit various response functions to identify the N fertilization rate at which biomass yields

are maximized: the agronomically optimum N rate (AONR). Examples of AONRs for

switchgrass managed for bioenergy in the Midwestern US ranged from 62 to 120 kg ha^{-1}

(Vogel *et al*. 2002, Boyer *et al*. 2012). However, many studies reporting AONRs do not

report statistical reliability with their estimates. Failing to include confidence intervals or

other measures of statistical uncertainty in AONR estimates can lead to over or under-

application of fertilizers and suboptimal crop production (Jaynes 2010). Methods to calculate uncertainty of AONRs have been reported for corn production (Hernandez and Mulla 2008).

Maximum theoretical ethanol potential can be estimated based on the concentration of fermentable sugars within biomass lignocellulose (Dien *et al*. 2006). Previous studies reported an average theoretical ethanol potential of 405 L Mg^{-1} in switchgrass harvested in North Dakota, USA (Schmer *et al*. 2012), 450 L Mg^{-1} in mixed-species biomass from conservation grasslands in Minnesota, USA (Jungers *et al*. 2013), and 388 L Mg^{-1} in C4 dominated grasslands in Minnesota, USA (Gillitzer *et al*. 2012). Furthermore, multiplying theoretical ethanol potential by biomass yield provides a measure of ethanol potential per unit area; hereafter referred to as land ethanol yield. Estimates of land ethanol yield range from 1125 L ha^{-1} from conservation grassland biomass (Jungers *et al*. 2013) to 5500 L ha^{-1} for fertilized C4 dominated grasslands (Jarchow *et al*. 2012) in the Upper Midwest, USA. The AONR for land ethanol yield is unknown for mixed-species grassland biomass in the Upper Midwest, USA.

Nutrients in biomass are removed annually during harvest. Over time, nutrient removal during biomass harvest may deplete nutrients from the soil and subsequently lower biomass yields. For example, available soil phosphorus (P) decreased at some sites in North and South Dakota after five years of annual switchgrass harvest, suggesting that P fertilizer may be necessary for long-term harvest sites (Schmer *et al*. 2011). Nitrogen in

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harvested biomass can be substantial in high-yielding, N fertilized systems as demonstrated by Guretzky *et al*. (2010); who reported harvest rates of 85 kg ha^{-1} of N in switchgrass biomass fertilized at 90 kg ha^{-1}. Although K harvest has been reported for switchgrass, big bluestem (Heggenstaller *et al*. 2009), and mixed-species grasslands (Tonn *et al*. 2010), the implications of K harvest from grasslands are less understood. Reports of nutrient removal through harvest of monoculture bioenergy crops vary by species (Kering *et al*. 2011) and fertilization rates (Heggenstaller *et al*. 2009). Therefore, determination of nutrient harvest from dedicated energy crops and mixed-species grasslands across locations and fertilizer gradients is essential for planning economically viable, long-term bioenergy operations.

Determining the AONR that maximizes land ethanol yield of mixed-species grasslands harvested after senescence will provide useful information to increase production efficiency. Our objectives were to measure the response of mixed-species grassland and switchgrass biomass and ethanol yields to a range of N fertilizer rates, determine whether responses were affected by P or K fertilization, and identify an AONR based on land ethanol yield for three regions of Minnesota, USA. Another objective was to measure the effect of fertilization on biomass nutrient harvest to determine nutrient removal and N use efficiency of harvested biomass across fertilizer treatments and environments.

2.2 Methods

2.2.1 Site description

Research was conducted on established stands of native perennial plants at sites in

Austin, Lamberton, and Rosemount, Minnesota in 2008 and 2009 (Table 2.1). The Austin

and Lamberton sites were restored in 2005 to a diverse mixture of native grasses and

forbs. The average canopy cover was 64% perennial grasses, 35% forbs, and 2% weeds

at Austin and 62% perennial grasses, 16% forbs, and 23% weeds at Lamberton. The most

prominent grass species at both polyculture sites were switchgrass, big bluestem, and

indiangrass (*Sorghastrum nutans* (L.) Nash). Common forbs at Austin were Canada

goldenrod (*Solidago canadensis* L.), yellow coneflower (*Ratibida pinnata* (Vent.)

Barnh.), and blackeyed Susan (*Rudbeckia hirta* L.). Common forbs at Lamberton were

Maximilian sunflower (*Helianthus maximilani* Schrad.), daisy fleabane (*Erigeron*

strigosus Muhl. ex Willd.), and blackeyed Susan. Common weeds at Austin and

Lamberton were green foxtail (*Setaria viridis* (L.) P.Beauv.), common ragweed

(*Ambrosia artemisiifolia* L.), and Canada thistle (*Cirsium arvense* (L.) Scop.). The

Rosemount site was seeded to a commercially-marketed switchgrass variety, 'Sunburst'

in 2005. Initial stands at all locations had >95% ground cover prior to fertilizer

application in 2008. All locations were rain-fed (Table 2.2).

2.2.2 Experimental design and field methods

The experimental design at each location was a randomized complete block with four

replications per location. Treatments were applied in a full factorial arrangement of either

N and P or N and K. Plots were 3 m × 3 m and received variable rates of N fertilizer (0,

56, 112, 168, and 224 kg N ha^{-1}) as ammonium nitrate (34-0-0) that were combined in a

factorial arrangement with variable rates of P or K fertilizer depending on initial soil fertility tests. For the low P soils at Austin and Lamberton, P was applied at rates of 0, 34, 67, 101 and 135 kg P_2O_5 ha^{-1} as triple super phosphate (0-46-0) and for the low K soil at Rosemount, K was applied at 0, 45, 90, 135, and 179 kg K_2O ha^{-1} as potassium chloride (0-0-60). Fertilizers were broadcast in May of 2008 and 2009.

Biomass yield was determined by harvesting and weighing a representative 1 m × 1 m area to a 1.5 cm stubble height within each plot in early November each year following a killing frost (-2º C). A subsample of the harvested material from each plot was oven-dried at 57º C to adjust biomass yields for moisture content, thus yields were expressed on a dry matter basis. Each subsample was then ground and analyzed for cell wall polysaccharides using a combination of wet chemistry (Theander *et al*. 1995) and near infrared reflectance spectroscopy (NIRS) (Vogel *et al*. 2010). Equations for NIRS were developed using the software program Calibrate (NIRS 3 version 4.0, Infrasoft International, Port Matilda, PA) with the modified partial least squares regression option (Shenk and Westerhaus 1991). Ethanol potential was calculated using the energy ethanol yield calculator (http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html), which was based on biomass 5- and 6-carbon sugar concentrations Equation 2.1:

$$Theoretical\ Ethanol\ Yield\ (L\ Mg^{-1})$$
$$= [(\%\ Arabinose + \%\ Xylose) \times 737.55]$$
$$+ [(\%\ Glucose + \%\ Galactose + \%\ Mannose) \times 720.66]$$

Land ethanol yield was calculated by multiplying ethanol potential by biomass yield.

Biomass N was determined by combustion, while P and K by inductively coupled plasma

spectrometry using standard procedures at a commercial laboratory (Agvise Laboratories,

Benson, MN). Nutrient harvest was calculated by multiplying biomass nutrient

concentrations by biomass yield.

2.2.3 Statistical analysis

Data were first analyzed as a factorial randomized complete block design. Data from each

location were analyzed separately due to variation in plant species composition and

fertilizer type. The effect of N, P, and K fertilizer, and year were determined using

analysis of variance (ANOVA) with $\alpha = 0.05$. Fisher's least significant difference (LSD;

$P = 0.05$) was used to identify differences in means between levels of significant factor

predictors. Fertilizers were analyzed as factored variables when used with ANOVA for

all response variables. When fertilizers were significant based on ANOVA, they were

analyzed as continuous responses using linear regression.

2.2.4 Agronomically optimum nitrogen rate

Agronomically optimum nitrogen rates were determined for land ethanol yield by fitting

five response models to the data. The five response models were linear (LR), quadratic

(QD), square root quadratic (SQD), linear plateau (LRP), and quadratic plateau (QDP;

Table 2.3). The use of these models for estimating optimum fertilizer rates for crops is

described by Cerrato and Blackmer (1990) and Bullock and Bullock (1994). The models

were reparameterized from their original form to include a parameter that identifies the optimum of each function (β_2; Table 2.3). The β_2 parameter is equivalent to the AONR. Reparameterization allowed estimation of standard errors and confidence intervals (CIs) of β_2, and thus AONR, directly from the regression analysis. This method is described in detail by Hernandez and Mulla (2008) and Jaynes (2010). Reparameterized models were analyzed using non-linear regression for each site-year environment using the `nls` function in the R 'stats' package (R Development Core Team 2010).

After fitting all functional response models to observed land ethanol yields, CIs were generated for the parameter estimates by bootstrapping the data ($n = 9999$) using the `nlsBoot` function in the R package 'nlstools' (Baty and Delignette-Muller 2012). Confidence intervals for β_2 and goodness of fit as determined by Akaike information criterion adjusted for small sample size (AICc) were used to select one model for reporting AONR (hereafter the predictor model). The AONR was used from the predictor model for each environment to estimate all other response variables (biomass yield, nutrient harvest, and nitrogen use efficiency) at this N rate. We used a two step process for selecting the predictor model; 1) ranked the candidate models by AICc score with the lowest score identifying the superior model (Burnham and Anderson 2002), and 2) assessed the CI of the AONR for reasonableness. In many cases, the difference in AICc among competing models was less than two points, which does not provide strong evidence of differentiation among a pair of non-nested models (Arnold 2010). If multiple top models were within two AICc points, we selected the model with the smallest

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CI/AONR ratio as the predictor model (Table 2.4). Figure 1 illustrates how multiple

models that fit the data similarly can generate AONRs and CIs that are considerably

different. Our two-step method for determining a predictor model is based on the

variation explained by the model (accuracy of parameter estimation) and confidence of its

predictive capabilities (precision of parameter estimation). Since the LR model does not

estimate an AONR, the LR model was selected if its AICc score was more than 2 points

less than any other model with a CI/AONR ratio less than 1. This method does not rely

on P values from a statistical test for model selection like methods used by Boyer *et al*.

(Boyer *et al*. 2012).

After selecting a predictor model to estimate an AONR and its associated CI for each

environment, we sequentially fit the same five models to all other response variables;

biomass yield, N, P, and K harvest, and N use efficiency (NUE). We selected the top

model for each of these responses at each environment based solely on lowest AICc. We

omitted the step of assessing CIs of the parameter estimates since we were less concerned

with parameter estimate precision than determining the best model fit. Instead, we

predicted the response at the level of the AONR based on land ethanol yield. This value

is not a predicted parameter in the modeled response. Confidence and prediction intervals

are not available for estimates other than the coefficients for non-linear models at this

time.

2.3 Results and Discussion

2.3.1 Biomass yield

Average biomass yield in unfertilized plots was 4.9, 3.7, and 4.6 Mg ha^{-1} at Austin, Lamberton, and Rosemount, respectively. At Austin, biomass yields declined from 2008 to 2009 (Table 2.5). This may be associated with a decrease in rainfall at that location (Table 2.2). Biomass yields and precipitation were similar between years at Lamberton (Table 2.2; Table 2.5). Rosemount experienced a 57% decline in biomass yield despite receiving more precipitation in 2009 than 2008. However, the precipitation that fell at Rosemount in 2009 was more intermittent, with heavy events in August and October. Except for Austin in 2008, all sites and years received less cumulative precipitation during each growing season than the 30-year average (Table 2.2).

Nitrogen fertilization increased biomass yield at all locations. At Austin and Rosemount, the effect of N differed by year (Table 2.6). Therefore, we analyzed the effect of N on biomass yields in 2008 and 2009 separately for all locations. In 2008, observed biomass yields peaked at the greatest applied N fertilizer rate of 224 kg N ha^{-1} at all locations. There was a 46, 30, and 44% increase in biomass yield at the largest N fertilization rate (224 kg N ha^{-1}) compared to unfertilized biomass at Austin, Lamberton and Rosemount, respectively. Compared to 2008, yield responses were similar in 2009 at Lamberton, but peaked at lesser N rates at Austin and Rosemount in 2009 (Table 2.5). In 2009, maximum biomass yields were 100, 49, and 79% greater than unfertilized yields at Austin, Lamberton, and Rosemount, respectively. Averaged across years, P and K fertilization did not affect biomass yield at any location (Table 2.6).

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In mixed-species grasslands at Austin, the biomass yield response to N fertilization was predicted by the LR model in 2008 and the SQD model in 2009. The best-supported model at Lamberton was SQD during both years. The SQD and LRP models were best-supported for the switchgrass monocultures at Rosemount in 2008 and 2009, respectively.

Variation in biomass yield responses to N fertilization across locations may have been related to species composition of the biomass. Other studies reported variation in biomass yield responses to N fertilization depending on grass species (Kering *et al*. 2011). In other experiments investigating N fertilizer effects on mixed-species grasslands, sites dominated by both C4 and C3 grasses responded positively to N fertilizer (Mulkey *et al*. 2006, Lee *et al*. 2013). The LR response we observed at Austin, where we tested mixed-species plantings, corroborate previous research (Berg 1995). It is notable that the response shifted from LR in 2008 to SQD in 2009, resulting in peak biomass at a lower N rate at Austin. Muir *et al*. (2001) observed a similar shift from a LR to QD response and noted that a LR response earlier in the experiment could have been caused by the relatively undeveloped root system which prevented complete utilization of the applied N. Heggenstaller *et al*. (2009) also observed this trend and predicted that more years of observation might lead to reduced N fertilization recommendations as responses may shift from linear to quadratic. The grassland plots at Austin were well established, so it is not clear if the immature root system hypothesis explains the shift from LR to SQD

response. A post-hoc analysis of this assumption was not possible because belowground biomass was not measured.

2.3.2 Theoretical ethanol potential

Average theoretical ethanol potential in unfertilized plots was 448, 435, and 479 L Mg^{-1} of biomass at Austin, Lamberton, and Rosemount, respectively. Theoretical ethanol potential was similar in both years at Austin, increased in 2009 at Lamberton, and decreased in 2009 at Rosemount (Table B.1). Other studies reported greater ethanol potential in grasslands dominated by C4 grasses compared to C3 grasses (Gillitzer *et al*. 2012, Zamora *et al*. 2013), likely because of a greater concentration of cell wall sugars in C4 grasses (Dien *et al*. 2006). Despite the presence of C3 grasses and forbs in the mixed-species grasslands at Austin and Lamberton, we did not consistently observe reduced ethanol potential at these sites compared to switchgrass monoculture at Rosemount.

Theoretical ethanol potential decreased where N fertilizer was applied at all locations except for at Lamberton in 2008, where no relationship was observed (Sindelar *et al*. 2012) (Table B.1). Phosphorus fertilization also affected theoretical ethanol potential at Austin and Lamberton (Table 2.6). When considered a categorical variable, a significant interaction between P fertilizer and year was apparent at Austin ($F = 2.72$, $P = 0.03$), but when P fertilizer was modeled as a continuous variable using linear regression, a weak, non-significant relationship was observed ($P = 0.07$, $R^2 = 0.03$). The response of theoretical ethanol potential to fertilization was relatively small compared to the response

of biomass yield. In light of this finding and its economic implications, we focused on land ethanol yield.

2.3.3 Land ethanol yield

Average land ethanol yield in unfertilized plots was 2197, 1619, and 2218 L ha^{-1} at Austin, Lamberton, and Rosemount, respectively (Table 2.7). At all locations, ethanol yield was strongly correlated to biomass yield (Pearson's correlation coefficient = 0.99, P < 0.001). Land ethanol yield declined from 2008 to 2009 by 20% at Austin and 59% at Rosemount, and was similar between years at Lamberton. Averaged across treatments, Rosemount had the greatest land ethanol yield in 2008 (4197 L ha^{-1}) followed by Austin (3348 L ha^{-1}) and Lamberton (1938 L ha^{-1}; LSD = 200 L ha^{-1}). This changed in 2009 as land ethanol yields ranked largest to smallest at Austin (2686 L ha^{-1}), Lamberton (2011 L ha^{-1}) and Rosemount (1722 L ha^{-1}; LSD = 227 L ha^{-1}). The relatively drastic decline in biomass yield at Rosemount translated to a significant decline in land ethanol yield from 2008 to 2009 (Table 2.7).

The relationship between N rate and land ethanol yield was positive at all locations in 2008 and 2009. At Austin, the predictor model used to estimate AONR was LR in 2008 and SQD in 2009 (Figure B.1). The predictor models were LRP at Lamberton and Rosemount during both years (Figures 2.1 and B.1, Table 2.7). Phosphorus and K fertilizers did not affect land ethanol yield at any location or year (Table 2.6).

Two or more models were similar in estimating variation in land ethanol yield at all environments except Austin in 2009. At environments where multiple models were similar in AICc, CIs were important for choosing the predictor model (Table 2.4, Figure 2.1). For instance, at Rosemount in 2009 the SQD, LRP, and QDP models differed in AICc by less than one (Table 2.7), and all three fit the data well based on visual assessment (Figure 2.1). The SQD model estimated an AONR with a relatively large CI (Figure 2.1; Table 2.4). The LRP and QDP models estimated AONRs that were similar, but the LRP had a smaller CI relative to its estimate; therefore, it was selected as the predictor model (Table 2.4). At Lamberton in 2009 the AICc score for the LRP model was more than 2 points less than the next lowest model score, indicating that it explained the most variation in the data. However, this model estimated an AONR of 1799.4 kg N ha^{-1}, which far exceeds a reasonable N fertilization rate. Small CIs are a desired trait for predicting AONR, but they should not be used to compare the accuracy among other models (Jaynes 2010). Nonetheless, small CIs are an appropriate qualitative measure for choosing a predictor model when multiple models do not generate similar distributions for AONR estimates (Jaynes 2010).

If a bioenergy industry grows and a market for biomass stabilizes, it will be necessary to factor in biomass prices to determine economically optimum nitrogen rates. Also, as cellulosic ethanol facilities expand to production capacity, realized conversion efficiency rates will be available and necessary for calculating economically optimum nitrogen

rates. In our analysis and others (Jungers *et al*. 2013), maximum theoretical ethanol potential was calculated because realized efficiencies are not yet available.

2.3.4 Nutrient harvest

Various interactions between fertilizers and time influenced nutrient harvest at all locations (Table 2.6). Since N was the only fertilizer that affected yields, we focus on the effects of N on nutrient harvest.

Nutrients harvested in aboveground biomass varied by location and year (Table 2.6). In 2008, average N harvest in unfertilized plots was similar at all locations averaging 28.9 kg ha^{-1} (Table 2.8). Nitrogen harvest declined at all locations in 2009, averaging 14.8, 15.4, and 8.2 at Austin, Lamberton, and Rosemount, respectively (Table 2.8). As expected, the patterns in nutrient harvest closely followed the patterns observed in biomass yield. Nitrogen fertilization affected N harvest at all locations and in all years (Table 2.6; Table 2.8). The positive relationship was LR at Lamberton and Rosemount during both years, LR at Austin in 2008, and QD at Austin in 2009 (Table 2.8). At environments where AONRs were identified for land ethanol yield, it is clear that the AONRs were well above the amount of N removed in the biomass at those locations (Table 2.8).

In 2008, average P harvest in unfertilized plots was 4.8, 1.9, and 8.6 kg ha^{-1} at Austin, Lamberton, and Rosemount, respectively. Phosphorus harvest declined at Austin and

Rosemount in 2009 (Table 2.8). The effect of N fertilization on P harvest varied by

location and year (Table 2.6). Averaged over both years, P harvest was 105, 32, and 64%

greater in plots fertilized with 224 kg N ha^{-1} compared to unfertilized plots at Austin,

Lamberton, and Rosemount, respectively. Nitrogen fertilization did not affect P harvest at

Lamberton in 2008 but did generate a LR response in 2009 (Table 2.8). The relationship

between N fertilization and P harvest was LRP during both years at Rosemount, LR at

Austin in 2008, and LRP at Austin in 2009 (Table 2.8).

In 2008, average K harvest in unfertilized plots was 17.4, 11.0, and 27.5 kg ha^{-1} at

Austin, Lamberton, and Rosemount, respectively. Potassium harvest declined at all sites

in 2009 (Table 2.8). Averaged over both years, K harvest was 133, 80, and 75% greater

in plots fertilized with 224 kg N ha^{-1} compared to unfertilized plots at Austin, Lamberton,

and Rosemount, respectively. At Austin a LR relationship was observed between N

fertilizer rate and K harvest in 2008, followed by a SRQ relationship in 2009. A LR

relationship was observed for both years at Lamberton, and a LRP relationship for both

years at Rosemount (Table 2.8).

Nutrient harvest can be considered a consequence of increased biomass growth from N

fertilization and assessed at the AONR for land ethanol yield. The N removed annually

with biomass harvest is replaced at the AONRs we identified. This is not the case for P

and K. Since our results suggest that P and K fertilizers do not affect biomass yields on

these soils in the short term, we do not recommend investing in their application

annually. In unfertilized plots, P and K harvest was low compared to other reported

values (Guretzky *et al.* 2010), however, we observed significant increases in P and K

harvest with N fertilization. Therefore, we suggest that P and K be monitored with soil

tests, and added to soils when needed. Phosphorus harvest was 4.5, 2.1, and 4.0 kg ha^{-1} at

AONRs identified for Austin, Lamberton and Rosemount in 2009 (Table 2.8), which are

low compared to other reported P harvest values between 7.9 and 13.0 kg ha^{-1} for four

different grass species fertilized at 140 kg N ha^{-1} (Heggenstaller *et al.* 2009). The effects

of nutrient removal from biomass harvest on soil properties were reported by Schmer *et

al.* (2011) who found an average annual decrease in soil available P of 1.5 kg P ha^{-1} yr^{-1}

after 5 years of switchgrass harvest. At this rate of decline, the authors stated that it was

unlikely that available P limited biomass yield during the study.

Far less research has been done on the effect of biomass removal on soil K. As an

essential mineral for plant physiological and biochemical function, K conservation is

critical in harvested grasslands (Kayser and Isselstein 2005). Potassium harvest at

AONRs ranged from 12.4 kg K ha^{-1} at Lamberton in 2009 to 42.2 kg K ha^{-1} at

Rosemount in 2008. Mineral harvest at Austin was similar to unfertilized C3 dominated

grasslands in Minnesota, while mineral harvest at Rosemount was similar to unfertilized

C4 grasslands reported from the same study (Gillitzer *et al.* 2012).

2.3.5 Nitrogen use efficiency

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In 2008, nitrogen use efficiency (NUE) did not change with N fertilization at Austin ($P =$ 0.06) and Lamberton ($P = 0.12$), where it averaged 15.2 and 7.6 kg biomass kg N^{-1}, respectively (Figure 2.2). At Rosemount in 2008, the SRQ model best explained the decrease in NUE, and predicted NUE of 30.8 kg biomass kg N^{-1} at the AONR. In 2009, the SQR model best explained the decrease in NUE in response to N fertilization at all locations. The predicted NUE at the AONR was 42.2, 15.7, and 27.3 kg biomass kg N^{-1} at Austin, Lamberton, and Rosemount, respectively.

Reduced NUE with increased N fertilization has been observed for orchardgrass (Zemenchik and Albrecht 2002) and other dryland forage grass species (Jacobsen *et al*. 1996) when grown in monoculture. Diminishing NUE with associated increases in N fertilization rates suggests that other resources, other than N, become the limiting resource for productivity in N fertilized systems (Jacobsen *et al*. 1996). Our results suggest that neither P nor K were limiting productivity following N fertilization at locations where N and P concentrations were low in the soil. Moisture could explain the observed relationship between NUE and N fertilization. Austin and Lamberton received more precipitation in 2008 compared to 2009, which may explain why NUE was constant across N fertilization rates in 2008, but not in 2009.

Comparing NUE of perennial crops to annual crops can be misleading since perennial crops invest more resources, including N, to belowground biomass. Nitrogen use efficiency measures the change in aboveground biomass (shoots) in response to N

fertilization, but does not account for changes in belowground biomass (roots). In a study of switchgrass and big bluestem grown in monoculture, root biomass and the concentration of N in the root biomass increased in response to N fertilizer (Heggenstaller *et al*. 2009). Although we did not measure root biomass, it is likely that the plants at Austin, Lamberton, and Rosemount used N to increase root biomass, which would explain relatively low values of NUE at these sites. Investment of N fertilizer to root biomass in perennial grasses managed for bioenergy is important for long-term crop management and carbon sequestration, thus should not be considered a negative consequence of fertilization.

2.4 Conclusions

In established mixed-species grasslands and switchgrass monocultures, N fertilization consistently increased biomass and land ethanol yield, while P and K fertilizers had no effect. We identified agronomically optimum N rates (AONRs) and associated confidence intervals based on land ethanol yield for five of six environments, which ranged from 61 to 87 kg N ha^{-1}. Averaged across years, N fertilizer applied at AONRs increased biomass yield by 49, 19, and 34% compared to controls at Austin, Lamberton, and Rosemount, respectively. Land ethanol yield increased similarly to biomass yield with N fertilization, and averaged 3161, 2090, 3182 L ha^{-1} at the AONR at Austin, Lamberton, and Rosemount, respectively. Our results show that multiple models can provide similar measures for goodness of fit, yet predict very different AONR for yield responses to N fertilization. In these situations, uncertainty measurements should be used

to select a model for predicting AONR. We show that confidence intervals can be calculated for AONRs and incorporated into model selection criteria.

Averaged across years, fertilizing grasslands at AONRs resulted in P harvest of 4.5, 2.1, and 8.1 kg P ha^{-1} and K harvest of 19.5, 13.3, and 27.7 kg K ha^{-1} at Austin, Lamberton, and Rosemount, respectively. Therefore, we recommend that P and K be monitored in soils under grasslands managed with N fertilizers for long-term bioenergy production. Nitrogen harvest was well below the AONR for land ethanol yield at all locations (averaged 38.5, 26.7, and 31.4 kg N ha^{-1} at Austin, Lamberton and Rosemount, respectively), therefore soil N depletion may not be an issue for grassland bioenergy systems fertilized at the AONR found in the study region. Nitrogen use efficiency was unaffected by N fertilization at Austin and Lamberton in 2008, and declined at Rosemount in 2008 and all locations in 2009. Declining NUE in response to N fertilization could be due to moisture limitation, reallocation of N to root production, or a decrease in N acquisition. Nitrogen use efficiency was best predicted with the SQD function, and was estimated at 30.8 kg biomass kg N^{-1} for Rosemount in 2008, and 42.2, 15.7, and 27.3 kg biomass kg N^{-1} for Austin, Lamberton, and Rosemount in 2009. More research is needed to determine the fate of N fertilizer in mixed-species grasslands managed for bioenergy.
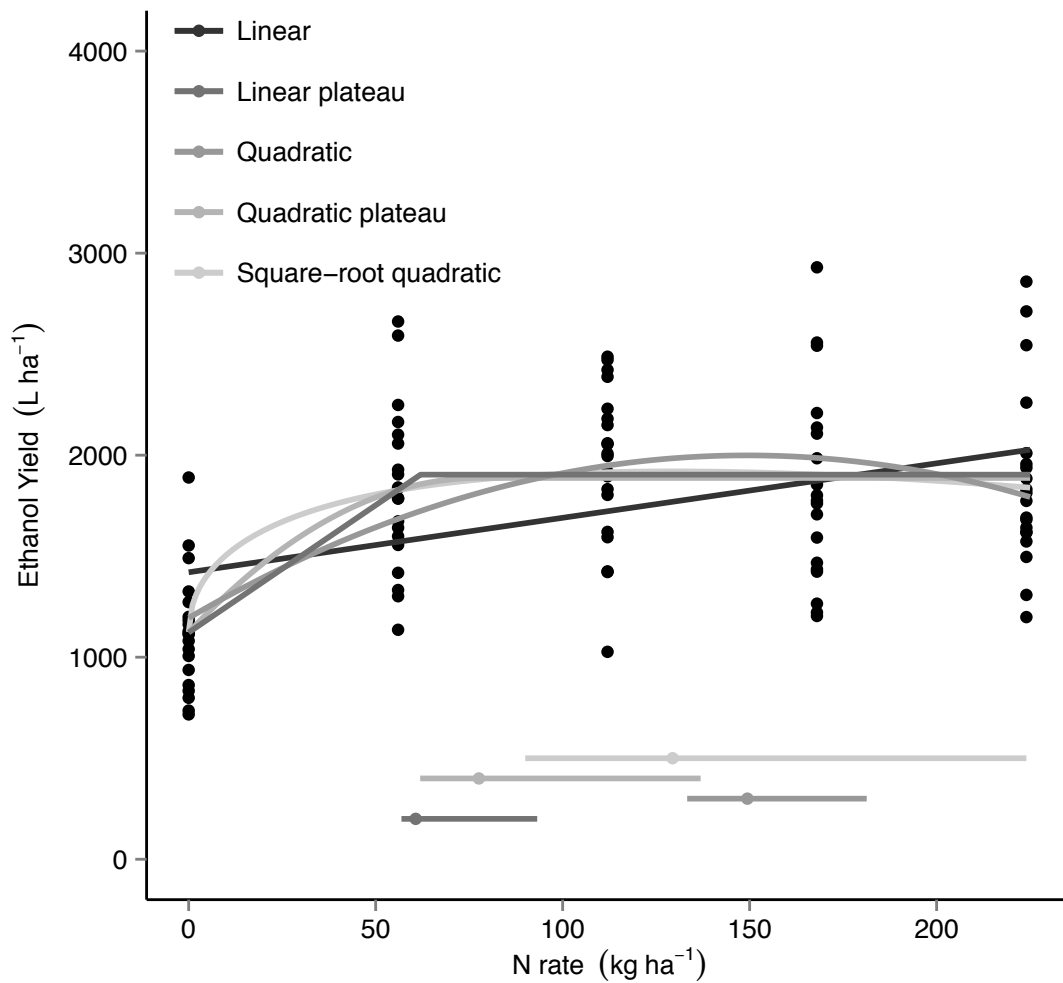
Figure 2.1. Measured land ethanol yield at five nitrogen fertilization rates (0, 56, 112, 168, 224 kg N ha^{-1}) at Rosemount in 2009. Also shown are model fit curves from five response functions along with the agronomically optimum nitrogen rate and 95% confidence intervals for each model.
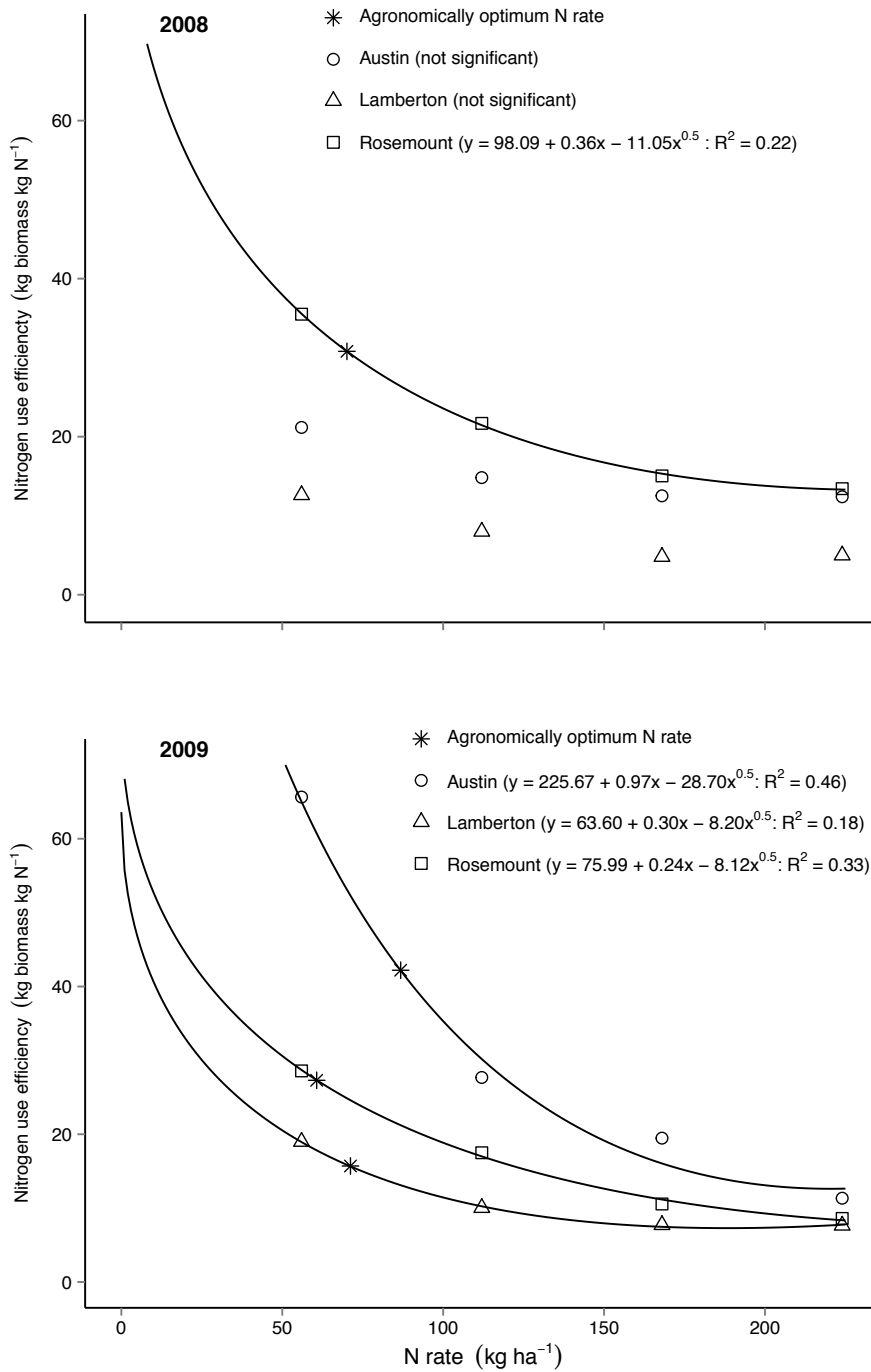
Figure 2.2. Average nitrogen use efficiency (NUE) at four N fertilization rates (56, 112, 168, 224 kg N ha^{-1}) for three locations in 2008 and 2009. Also shown is the best-supported model fit for NUE at each site-year environment, with the agronomically optimum nitrogen rate based on land ethanol yield for each environment.

Table 2.1. Site description of three experimental locations in Minnesota, USA.

Location	GPS corrdinates	Soil description	Grassland type	pH	Organic matter (%)	P (ppm)	K (ppm)
Austin	43º 40" N 92º 58" W	Sargeant silt loam (Fine-loamy, mixed, superactive, mesic Aquic Glossudalfs)	Mixed-species	5.9	3	12	126
Lamberton	44º 14" N 95º 18" W	Ves Clay Loam (fine-loamy, mixed superactive mesic Calcic Hapludolls)	Mixed-species	7.2	3.8	8	172
Rosemount	44º 44" N 93º 7" W	Waukegan silt loam (fine-silty over sandy, mixed mexic, Typic Argiudoll)	Switchgrass monoculture	6.8	4.3	49	160

Table 2.2. Precipitation and 30-year averages for each month of the growing season from 2008 and 2009 at three locations in Minnesota, USA.

Month	Austin			Lamberton			Rosemount		
	2008	2009	30-year average	2008	2009	30-year average	2008	2009	30-year average
	Precipitation (mm)								
April	155	74	90	75	38	75	118	57	74
May	100	111	110	82	41	83	68	34	103
June	216	149	124	91	82	106	117	100	120
July	79	60	121	85	42	95	71	47	114
August	74	86	112	15	88	93	77	198	120
September	41	30	88	54	71	84	58	15	92
October	57	191	60	107	138	52	51	160	73
Total	722	701	705	509	500	588	560	611	622

Table 2.3. Equations for original response functions and reparameterized response functions from five response models used to predict AONR for land ethanol yield.

Model	Abbreviation	Reparameterized response function[a]	Original response function
Linear	LR	No reparameterization required	$Y = \beta_0 + \beta_1 X$
Quadratic	QD	$Y = \beta_0 - 2\beta_2\beta_3 X + \beta_2 X^2$	$Y = \beta_0 + \beta_1 X + \beta_3 X^2$
Square root quadratic	SQD	$Y = \beta_0 - (0.5\beta_2/\beta_3^{0.5})X + \beta_2 X^{0.5}$	$Y = \beta_0 + \beta_1 X + \beta_3 X^{0.5}$
Linear plateau	LRP	$Y = \beta_0 + \beta_1 X$ for $X < \beta_2$	$Y = \beta_0 + \beta_1 X$ for $X < k$
		$Y = \beta_0 + \beta_1\beta_2$ for $X > \beta_2$	$Y = \beta_0 + \beta_1 k$ for $X > k$
Quadratic plateau	QDP	$Y = \beta_0 + \beta_1 X + (-\beta_1/2\,\beta_2)X^2$ for $X < \beta_2$	$Y = \beta_0 + \beta_1 X + \beta_2 X^2$ for $X < k$
		$Y = \beta_0 + (\beta_1\beta_2)/2$ for $X > \beta_2$	$Y = \beta_0 + \beta_1 k + \beta_2 k^2$ for $X > k$

[a] Reparametarized models include β_2, which represents the AONR. For the QD and SQD models, β_2 was determined by setting the derivative of the original response function to 0 and solving for β_1.

Table 2.4. Akaike information criterion (AICc; adjusted for small sample size), agronomically optimum nitrogen rate (AONR), and 95% confidence intervals from five models based on different response functions used to select predictor models to estimate AONR for six site-year environments.

Location[a]	Function	AICc	AONR	2.50%	97.50%	Range	Range/AONR
					—kg N ha^{-1}—		%
Aus08	Linear[b]	1516.54	na	na	na		
	Quadratic	1516.91	285.2	182.2	2276.8	2094.65	734.4
	SR Quadratic	1515.42	>224	-[c]	-		
	Linear plateau	1523.09	91.3	67.3	141.4	74.1	81
	Quadratic plateau	1516.91	299	169.6	1617.2	1447.6	480
Aus09	Linear	1539.91	na	na	na		
	Quadratic	1518.43	131.1	118.9	151.8	32.9	25.1
	SR Quadratic[b]	1509.15	86.8	70.6	122.4	51.8	59.7
	Linear plateau	-	-	-	-		
	Quadratic plateau	-	-	-	-		
Lam08	Linear	1490.84	na	na	na		
	Quadratic	1490.14	177.5	130.9	660.5	529.6	298.4
	SR Quadratic	1489.2	272.8	142.5	22860.3	22717.8	8328.9
	Linear plateau[b]	1489.36	73.0	59.1	148.1	89	121.9
	Quadratic plateau	1489.36	108.2	67.2	439.2	372	343.8
Lam09	Linear	1445.8	na	na	na		
	Quadratic	1445.67	242.1	168.1	1970.3	1802.2	744.4
	SR Quadratic	1443.07	1799.4	-	-		
	Linear plateau[b]	1446.74	71.2	58.1	112	53.9	75.7
	Quadratic plateau	1446.74	104	64.3	231.9	167.6	161.2
Ros08	Linear	1569.5	na	na	na		
	Quadratic	1559.43	174.9	148.5	242.7	94.2	53.9
	SR Quadratic	1554.72	244.7	137.4	2698.4	2561	1046.6
	Linear plateau[b]	1555.3	70.1	58.4	101.6	43.2	61.6
	Quadratic plateau	1555.3	101.6	66.7	173.2	106.5	104.8
Ros09	Linear	1510.3	na	na	na		
	Quadratic	1492.68	149.4	133.3	181.3	48	32.1
	SR Quadratic	1486.89	129.4	90	280.5	190.5	147.2
	Linear plateau[b]	1486.93	60.7	56.9	83.2	26.3	43.3
	Quadratic plateau	1486.93	77.6	61.9	136.9	75	96.6

[a] Site-year environments include Austin in 2008 (Aus08), Austin in 2009 (Aus09), Lamberton in 2008 (Lam08), Lamberton in 2009 (Lam09), Rosemount in 2008 (Ros08), and Rosemount in 2009 (Ros09).
[b] Model selected as predictor model
[c] Models did not converge

Table 2.5. Average (standard error) biomass yield by N fertilizer rates, best-fit model and parameter estimates explaining variation in biomass yield, agronomically optimum N fertilizer rate (AONR), and predicted yield at AONR for grassland biomass at three locations in 2008 and 2009.

| | | Biomass yield (Mg N ha^{-1}) | | | | | | Regression analysis | | | | | |
| | | N fertilizer rate (kg N ha^{-1}) | | | | | | Parameter estimates | | | | | Biomass |
Location	Year	0	56	112	168	224	Mean	Model[a]	β_0 (intercept)	β_1	β_2 (maximum)	AONR[b] (kg N ha^{-1})	yield at AONR
Austin	2008	6.1 (0.1)	7.3 (0.3)	7.8 (0.2)	8.2 (0.3)	8.9 (0.2)	7.7 (0.1)	LR	6.35	0.01	ns	-	-
	2009	3.7 (0.3)	7.4 (0.4)	6.8 (0.4)	7.0 (0.4)	6.2 (0.4)	6.2 (0.2)	SQD	3.76	0.73	92.9	86.80	7.3
	Mean	4.9 (0.2)	7.3 (0.2)	7.3 (0.2)	7.6 (0.3)	7.6 (0.3)	6.9 (0.1)						
Lamberton	2008	4.0 (0.3)	4.7 (0.2)	4.9 (0.2)	4.8 (0.2)	5.2 (0.3)	4.7 (0.1)	SQD	4.05	0.11	414.70	72.98	4.8
	2009	3.5 (0.2)	4.5 (0.1)	4.6 (0.2)	4.8 (0.1)	5.2 (0.2)	4.8 (0.1)	SQD	3.48	0.13	1243.63	71.17	4.4
	Mean	3.8 (0.2)	4.6 (0.1)	4.8 (0.1)	4.8 (0.1)	5.2 (0.2)	4.6 (0.1)						
Rosemount	2008	6.8 (0.3)	8.8 (0.3)	9.3 (0.3)	9.4 (0.3)	9.8 (0.2)	8.8 (0.2)	SQD	6.85	0.31	374.50	70.11	8.9
	2009	2.4 (0.1)	4.0 (0.2)	4.3 (0.2)	4.1 (0.2)	4.3 (0.2)	3.8 (0.1)	LRP	2.38	0.03	66.02	60.69	4.2
	Mean	4.6 (0.4)	6.4 (0.4)	6.8 (0.4)	6.8 (0.5)	7.1 (0.5)	6.3 (0.2)						

[a] Response function abbreviations: LR = linear; SQD = square root quadratic; LR*P* = linear plateau
[b] Agronomically optimum nitrogen rate (AONR) based on biomass yield.

Table 2.6. P values from analysis of variance for fertilizer and year effects on biomass yield, theoretical ethanol potential, land ethanol yield, biomass nutrient concentrations and nutrient harvest. Fertilizers were analyzed as factor variables for this analysis.

	Treatment	Biomass yield	Eth potential[a]	LEY[b]	Nutrient concentrations			Nutrient harvest		
					N	P	K	N	P	K
Austin	N	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	P	0.300	0.088	0.521	**0.032**	**0.002**	0.077	**0.018**	**0.001**	**0.017**
	Year	**< 0.001**	**0.037**	**< 0.001**	**< 0.001**	**0.006**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	N:P	0.108	0.060	0.066	0.275	0.061	0.062	**0.032**	0.118	0.100
	N:Year	**< 0.001**	0.603	**< 0.001**	0.215	0.472	0.078	**0.001**	0.057	**< 0.001**
	P:Year	0.183	**0.032**	0.530	0.338	0.058	0.918	0.062	**0.025**	0.211
	N:P:Year	0.945	0.290	0.973	0.879	0.816	0.660	0.879	0.847	0.275
Lamberton	N	**< 0.001**	**0.011**	**< 0.001**	**< 0.001**	0.261	**0.011**	**< 0.001**	**0.002**	**< 0.001**
	P	0.217	**0.021**	0.345	0.421	**< 0.001**	**0.036**	0.146	**< 0.001**	**0.020**
	Year	0.054	**< 0.001**	0.188	**< 0.001**	0.339	0.504	**< 0.001**	0.650	0.186
	N:P	0.864	0.144	0.846	0.225	0.109	0.217	0.482	0.242	**0.037**
	N:Year	0.639	0.065	0.692	0.282	0.541	0.889	0.327	0.198	0.856
	P:Year	0.855	0.129	0.654	0.516	0.906	0.921	0.730	0.796	0.984
	N:P:Year	0.964	0.362	0.941	0.192	0.657	0.808	0.206	0.477	0.917
Rosemount	N	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	K	0.141	0.584	0.129	0.307	0.527	**< 0.001**	0.961	0.629	**< 0.001**
	Year	**< 0.001**	**< 0.001**	**< 0.001**	**0.012**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	N:K	0.565	0.715	0.654	0.505	0.257	**< 0.001**	0.507	0.155	**0.011**
	N:Year	0.194	**< 0.001**	0.166	**< 0.001**	0.076	0.267	**0.002**	0.100	**< 0.001**
	K:Year	0.322	0.778	0.295	0.989	0.436	0.165	0.933	0.529	0.390
	N:K:Year	0.410	0.852	0.529	0.806	0.904	0.721	0.174	0.465	0.393

[a] Eth potential is theoretical ethanol potential
[b] LEY = Land ethanol yield

Table 2.7. Treatment averages (standard error), best-supported predictor model and parameter estimates, and land ethanol yield at AONR for three locations in 2008 and 2009.

| | | Land ethanol yield (L ha^{-1}) | | | | | | Regression analysis | | | | |
| | | N fertilizer rate (kg N ha^{-1}) | | | | | | Parameter estimates | | | | |
Location	Year	0	56	112	168	224	Mean	Model[a]	β_0 (intercept)	β_1	β_2 (AONR)	LEY at AONR[b]
Austin	2008	2733.1	3254.8	3380.5	3567.9	3801.9	3347.6	LR	2857.45	4.38	ns	-
		(53.1)	(131.4)	(83.7)	(125.6)	(103.7)	(57.9)					
	2009	1600.6	3246.4	2936.5	2988.7	2619.4	2686	SQD	1621.19	330.59	86.80	3161.1
		(114.6)	(174.5)	(193.2)	(216.1)	(194.1)	(99.2)					
	Mean	2196.6	3250.7	3158.5	3301.8	3225.8	3028.8					
		(110.7)	(107.0)	(109.8)	(127.9)	(143.7)	(61.2)					
Lamberton	2008	1636.1	1943.5	2016.8	2017.3	2075.9	1937.9	LRP	1636.11	5.49	72.98	2036.8
		(100.3)	(74.6	(83.9)	(70.6)	(117.2)	(42.9)					
	2009	1601.3	2026.7	2018.7	21329	2274.1	2010.6	LRP	1601.35	7.60	71.17	2142.2
		(72.6)	(70.8)	(85.1)	(61.5)	(92.7)	(40.9)					
	Mean	1618.7	1984.0	2017.8	2075.1	2175.0	1974.1					
		(61.2)	(51.3)	(59.0)	(47.1)	(75.6)	(29.7)					
Rosemount	2008	3312.7	4243.0	4416.1	4429.1	4587.4	4197.7	LRP	3312.65	16.61	70.11	4477.2
		(140.4)	(135.7)	(125.8)	(140.9)	(75.7)	(71.8)					
	2009	1122.4	1828.1	1949.1	1842.1	1870.4	1722.4	LRP	1122.39	12.60	60.69	1887.1
		(65.1)	(89.9)	(86.7)	(105.1)	(97.1)	(49.7)					
	Mean	2217.5	3035.6	3182.6	3135.6	3228.9	2960.0					
		(191.3)	(209.4)	(211.4)	(224.6)	(225.9)	(97.9)					

[a] Response function abbreviations: LR = linear; SQD = square root quadratic; LR*P* = linear plateau
[b] Land ethanol yield (LEY) at the agronomically optimum nitrogen rate (AONR)

Table 2.8. Treatment averages (SE), agronomically optimum N fertilizer rate (AONR), and nutrient harvest at AONR for grassland biomass at Austin (Aus), Lamberton (Lam), and Rosemount (Ros) in 2008 and 2009.

		N fertilizer rate (kg N ha^{-1})						Model[a]	AONR[b] (kg N ha^{-1})	Removal at AONR
Location	Year	0	56	112	168	224	Mean			
				Biomass N harvest						
Austin	2008	33.2 (1.6)	39.8 (2.3)	52.3 (2.4)	64.3 (3.8)	80.1 (3.9)	53.7 (2.1)	LR	-	-
	2009	14.8 (1.4)	31.1 (1.8)	43.9 (3.9)	51.3 (3.2)	49.8 (3.3)	38.1 (1.9)	QD	86.8	38.5
	Mean	24.2 (1.8)	35.5 (1.6)	48.1 (2.4)	58.2 (2.7)	64.9 (3.5)	46.0 (1.5)			
Lamberton	2008	23.3 (3.1)	33.2 (3.5)	32.8 (1.8)	35.2 (2.1)	40.5 (2.7)	33.0 (1.3)	LR	73.0	30.2
	2009	15.4 (0.7)	22.8 (1.1)	26.2 (1)	31.6 (1.6)	37.6 (1.7)	26.8 (1.0)	LR	71.2	23.2
	Mean	19.3 (1.7)	28.2 (2)	29.4 (1.1)	33.4 (1.3)	39 (1.6)	29.9 (0.8)			
Rosemount	2008	29.8 (1.9)	43.1 (2.1)	54.5 (2.5)	61.9 (2.8)	80.2 (2.9)	54.0 (2.1)	LR	70.1	44.7
	2009	8.2 (0.6)	17.1 (1)	28 (1.3)	32.4 (1.6)	42.2 (2.7)	25.6 (1.4)	LR	60.7	18.0
	Mean	18.7 (2)	30.1 (2.4)	41.3 (2.5)	46.4 (2.9)	61.2 (3.6)	39.6 (1.6)			
				Biomass P harvest						
Austin	2008	4.8 (0.2)	6.1 (0.4)	6.9 (0.3)	8.4 (0.6)	9.4 (0.7)	7.1 (0.3)	LR	-	
	2009	2.7 (0.3)	5 (0.3)	5.6 (0.4)	6 (0.4)	6.1 (0.3)	5.1 (0.2)	LRP	86.8	4.5
	Mean	3.8 (0.2)	5.6 (0.3)	6.2 (0.3)	7.3 (0.4)	7.8 (0.5)	6.1 (0.2)			
Lamberton	2008	1.9 (0.2)	2.3 (0.2)	2.3 (0.2)	2.1 (0.1)	2.4 (0.2)	2.2 (0.1)	NS	73.0	-
	2009	1.9 (0.2)	2.1 (0.2)	2 (0.1)	2.3 (0.2)	2.5 (0.2)	2.2 (0.1)	LR	71.2	2.1
	Mean	1.9 (0.1)	2.2 (0.1)	2.2 (0.1)	2.2 (0.1)	2.5 (0.1)	2.2 (0.1)			
Rosemount	2008	8.6 (0.4)	11.5 (0.6)	13.2 (0.5)	12.1 (0.4)	12.5 (0.5)	11.6 (0.3)	LRP	70.1	12.1
	2009	2.2 (0.1)	4.1 (0.2)	4.7 (0.2)	4.6 (0.2)	4.9 (0.4)	4.1 (0.1)	LRP	60.7	4.0
	Mean	5.3 (0.6)	7.8 (0.7)	9 (0.7)	8.1 (0.7)	8.7 (0.7)	7.8 (0.3)			
				Biomass K harvest						

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Location	Year							Response function[a]		AONR[b]
Austin	2008	17.4 (1.0)	23.5 (1.2)	28.2 (1.5)	33.2 (2.0)	44.0 (4.6)	29.1 (1.4)	LR	-	-
	2009	10.5 (1.1)	18.7 (1.4)	19.7 (1.9)	20.7 (1.8)	21.4 (1.6)	18.2 (0.8)	SRQ	86.8	19.5
	Mean	14 (0.9)	21.2 (1)	23.9 (1.3)	27.2 (1.7)	32.7 (3)	23.8 (0.9)			
Lamberton	2008	11.0 (0.9)	14.2 (0.9)	16.3 (1.4)	14.7 (0.8)	18.3 (1.8)	14.9 (0.6)	LR	73.0	14.1
	2009	9.0 (0.6)	12.3 (0.9)	15.0 (1.6)	15.1 (1.2)	17.8 (2.3)	13.9 (0.7)	LR	71.2	12.4
	Mean	10 (0.5)	13.3 (0.6)	15.7 (1.1)	14.9 (0.7)	18 (1.4)	14.4 (0.5)			
Rosemount	2008	27.5 (1.6)	39.0 (2.3)	44.3 (2.0)	40.6 (2.1)	45.5 (2.0)	39.5 (1.1)	LRP	70.1	42.2
	2009	6.4 (0.3)	13.0 (0.7)	14.1 (0.9)	12.0 (0.6)	13.1 (0.8)	11.7 (0.4)	LRP	60.7	13.1
	Mean	16.7 (1.9)	26 (2.4)	29.2 (2.7)	25.6 (2.6)	29.3 (2.8)	25.4 (1.1)			

[a] Response function abbreviations: LR = linear; SQD = square root quadratic; LR*P* = linear plateau; QD = quadratic; NS = not significant
[b] Agronomically optimum nitrogen rate (AONR) based on land ethanol yield

Chapter 3

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

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 post-harvest 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 at the central location. Ordination revealed patterns in species composition due to location, but not due to harvest treatment. Therefore, habitat and bioenergy characteristics

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related to grassland plant communities are not expected to change due to short-term or intermittent late-season biomass harvest.

3.1 Introduction

Achieving renewable energy targets with biomass (USDOE, 2011) requires measuring bioenergy production potential and various ecological implications of multiple feedstock production systems in regions throughout the US. 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, 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*. 2011a). 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*. 2011a).

Managing mixed-species grasslands for bioenergy has benefits over conventional bioenergy crops and grassland plant monoculture. Bioenergy from cellulose of grassland biomass has greater net-energy benefits than biofuels from conventional food crops (Adler *et al*. 2007). 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 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 U.S. 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 U.S. 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 U.S. states like Minnesota maintain Wildlife Management Areas (WMAs).

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 the goals of conservation grassland programs. 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 and Turkington 2007) and to promote forb establishment (Williams *et al.* 2007).

We determined if harvesting biomass from conservation grasslands, with production-scale equipment in late autumn/early winter, could achieve 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 such changes would have 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).

3.2 Methods

3.2.1 Site description and experimental design

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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, Figure 3.1). Experimental plots, each about 8 ha, were delineated within previously restored grasslands planted to mixes of perennial grasses and forbs. The grasslands were enrolled as WMAs, WPAs, or CRP land and were established at least five years prior to the start of our study. Twenty-eight plots were studied, 8 in 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 replicates per location. Two harvest treatments were applied in each block. Treatments included 1) harvested (in late fall) and 2) unharvested (control). One additional harvest plot was added to each replicate 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 1.1). 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 five days before baling. For further details on biomass harvest methods, see Jungers *et al*. (2013). Plots were harvested in 2009, 2010, and 2011from 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).

3.2.2 Plant community measurements

Plant community data was collected before initiation of harvest treatments and each year

of the harvesting from sample quadrats within each plot. The number and size of sample

quadrats varied by year due to labor and resource availability (Table 1.1). 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.

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: C4 grasses, C3 grasses, legumes, and non-legume 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 non-game birds (Delisle and Savidge 1997), mammals (Schweitzer *et al*. 1993), and invertebrates (Doxon and 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 percent.

3.2.3 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 `isoMDS` 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.

The Shannon diversity index ($H' = -\sum p_i \log p_i$) was calculated for each quadrat to

determine species diversity, where p_i is 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 m^2 per plot, which was equivalent to the area of five 1.0 X 1.5 m sample

quadrats used during the following 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 m^2.

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

2009, 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, forb, and non-legume 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 3.3). 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 two percent 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 post-treatment was used as the effect size (Hedges *et al.* 1999). A negative effect size indicates that the percent cover of a functional group decreased from pre-treatment to either two years (north location) or three years (south and central locations) post-treatment. 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.

3.3 Results

3.3.1 Characterization of plant communities

The average percent cover for the main functional groups in sample quadrats was 23% C4 grasses, 19% C3 grasses, 4% non-legume 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 3.2). 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 m^2 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 quadrats respectively, while bull thistle and purple loosestrife were both observed in less than 0.01%. When present, bull thistle and common sowthistle covered, on average, 3 and 4% of the quadrat, respectively.

3.3.2 Variation in plant community composition by location

Ordination plots indicated that plant community types were similar among plots within the same location (Figure 3.2). 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 ($R^2 = 0.34$, $P = 0.007$).

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 two years of harvest, plots in the north location were correlated with higher species diversity (Figure 3.2).

Changes in the C4 functional group were explained by the best-supported model which included both a Location × Year and Location × Treatment interaction (Table 3.3). 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 3.4, Figure 3.3). The Location × 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 3.4, Figure 3.3). Forb cover was greater in the central location compared to the south (Table 3.4, Figure 3.3g, h, and i), while legume cover was greatest in the south compared to both the central and north locations (Table 3.4, Figure 3.3j, k, and l).

A Location × Year interaction was retained in the best-supported model for species diversity and weed proportion (Table 3.3). Averaged across time, species diversity was similar at all locations, but decreased in the south and north locations (Table 3.4, Figure 3.3). The proportion of noxious weeds was greater in the central location compared to the south, but this decreased through time (Table 3.4). Averaged across time, species

richness, functional group diversity, and the proportion of non-native species were similar across locations (Table 3.3, Figure 3.4).

3.3.3 Changes in plant community composition through time

A comparison of the ordination plots from pre- and post-treatment application can be used to identify potential changes in plant community composition due to biomass harvest (Figure 3.2). There was no discernible pattern in the distribution of plant community types by harvest treatment in the pre-treatment ordination space. The ordination plot for post-treatment was similar to that of pre-treatment in that there were no obvious differences in plant community types between harvested and unharvested plots.

The cover of C3 grasses decreased with time at all locations and in all treatments (Table 3.3, Table 3.4). 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 3.3). As with the cover of C4 grasses, species diversity and the proportion of weeds changed with time, but uniquely at each location (Table 3.3). There were no Year × Treatment or Year × Treatment × Location interactions for any response variables (Table 3.3).

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 (Figure 3.5). 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 overlap for all functional groups at all locations (Figure 3.5). 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.

3.4 Discussion

3.4.1 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 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 some variation in initial cover of the functional groups, 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*. 2013), 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 pre-treatment community composition. Similar to our results, changes in the relative abundance of native C4 grasses and the non-native C3 Kentucky bluegrass were the same in harvested and unharvested grasslands following three years of biomass harvest in areas dominated by native C4 grasses (Hendrickson and 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 and 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.

3.4.2 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 (NRCS 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 and Gross 2013). 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.

3.4.3 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 three years

after treatment initiation (Tarmi *et al*. 2011). Hansson and 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 and 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 and Olsson 2005). The lack of an affect 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 post-senescence (October-December) harvest time of our study. Mid-growing 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, mid-growing 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.

3.5 Conclusions

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 activities, 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 four years. We expect that many habitat and bioenergy characteristics related to plant composition will remain the same where late-season biomass harvest is implemented.
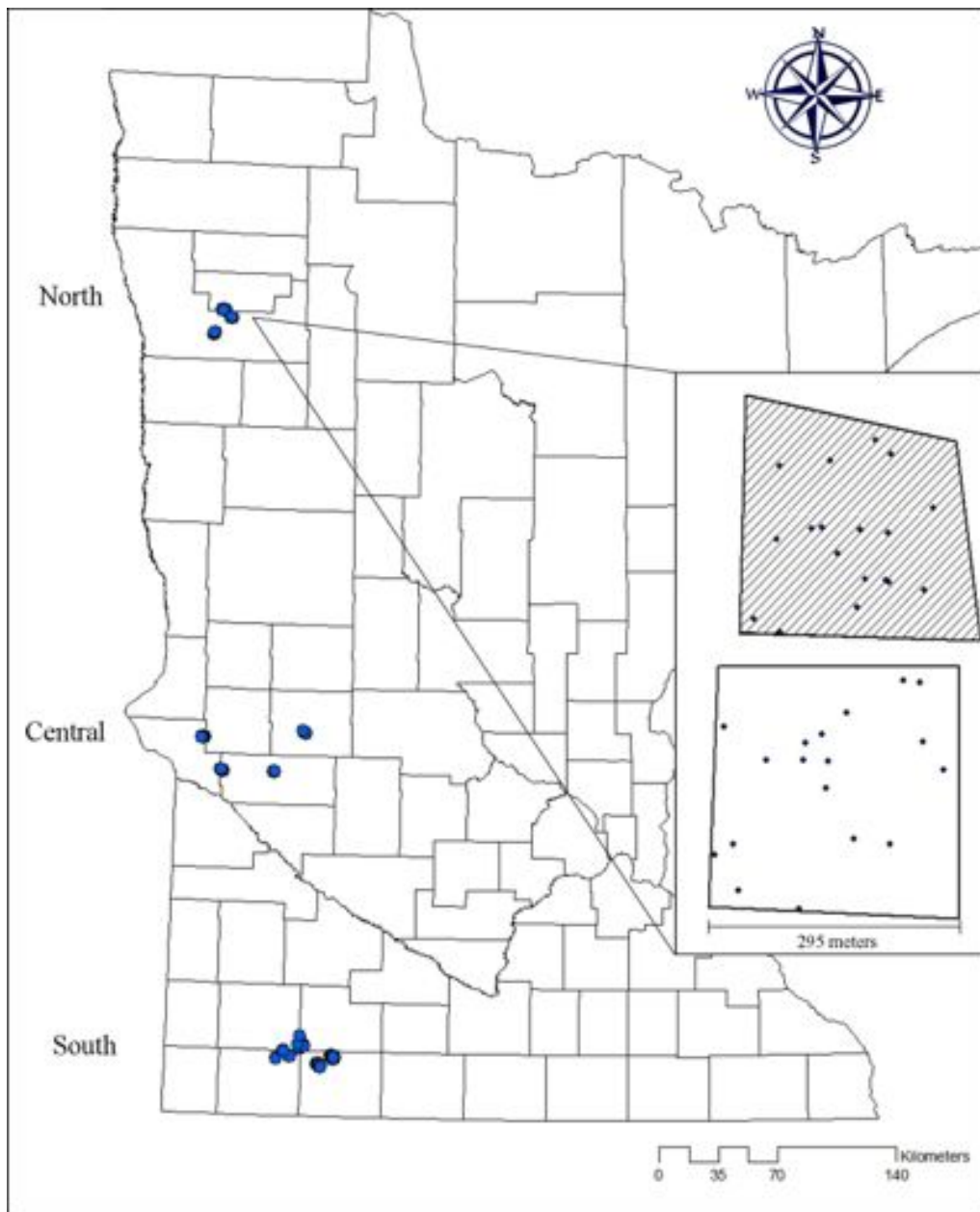
Figure 3.1. Map of the study area in Minnesota, USA. Inset shows 100% harvest plot and an unharvested control plot with randomly distributed sample quadrats where plant community composition was measured in 2011.

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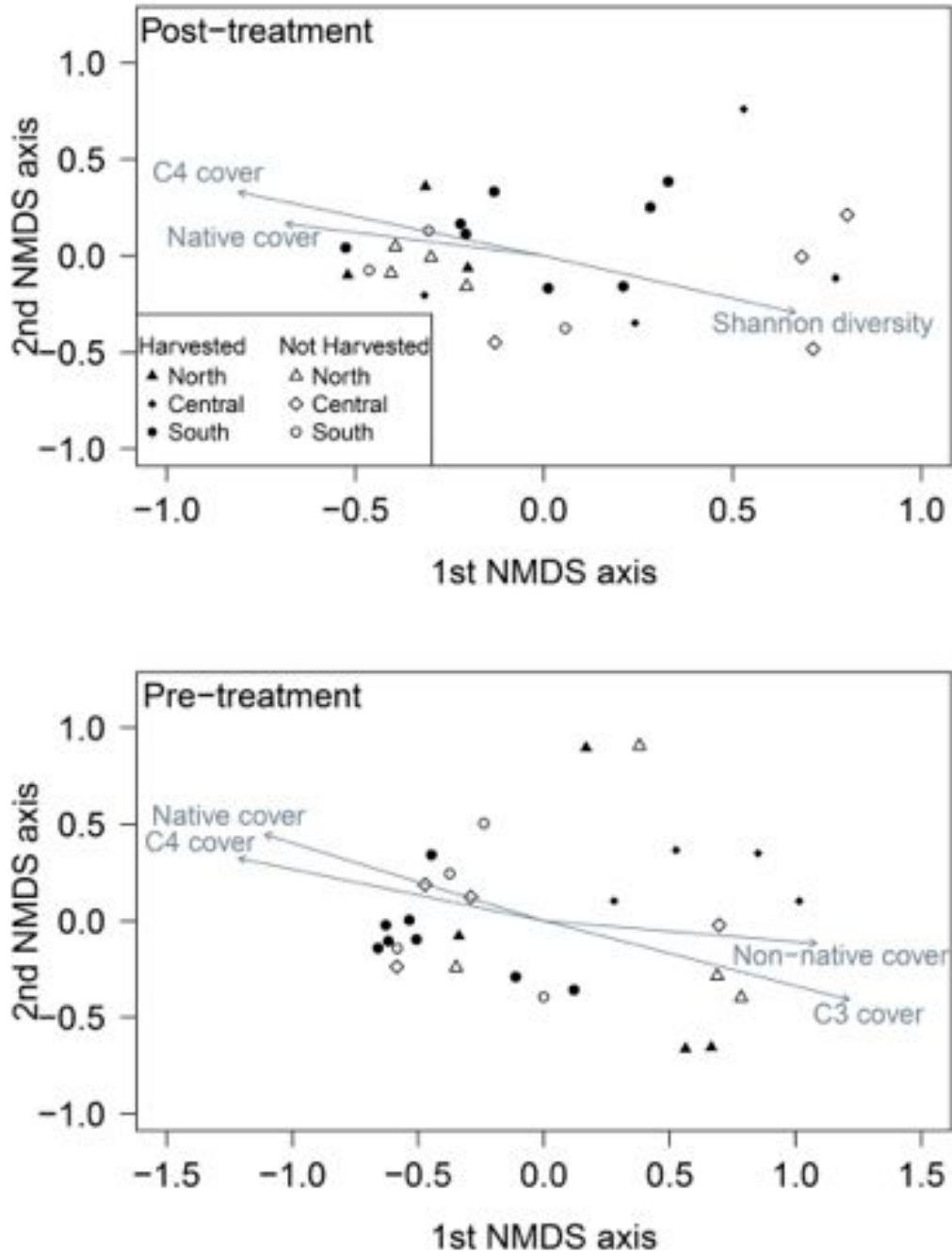
Figure 3.2. Non-metric multidimensional scaling ordination of plant communities in grasslands prior to biomass harvest (Pre-treatment) and following two (North) and three (Central and South) year of biomass harvest (Post-treatment). Lines represent gradients for metrics of plant community composition, with the length of the line representing strength of correlation to axes.
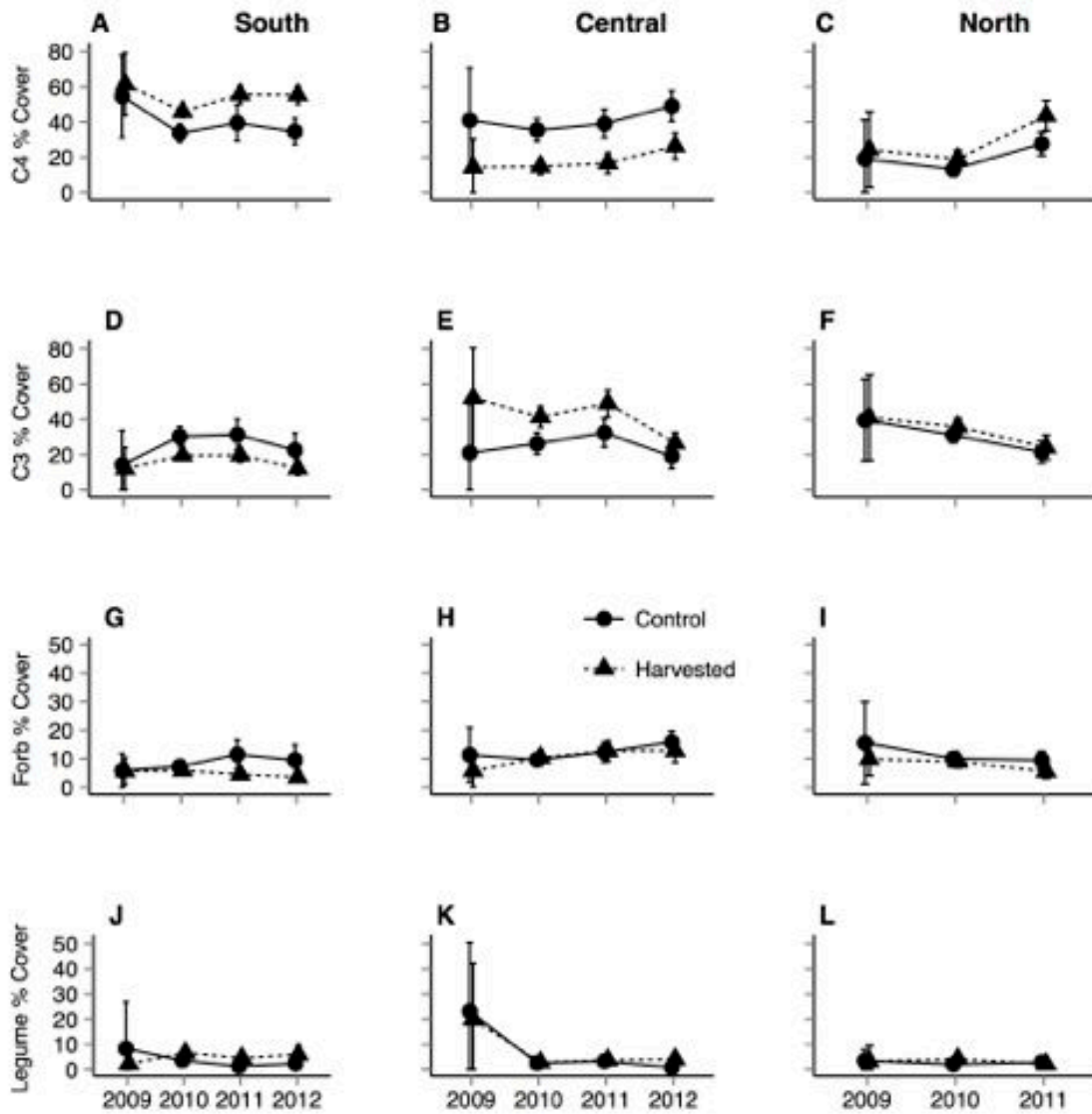
Figure 3.3. Average percent cover of the four major plant functional groups in harvested and unharvested plots located in the south, central and north locations from 2009 (pre-treatment) to 2012.
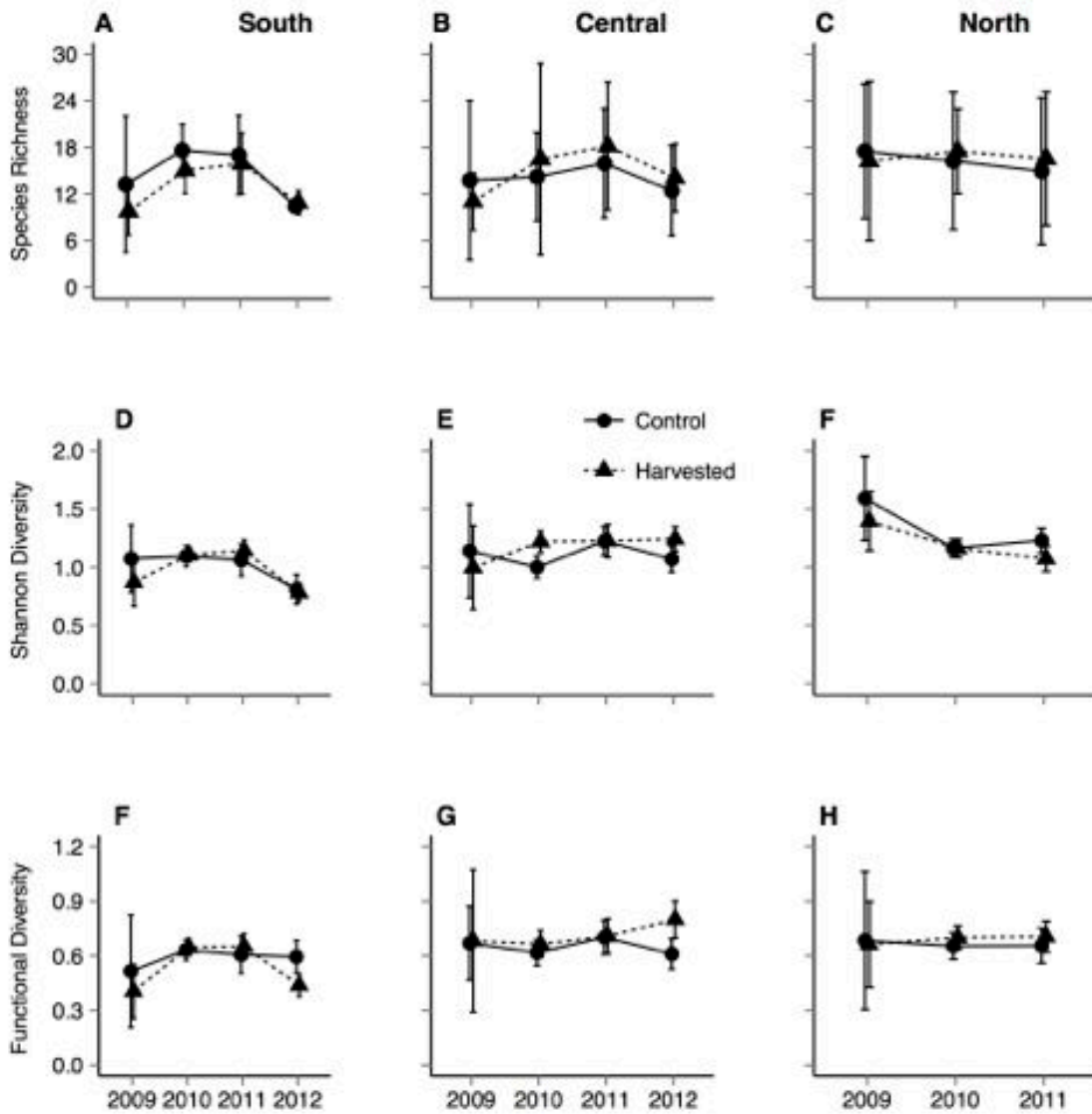
Figure 3.4. Average species richness, species, and functional group diversity in harvested and unharvested plots located in the south, central and north locations from 2009 (pre-treatment) to 2012.
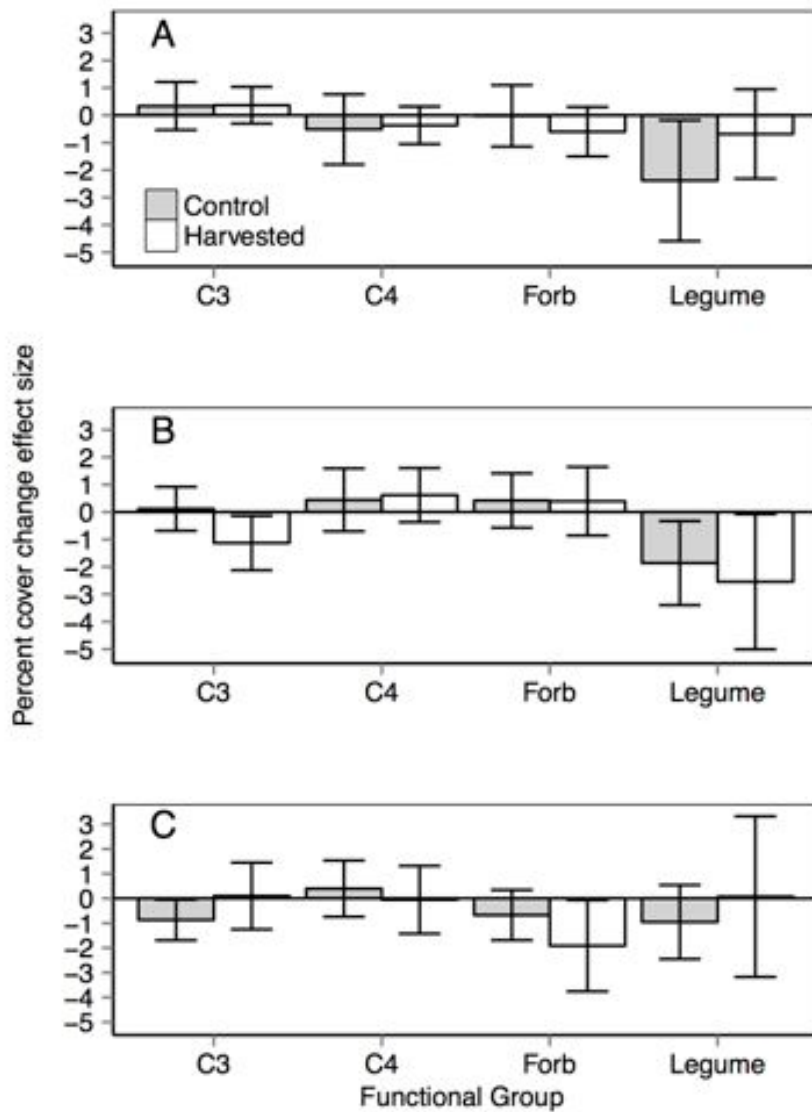
Figure 3.5. Effect sizes (Hedges' *g*) and associated 95% confidence intervals for the change in functional group cover from pre-treatment to final year post-treatment conditions in the south (A), central (B), and north (C) locations in Minnesota, USA.

Table 3.1. 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 X 5.0
2010	12	6	8	24	1.0 X 1.5
2011	9	8	7	12	1.0 X 1.5
2012	11	8	0	12	1.0 X 1.5

Table 3.2. Top five plants in terms of frequency observed and their associated average percent cover for four major functional groups – C4 grasses, C3 grasses, non-legume forbs, and legumes in Minnesota, USA.

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

Table 3.3. 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[a]	K	Δ AIC	Wi
C4 cover	Best supported	I + Y + H + L + Y:L + H:L	13	0	0.92
	Global[b]		16	4.88	0.08
	Null[c]		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

[a] I = intercept; Y = year; L = location; H = harvest treatment
[b] Parameters for all Global models: I + Y + H + L + Y:L + H:L + Y:H
[c] Parameters for all Null models: I

Table 3.4. Parameter estimates, standard errors, t-statistics, and p-values for best-supported models.

Response	Parameters	Value[a]	Std. Error	t	P
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
	Harvested × North	-0.568	0.727	-0.782	0.443
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

[a] Values not back transformed

Chapter 4

Title: Effects of grassland biomass harvest on nesting pheasants and ducks.

Grasslands enrolled in conservation programs provide important habitat for nesting game birds and waterfowl, but conservation grasslands have been targeted as a source of biomass for bioenergy and this could impact nesting birds. We studied the effects of biomass harvest on nest success and density in southwestern Minnesota using a before-after control-impact (BACI) study design. We located and monitored 109 nests during 2009 (pre-treatment) and 2010 (post-treatment). Biomass was harvested in late autumn of 2009 with production-scale machinery. Harvest treatments included 0, 50, 75, and 100% biomass removal from 8 ha plots. Nest success averaged 24% for waterfowl species (blue-winged teal (*Anas discors*) and mallard (*Anas platyrhynchos*)), and 59% for ring-necked pheasant (*Phasianus colchicus*). Nest success was similar across harvest treatments. Estimated total nest density (0.43 nests ha^{-1}; corrected for survivorship) was similar across harvest treatments, but within-plot analysis revealed that nest density was greater in unharvested refuge regions. Estimated nest density was positively correlated with vegetation height and the spatial extent of wetlands surrounding each plot. Harvesting relatively small-scale patches of conservation grasslands in late autumn does not appear to be detrimental to nesting ducks and pheasants the following spring, but managers should consider leaving unharvested refuges near wetlands when harvesting large, continuous tracts.

4.1 Introduction

State and federal governments have instituted numerous programs to expand and manage native grasslands as wildlife habitat for grassland birds, including several ecologically and economically important game and non-game bird species (Herkert *et al.* 1996). For example, the Minnesota Department of Natural Resources manages restored grasslands in the Wildlife Management Area (WMA) program, which is publically accessible for hunting. WMAs cover more than 1.1 million acres of Minnesota and some require regular maintenance to sustain early-successional herbaceous plants. Minnesota agencies plan to expand WMA acreage by 64% by 2050 (Yunker 2010), but increased land value due to rising crop prices (Rashford *et al.* 2011) and increased management costs could hinder expansion goals. Land acquisition and management has been primarily funded by hunting license fees and state funds, but it is not known if these sources alone can support future habitat goals.

Biomass from conservation grasslands can be harvested and sold to bioenergy producers or other markets to potentially finance the expansion and maintenance of conservation grasslands (Fargione *et al.* 2009). Biomass yields from WMAs in southwest Minnesota were about 3 Mg ha^{-1} (Jungers *et al.* 2013), which could bring revenues for achieving expansion goals. Moreover, biomass harvest could be used as an alternative to more resource-intensive prescribed burning to maintain early-successional plant communities (Devries and Armstrong 2011). If resulting habitat characteristics and wildlife benefits

are similar for both management operations, biomass harvest could provide funds through sales of biomass and also conserve funds by reducing costs of prescribed burning.

Conservation grasslands, such as WMAs, provide productive breeding habitat for upland-nesting waterfowl and pheasants (Kantrud 1994, Reynolds *et al*. 2001). It is unclear how this habitat might be impacted by biomass harvest, and even though the effects of other land management activities on nest success and density have been well studied, results are inconsistent. For instance, spring grazing and prescribed burning decreased the density of blue-winged teal (*Anas discors*) nests in North Dakota, but did not influence nest success (Kruse and Bowen 1996). Positive effects of biomass removal were evident when waterfowl nest success and density increased after mowing and burning of restored grasslands in the Canadian prairies (Devries and Armstrong 2011). The mechanisms underlying the varying effects of other biomass removal techniques on nest success and distribution are related to both local and landscape characteristics. Increases in nest success have been associated with nest-scale habitat variables such as vegetation height (Luttschwager *et al*. 1994), field-scale variables such as legume cover (Arnold *et al*. 2007), and landscape-scale variables such as surrounding grassland cover (Stephens *et al*. 2005, Thompson *et al*. 2012) and fragmentation (Horn *et al*. 2005). Therefore, analysis at multiple spatial scales is important for understanding the effects of management activities on reproductive rates (Koper and Schmiegelow 2006).

Our primary objective was to assess the effect of autumn biomass harvest on nesting biology of upland-nesting ducks and pheasants. We hypothesized that harvesting biomass in autumn for bioenergy would have limited effects on nest success and density compared with other grassland management techniques such as burning, mowing, and grazing treatments that often occur during the nesting season. We modeled densities and daily survival rates of duck and pheasant nests at two spatial scales to identify responses across harvest treatments. As a secondary objective, we tested the influence of habitat covariates on nest success and density.

4.2 Methods

4.2.1 Study site

We conducted our study on WMAs in Cottonwood, Jackson, and Nobles counties of Minnesota, U.S.A. (from 43.76º to 43.92º N, 95.15º to 95.63º W; Figure 4.1). In 2008, we delineated 28 plots within existing fields of restored grassland established > 5 y before the project started. Each plot was approximately 8 ha and included a variety of warm- and cool-season grasses, legumes, and other forbs. Plots were selected to be dry enough to operate farm equipment during the autumn months.

Each plot was randomly assigned one of six harvesting treatments: 1) control at 0% harvest, 2) 100% full harvest, 3) 25% partial block harvest, 4) 25% partial strip harvest, 5) 50% partial block harvest, and 6) 50% partial strip harvest (Figure 4.1). Partial-harvest plots contained refuges of unharvested vegetation. For some analyses, we compared

response variables among harvested and refuge regions, where refuge regions were unharvested areas within partially harvested plots and control plots. Harvested regions were the harvested areas within partially harvested plots and 100% fully harvested plots. The experiment was replicated in four blocks, each block further containing two replicates of the full harvest treatment and one replicate of all other treatments. In mid-November of 2009, a contracted harvester cut biomass with a self-propelled windrower to a minimum stubble height that prevented equipment damage (mean = 15 cm). Biomass was removed from the plot with a large round baler. One plot scheduled for harvest was not cut due to inclement weather and was treated as a control.

4.2.2 Data collection

We searched for nests from 20 May 2009 to 18 June 2009 and from 20 May 2010 to 8 July 2010 using the chain drag method (Klett *et al.* 1986). We searched each plot twice per year at three-week intervals. Crews of three (two drivers, one spotter) pulled a 30 m chain between a pair of all-terrain vehicles to flush nesting females from nests. Upon flushing a female, we recorded the nest location, if one was found, with a global positioning system and a flag placed 3 m north of the nest. At discovery and each subsequent visit, we estimated nest age and initiation date by counting eggs (assuming females laid one egg per day) and estimating embryo development by candling (Weller 1956). We estimated the hatch date for each nest by adding the clutch size to the expected 26 d incubation period. We revisited marked nests every 7 d until nests hatched, were abandoned, or were destroyed. For nests that had an expected hatch date that was

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scheduled to occur between the 7 d interval, we visited those nests on the expected hatch date or when possible daily thereafter to determine nest fate. We considered a nest successful if at least one egg successfully hatched. We took digital photographs of nest bowls and collected nest remains to assist in determining final nest fate.

We conducted post-harvest vegetation surveys in 2010 to test the effect of habitat covariates on nest density; which included vegetation height, biomass, species richness, and the relative abundance of grasses and forbs. These habitat covariates were fit to nest density models only. We measured vegetation height between 27 May 2010 and 10 June 2010 by visually assessing the distance above ground in which 80% of biomass occurred (Stewart *et al.* 2001). We conducted this measurement at eight random locations in each plot and averaged the eight measurements to generate a mean vegetation height for the plot. We determined the relative abundance of grasses and fobs by visually assessing plant cover within a 1.5 m^2 quadrat frame placed over vegetation. At 12 randomly selected points within each plot, we counted all plant species and assigned a score of relative abundance based on the percentage of the quadrat area covered. To assess the power and within-plot variability, we measured 12 more quadrats (totaling 24) in the control and 100% harvest treatments. We then categorized each plant species as either a grass or forb and summed the percent cover for all species in each category. The average cover of grasses and forbs was determined for each plot. To estimate biomass, we hand-clipped vegetation to a height of 2.5 cm in each quadrat. Clipped biomass was weighed wet, dried at 45 C for four days, and reweighed.

We quantified the amount of grassland and wetland in the surrounding landscape using ArcGIS (version 9.3.1, ESRI, Redlands, CA). We reclassified the GAP Land Cover layer from the Minnesota Department of Natural Resources into grassland and wetland areas (USGS 2011). We calculated the amount of grassland and wetland areas that were within a 500 m radius from the plot center and outside of the plot boundary to be used as a plot-scale habitat covariate for examining variation in nest density (Figure C.1). We also measured the distance from nearest wetland for each individual nest using the same data layers, which we used in modeling daily survival rate. Distance to the nearest wetland was the only habitat covariate used for modeling nest daily survival rate.

4.2.3 Nest survival analysis

We modeled daily survival rate (DSR) of nests with program MARK (White and Burnham 1999) using procedures described by Dinsmore *et al.* (2002). We tested for variation in DSR in relation to harvest treatment, year, species (waterfowl and pheasants), nest initiation date, and proximity to wetlands (Table 1). Only nests for which fate was determined were used for this analysis. The effect of biomass harvest on DSR was measured at two scales. The plot-scale predictor labeled "Harvest treatment" indicated the assigned harvesting treatment to the plot for each discovered nest. For partially harvested plots (those treatments with a refuge), nests could either have been initiated in harvested or refuges areas. Therefore, we also included a nest-scale predictor labeled

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"Cut area" for this distinction (Table 1). We assessed models based Akaike's information criterion adjusted for small sample size (AIC$_c$) (Burnham and Anderson 1998). First, we tested to see if year explained variation in DSR. A model that included the predictor "Year" was less supported (AIC$_c$ = 178.5) than the intercept-only model (null model; AIC$_c$ = 176.7), therefore we tested the effect of the remaining predictors using nests from both years combined. We treated all data from 2009 (before experimental biomass harvest) as unharvested controls. Next, we built five models, one for each predictor listed in Table 1. Each model in the set estimated two coefficients, one for the y-intercept and one for the effect of the predictor. Each was ranked based on AIC$_c$ and then compared to the null model (y-intercept only). We estimated nest success as DSR^{35} (Klett *et al.* 1986).

4.2.4 Nest density analysis

We considered apparent nest density as the total number of nests found per plot. To account for nests that failed before discovery, we used a Horvitz-Thompson estimator of total nests initiated per plot based on model-estimated DSR and average nest age at discovery (Arnold *et al.* 2007):

Equation 4.1

$$NEST = \frac{N_i}{DSR^{d_i}}$$

where *N* is apparent nest density, DSR is estimated daily survival rate for all species from the best-supported model, and *d* is the average nest age at time of discovery in plot *i*. We rounded NEST (nest abundance corrected for survivorship) to the nearest integer, and

because all plots were similar in size (mean = 7.9 ha ± 0.4 SD), we regard NEST as a measure of nest density (nests plot^{-1}).

We modeled estimated nest density using negative binomial generalized linear regression from the 'MASS' package in R (R Development Core Team, 2010). We developed a global model with all possible plot-level predictors including habitat covariates to explain variance in estimated nest density (Table 4.1). The predictor variable "Harvest treatment" was treated as the main effect. Although all plots were similar in size, we included plot area as a precautionary variable to control for any potential effect of plot size. The remaining variables were habitat covariates that have been used to describe variation in nest density and survival in previous studies (Reynolds *et al*. 2001, Stephens *et al*. 2005, Arnold *et al*. 2007, Kruse and Bowen 1996).

The global model (all predictor variables) was tested and then reduced by removing the least significant predictor based on the P value of the z statistic. The following reduced model was then tested and further reduced using the same criteria. This iterative process continued until all predictors were absent (null model; intercept-only model). All models were then compared and ranked based on AIC$_c$. Because most of the habitat covariates were only measured in 2010, we restricted this analysis to nests located in 2010.

In partially harvested treatment plots, nests were found in both harvested and refuge regions. Because we generated nest density estimates at the plot scale, we could not use

these estimates to examine density differences between refuge and harvested regions. To compare nest densities in refuge and harvested regions within plots, we used a chi-square test. We divided the total number of nests found by the total area searched in 2009 to calculate the expected number of nests ha^{-1}. We then multiplied this fraction by the total number of hectares searched in 2010 for both refuge and harvested regions to generate the number of nests we expected to find. All nests found in control plots were included with those analyzed in the refuge region group, and all nests found in the 100% harvest plots were included with those in the harvested region group. We compared observed and expected numbers of nests found in each region with a chi-square test with 1 df

We explored variation in nest initiation date for the 2010 data using analysis of variance (ANOVA). We tested if initiation date varied by species and nest location (harvested or refuge region) and tested for an interaction between species and nest location. We determined significance for all tests at $\alpha = 0.05$.

4.3 Results

We found 109 nests, including 62 blue-winged teal (*Anas discors*), 32 mallard (*Anas platyrhynchos*), and 15 ring-necked pheasant (*Phasianus colchicus*) from 28 plots (totaling 221 ha) during both years of the study. We were able to determine nest fate for 74 nests, 40 in 2009 and 34 in 2010.

4.3.1 Nest survival

Daily survival rate of nests did not vary by year, so we combined nests from both years for analysis. The best-supported model (Table 4.2) identified a greater DSR for pheasants (0.9848 ± 0.0106 SE) than for waterfowl (0.9603 ± 0.0064 SE). Daily survival rate for all species combined was 0.9634 ± 0.0058 SE. Daily survival rates translated to nest success rates of 24.2% for waterfowl, 58.5% for pheasants, and 28.0% for all species combined. DSR was not affected by harvest treatment, nor did it differ between harvested and refuge regions.

4.3.2 Nest density

We found an average of 1.9 nests plot^{-1} ± 0.04 SE, which translates to an apparent nest density of 0.25 nests ha^{-1} ± 0.01 SE. Estimated nest density corrected for survivorship averaged 0.43 nests ha^{-1} ± 0.01 SE across all treatments and years. The best-supported model for explaining variation in estimated nest density at the plot level included vegetation height, amount of surrounding grassland, and amount of surrounding wetland (Table 4.2). Another competitive model also included plot area, and together, these two models accounted for 71% of the model weights (Table 4.2). Vegetation height and the amount of wetland (m^2) within a 500 m radius of the plot center were positively associated with estimated nest density, whereas the amount of grassland in the same area was negatively associated with estimated nest density (Table 4.3). The harvest treatments did not explain variation in estimated nest density at the plot level.

In 2010, nest searches found 17 nests within 140 harvested ha for an apparent density of 0.12 nests ha^{-1}, versus 30 nests within 84 ha of refuge regions for an apparent density of 0.36 nests ha^{-1} ($\chi^2 = 16.2$; df $= 1$; $P < 0.001$). Average nest age at detection was greater in refuge regions ($F = 19.7$; df $= 1$; $P < 0.001$). When we used this to adjust nest density for nests that failed before detection, it led to an increase in the estimated difference in density between harvested and refuge regions. Estimated nest density was 0.17 nests ha^{-1} in harvested regions versus 0.65 nests ha^{-1} in refuge regions.

Nest initiation date was earlier for all species in the refuge regions, but also varied by species ($F = 7.28$; df $= 2$; $P = 0.002$). Pheasants initiated nests about 14.6 days earlier than waterfowl (LSD $= 10.2$), but initiation dates were similar for blue-winged teal and mallards. The interaction between species and harvest treatment was not significant for initiation date ($F = 0.04$; df $= 2$; $P = 0.95$).

4.4 Discussion

Harvesting biomass from conservation grasslands in autumn did not decrease the number of nesting game birds, nor did it increase the risk of nest failure in 8 ha plots the following year. However, we observed fewer nests per hectare in harvested regions compared with refuge regions. Our results suggest that when ducks and pheasants have access to unharvested refuge regions for nesting, local nesting densities will not decline due to biomass harvest, even though birds avoided nesting in recently harvested portions of WMAs.

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Other studies have also found that waterfowl preferentially select nest sites with some residual grass. Kruse and Bowen (1996) recorded species-specific declines in nest density in response to vegetation removal (burning and grazing), and associated these declines with differences in vegetation height among removal treatments. Likewise, Luttschwager *et al.* (1994) measured lower nest densities in hayed fields compared to idle fields after the earliest nest search the year after management, which they attributed to decreased vegetation height.

Other studies on the impacts of haying on waterfowl production observed a decline in nest success as a result of direct nest destruction by harvesting machinery, which can be mediated by delaying harvest until after waterfowl nesting occurs (McMaster *et al.* 2005). Although the mechanical techniques for harvesting biomass for energy are similar to those for haying, the timing of biomass harvest is considerably later. As anticipated, fall biomass harvest did not cause direct nest losses in our study. Delaying biomass harvest of perennial grasslands until after plant senescence also permits the translocation of nutrients from shoots to roots (Vogel *et al.* 2002), thus conserving resources for growth in following years and limiting emissions during combustion for energy (Ogden *et al,* 2010).

Our estimate of waterfowl nest success (24.2%) was substantially greater than the 5-15% nest success observed in Canadian grasslands under delayed haying management (Emery

et al. 2005), and was also greater than the 13% nest success rate observed by Thompson

et al. (2012) in unharvested conservation grasslands about 200 km north of our sites.

Besides a difference in DSR between pheasants and ducks, our models did not identify

any other predictors that explained variation in DSR. Other studies measured a greater

DSR of nests in landscapes with more grassland and less surrounding wetland (Stephens

et al. 2005, Thompson *et al.* 2012), but these variables were unimportant in our analysis.

Estimated nest density was relatively low (0.43 nests ha^{-1}) compared with those reported

by Arnold *et al.* (2007; 1.5 nests ha^{-1}) and Devries and Armstrong (2011; 1.33 nests ha^{-1}),

who recorded waterfowl nest densities in other areas of the prairie pothole region, where

waterfowl densities are typically greater. Because we chose our research plots for

bioenergy potential rather than waterfowl productivity, it was not surprising that we

recorded lower nest densities. Modeling nest density as the number of nests per plot

required measuring predictors at the plot scale, and the most important predictors were

related to vegetation height in the plot and habitat surrounding the plot, with both

vegetation height and the area of wetlands within 500 m of each plot center being

positively correlated with nest density. Typically, mallard and blue-winged teal densities

are greater in habitats with greater wetland densities (Johnson and Grier, 1988), and our

study supports previous findings that nest density is positively correlated to the proximity

of wetlands (Arnold *et al.* 2007, Devries and Armstrong 2011). Biomass harvesting

equipment is vulnerable to damage and not efficient when operated near wetlands and on

wet ground when used to harvest biomass in late autumn (Williams *et al.* 2012).

Therefore, until harvesting equipment is improved, harvesting operations will not likely occur on fields with greater relative densities of waterfowl nests.

4.5 Conclusion

Our data suggest that autumn biomass harvest does not decrease the number of nesting ducks and pheasants, nor is it detrimental to nest survival following one year of management. Nest density was greater in refuge regions compared with harvested regions, which is evidence that the refuge regions provided important sanctuaries for nesting waterfowl and pheasants when grasslands were managed for bioenergy. Female ducks and pheasants appeared to avoid nesting in harvested regions early in the spring, but this had no measureable effect on nest survival. Selecting perennial grassland sites for harvest that are further from wetlands, which may increase bioenergy potential of the site, would alter habitat at sites less preferential for nesting waterfowl. Although more data are required to determine how much refuge is necessary to optimize the joint production of waterfowl and bioenergy, we recommend orienting refuges closer to wetlands to support nesting waterfowl. Similar studies are needed to record nest survivorship and density for two or more years following biomass harvest (Devries and Armstrong 2011) and to expand the spatial scale beyond 8 ha plots.

Figure 4.1. Distribution of 28 conservation grassland plots on Wildlife Management Areas in southwest Minnesota. Inset is a graphical depiction of the six biomass harvest treatments randomly assigned to each 8 ha plot (stippled regions indicate harvest).
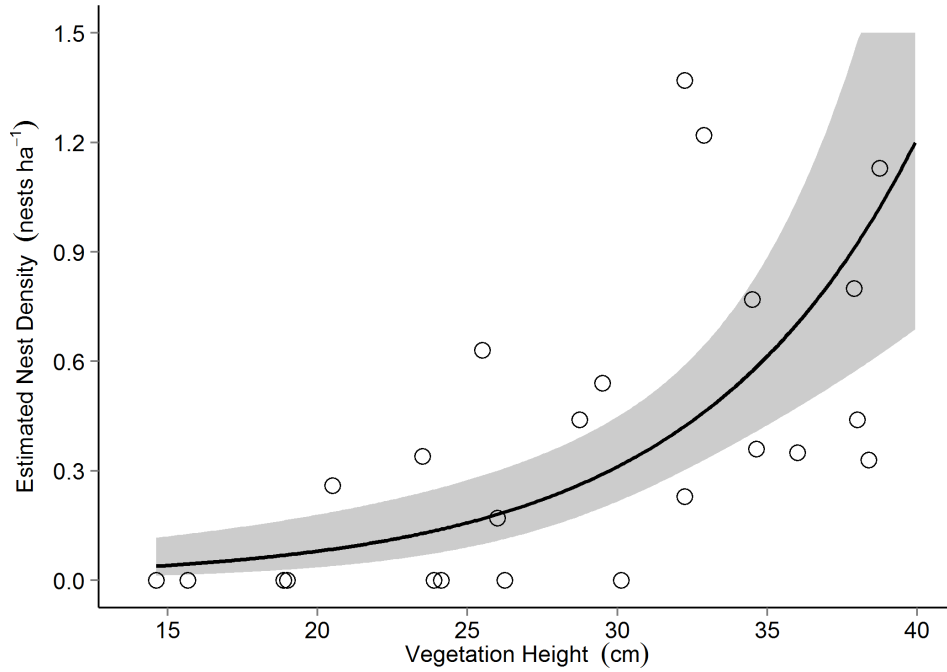
Figure 4.2. Model-based estimates of post-harvest nest density in relation to vegetation height (regression line; ±95% prediction intervals), with percent grassland and wetland held constant at their mean values of 26% and 2%, respectively. Data points are observed values from each nesting field, corrected for nests that failed prior to discovery.

Table 4.1. List and description of all tested model predictors for nest survival and density models.

Predictors[1]	Description	Scale[2]
Nest Survival		
Year	Categorical: Indicates if the nest was found in 2009 or 2010	nest-level
Cut area	Categorical: Indicates if the nest was in a cut area or refuge within the plot	nest-level
Harvest treatment	Categorical: Indicates the harvest treatment applied to the plot surrounding the nest	plot-level
Species	Categorical: Indicates which species initiated the nest	nest-level
Nest initiation date	Continuous: Julian day on which the nest was initiated	nest-level
Nearest wetland	Continuous: Distance (m) of the nest to the nearest wetland	nest-level
Nest Density		
Harvest treatment	Categorical: Indicates which harvest treatment was applied to the plot	plot-level
Area	Continuous: Area (ha) of the plot	plot-level
Vegetation height	Continuous: Mean height (cm) of vegetation within plot	plot-level
Plant species richness	Continuous: Mean number of species found from sample quadrats	plot-level
Grass cover	Continuous: Mean cover (%) of grasses from sample quadrats	plot-level
Forb cover	Continuous: Mean cover (%) of forbs from sample quadrats	plot-level
Plant biomass	Continuous: Mean biomass (g m^{-1}) sampled from sample quadrats	plot-level
Grassland	Continuous: Amount (m^2) of grassland within 500 m radius of plot center	plot-level
Wetland	Continuous: Amount (m^2) of wetland cover within 500 m radius of plot center	plot-level

[1] For each response variable, all listed parameters were included in the global model.

[2] Indicates if the parameters were measured at the scale of plot- or nest-level.

Table 4.2. Akaike Information Criteria based on small sample size (AIC$_c$), differences in AIC$_c$ between top ranked and null models (Δ AIC$_c$), Akaike weights (ω), and number of parameters (k) for models estimating nest daily survival rate (DSR) and density in conservation grasslands.

Model parameters	AIC$_c$	Δ AIC$_c$	ω	k
DSR Model				
Species	176.2	0	0.19	2
Null	176.7	0.4	0.15	1
Harvest treatment	177.4	1.2	0.10	2
Density Model				
Vegetation height + Grassland + Wetland	101.2	0	0.39	4
Area + Vegetation height + Grassland + Wetland	101.7	0.5	0.32	5
Vegetation height	103.5	2.3	0.12	3
Null	267.0	165.8	0.00	2

Table 4.3. Parameter estimates from the best-supported model for predicting the natural logarithm of nest density as a function of vegetation height (cm) and the amount of surrounding grassland (m^2) and wetland (m^2) within a 500 m radius from the plot center.

Model parameter	Estimate	SE	P value
Intercept	-2.76	0.97	0.004
Vegetation height	0.14	0.03	< 0.001
Grassland	-3.14×10^{-6}	1.36×10^{-6}	0.021
Wetland	1.28×10^{-5}	4.89×10^{-6}	0.009

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Appendix A

Chapter 1 Supporting Information

Table A.1. Ten most frequently observed species and their average percent cover in sample quadrats.

	South		Central		North	
Frequency Ranking	Species	Average Cover (%)	Species	Average Cover (%)	Species	Average Cover (%)
1	Andropogon geradii	34.8	Poa pratensis	20.3	Poa pratensis	27.2
2	Panicum virgatum	14.6	Solidago spp.	8.9	Solidago spp.	8.9
3	Poa pratensis	15.6	Andropogon geradii	30.4	Panicum virgatum	17.9
4	Asclepias syriaca	3.1	Cirsium arvense	2.9	Cirsium arvense	2.1
5	Cirsium arvense	2.5	Panicum virgatum	10.6	Andropogon geradii	38.9
6	Bromus inermis	25.2	Phalaris arundinacea	33.3	Phleum pratense	4.8
7	Schizachyrium scoparium	11.8	Bromus inermis	23.4	Taraxacum officinale	1.5
8	Solidago spp.	7.1	Sonchus oleraceus	4.3	Sporobolus heterolepis	22.9
9	Melilotus alba	14.1	Schizachyrium scoparium	24.7	Dalea purpurea	3.5
10	Elymus canadensis	8.2	Melilotus alba	12.2	Agropyron repens	9.6

Table A.2. Calibration statistics for NIRS prediction of forage characteristics and plant cell polysaccharides.

Perten Only	NDF	IVTD	Klausen Lignin	Rhamnose	Arabinose	Xylose	Mannose	Galactose	Glucose
						——mg/g——			
Factors	7	8	13	7	6	8	12	7	8
SEC	1.18	1.54	6.56	1.33	3.74	19.70	1.60	1.21	13.83
SECV	2.15	1.85	11.02	1.55	4.23	22.87	2.33	1.51	16.34
R	0.885	0.906	0.783	0.862	0.763	0.895	0.916	0.907	0.927
Range	63.5 – 81.6%	31.8 – 49.4%	153 – 220	1 – 12	14 – 40	45 – 203	1 – 25	4 – 21	185 – 378
N	76	66	66	73	72	78	75	70	77
Perten + Foss									
Factors	7	8	9	6	10	5	8	6	4
SEC	2.07	1.82	11.51	1.6	3.88	27.70	2.78	2.30	24.67
SECV	2.18	2.07	12.48	1.51	3.59	20.79	2.52	2.09	21.29
R	0.864	0.891	0.652	0.885	0.825	0.872	0.898	0.844	0.871
Range	63.5 – 81.6%	31.8 – 49.7%	153 – 260	1 – 12	12 – 43	45 – 242	1 – 25	4 – 27	185 – 424
N	123	107	374	394	373	383	397	407	377

Equation A.1. Equation developed by the US Department of Energy to estimate theoretical ethanol conversion efficiency from sugar concentrations; http://www1.eere.energy.gov/bioenergy/ethanol_yield_calculator.html

(((glucan + galactan + mannan) * 172.82) + ((xylan + arabinan) * 176.87)) * 0.01

Text A.1. Assessment of bale weight variability for large round bales of biomass harvested from conservation grasslands.

Using the information from multiple trailer loads, an assessment of variability was measured. The standard deviation of average bale weights from 13 trailer loads in 2010 was 45 kg. This was similar to published variance values of large round bales of switchgrass (sd = 36 kg; Monti *et al.* 2009).

Text A.2. Calculations for estimating residential power production from conservation grasslands in SW Minnesota. Area estimates for each conservation grassland type were calculated from state and federal data layers.

Total CRP in SW 80 mile radius = 185626 acres, WMA = 66337, WPA = 13853; SUM = 265816 * 0.75 = 199362 acres = 80678 ha
80678 ha * 2.5 Mg / ha = 201695 Mg
201695 Mg * 18.5 GJ / Mg[1] = 3731357 GJ
3731357 GJ * 0.278 MW*h = 1037317 MW*h
Average U.S. household electricity consumption[2] = 10.8 MW*h/year
1037317 MW*h / 10.8 MW*h/house = 96047 homes

[1] From bomb calorimetry estimates of biomass samples (unpublished data)
[2] http://www.eia.gov/tools/faqs/faq.cfm?id=97&t=3

Appendix B

Chapter 2 Supporting Information

Table B.1. Treatment averages and model response of theoretical ethanol potential to N fertilization rates for grassland biomass at three locations in 2008 and 2009.

Location	Year	N fertilizer rate (kg N ha^{-1})						
		0	56	112	168	224	Mean	Model
Austin	2008	448	447	435	435	428	439	LR
	2009	447	446	429	428	417	433	LR
	Mean	448	447	432	432	423	436	
Lamberton	2008	407	412	408	416	402	409	ns
	2009	463	453	441	449	440	449	LR
	Mean	435	432	425	433	421	429	
Rosemount	2008	485	481	477	473	466	476	LR
	2009	473	460	450	443	435	452	SQD
	Mean	479	471	463	458	451	464	
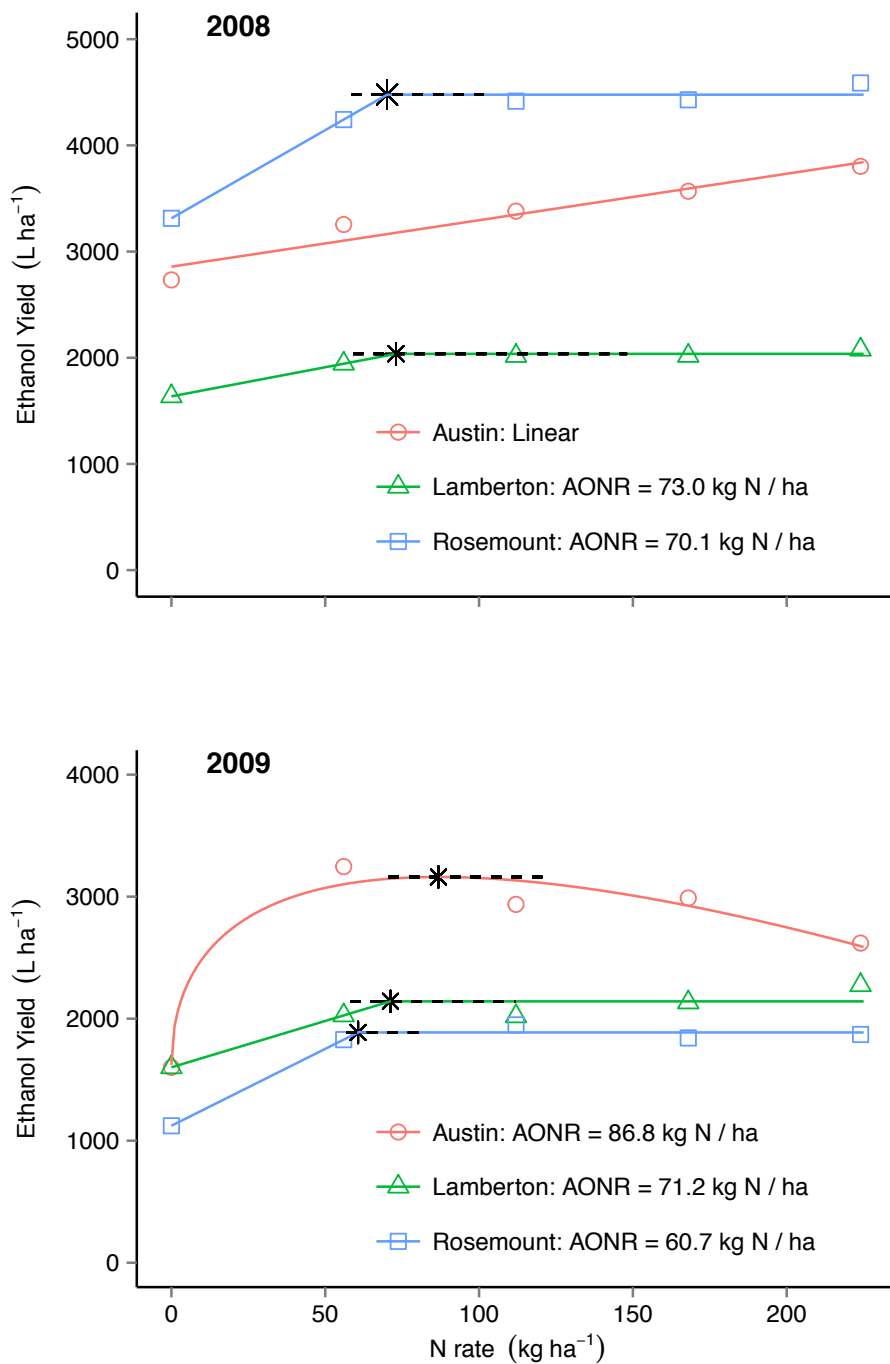
Figure B.1. Average land ethanol yield for five nitrogen fertilization rates at three locations in 2008 and 2009. Regression lines for each site:year combination are from best-supported models. Asterisks indicate agronomically optimum nitrogen rates (AONR) and 95% confidence interval (dashed lines) based on model estimates.

Appendix C

Chapter 4 Supporting Information
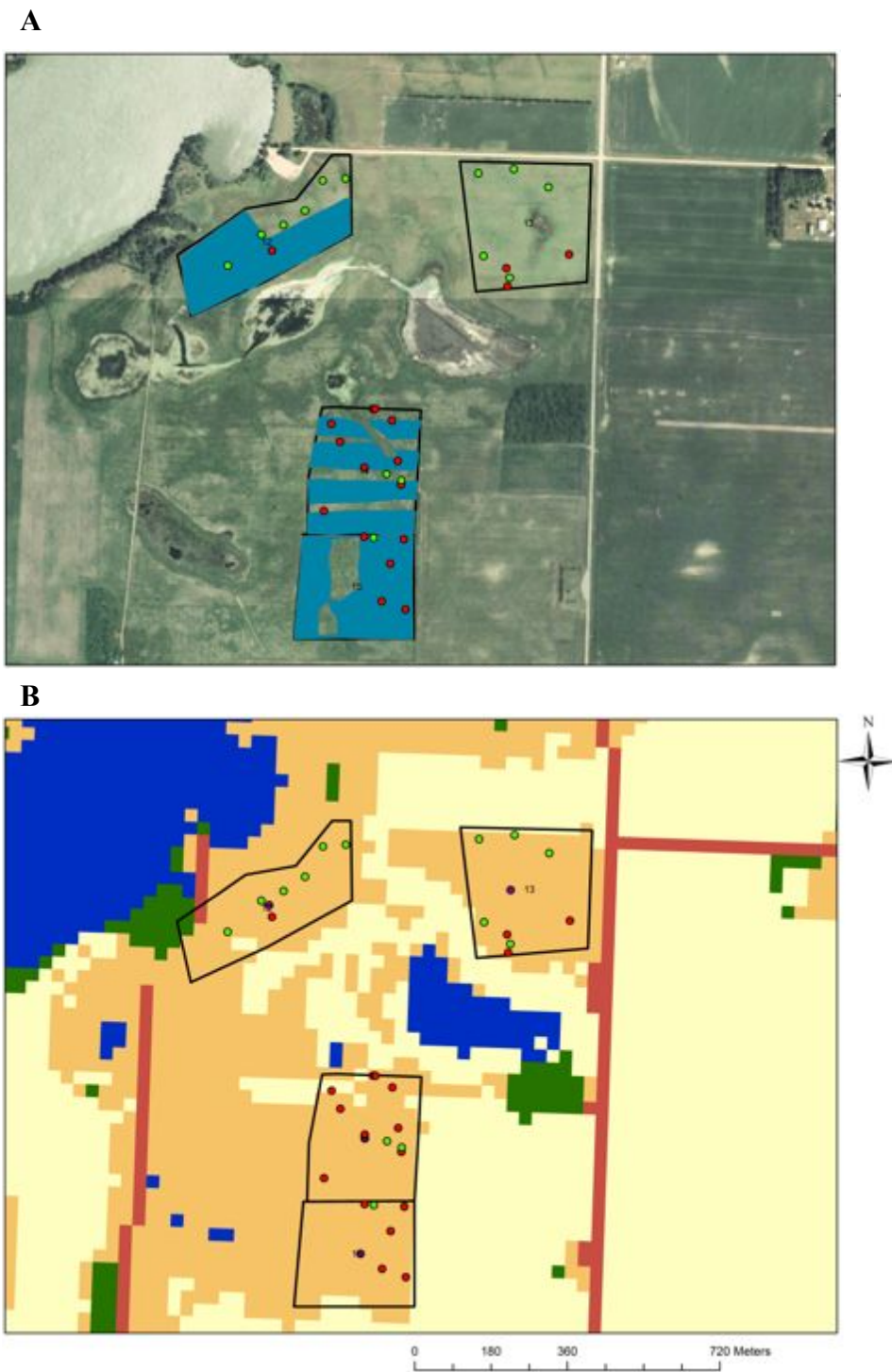
Figure C.1. Plot outlines, harvested areas (blue shading), and nest locations in 2009 (pre-harvest; red) and 2010 (post-harvest; green) transposed to an aerial photograph of the south research location (A) and a digitized land cover map characterizing grassland (light brown) and wetland regions (blue) (B).