Managing Conservation Grasslands for Bioenergy and Wildlife

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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

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 $N\text{-}NO_3$, 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

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,

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 $N\text{-}NO_3$ will produce biomass with greater concentrations of tissue N (Table 1.4). Elevated levels of soil $N\text{-}NO_3$ 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.

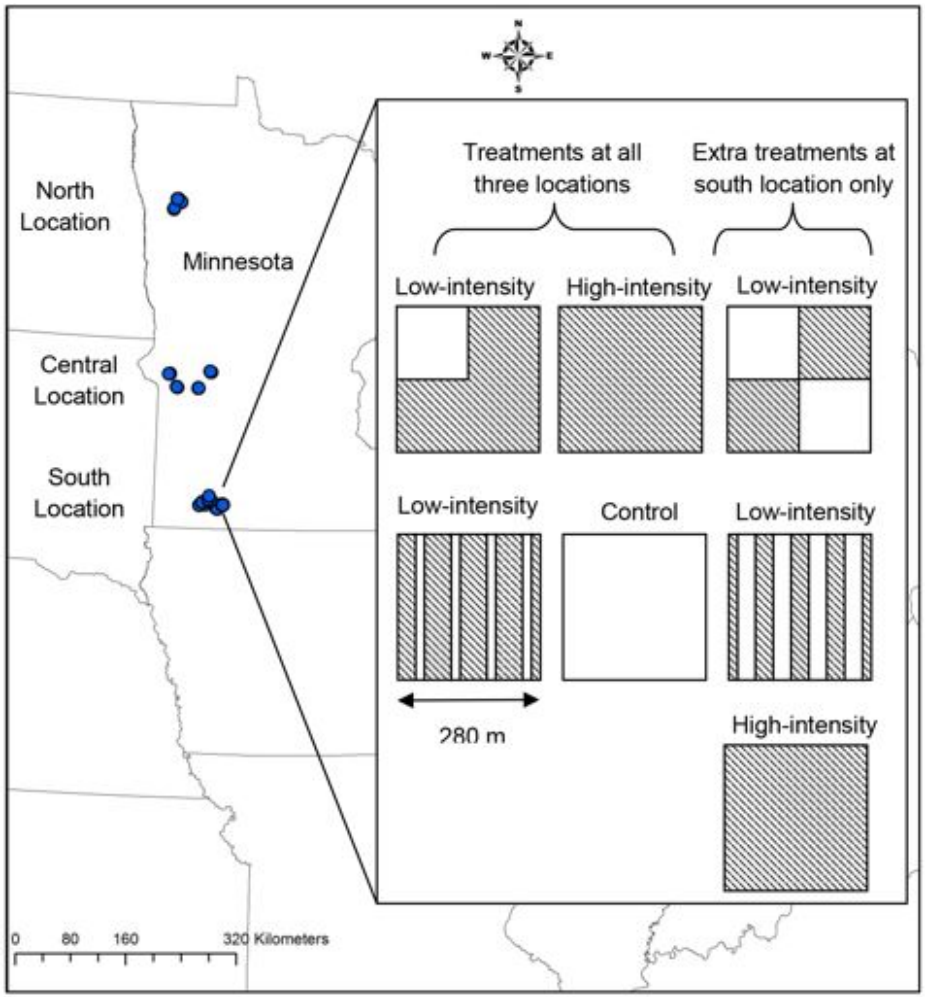

Figure 1.1. Study areas in Minnesota, located in the Upper Midwest, USA. Research blocks are indicated by circles within the outline of Minnesota in north, central, and south locations. Inset outlines treatments within blocks.

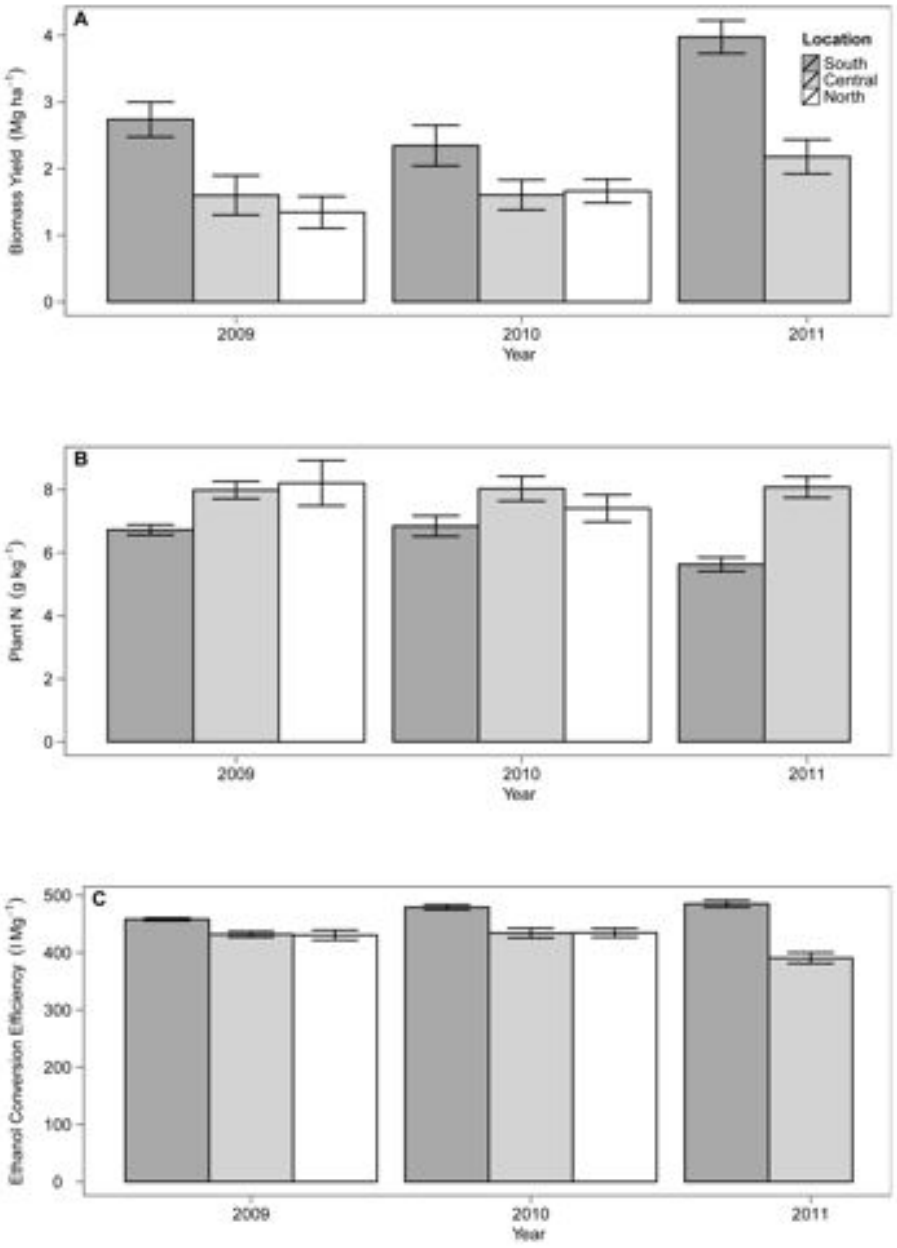

Figure 1.2. Average values (SE) of response variables by location and year. Mean values of biomass yield (A), plant tissue N (B), and ethanol conversion efficiency (C). Black, gray and white bars are mean values from plots harvested in south, central and north locations respectively.

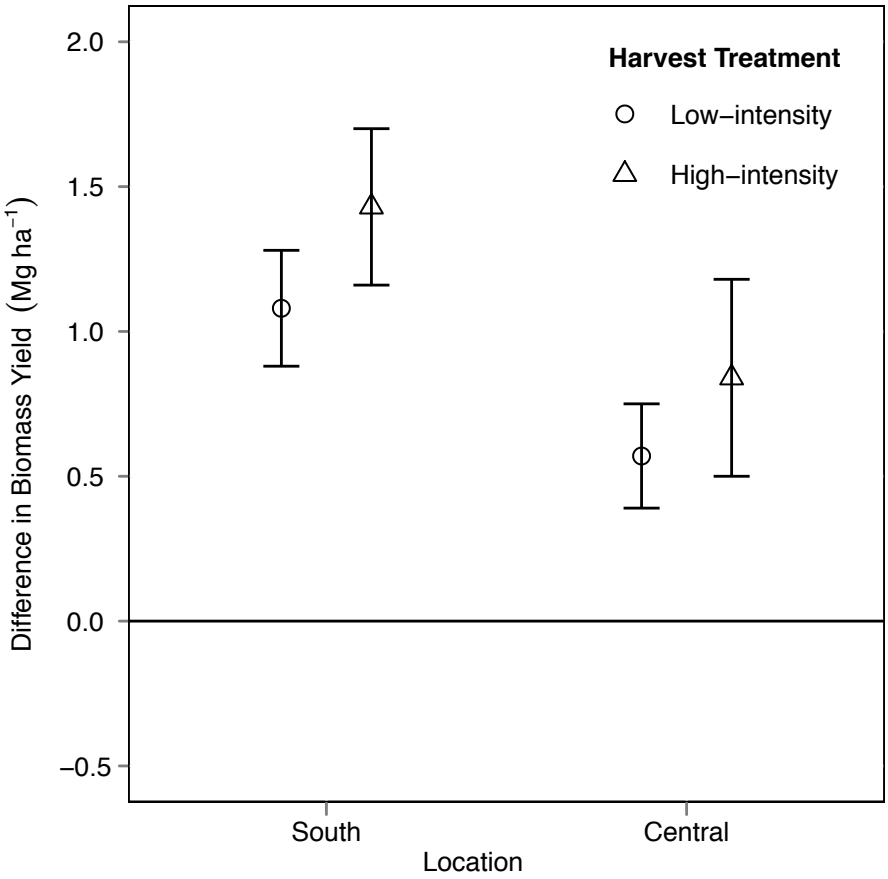

Figure 1.3. Change in biomass yield from 2009 to 2011 in low- and high-intensity harvest treatments by location. Average change in biomass yield (± 90% CI). In low-intensity plots, one third to one half of the annually harvested biomass was from an area not previously harvested. High-intensity harvest plots included biomass from the same area harvested annually.

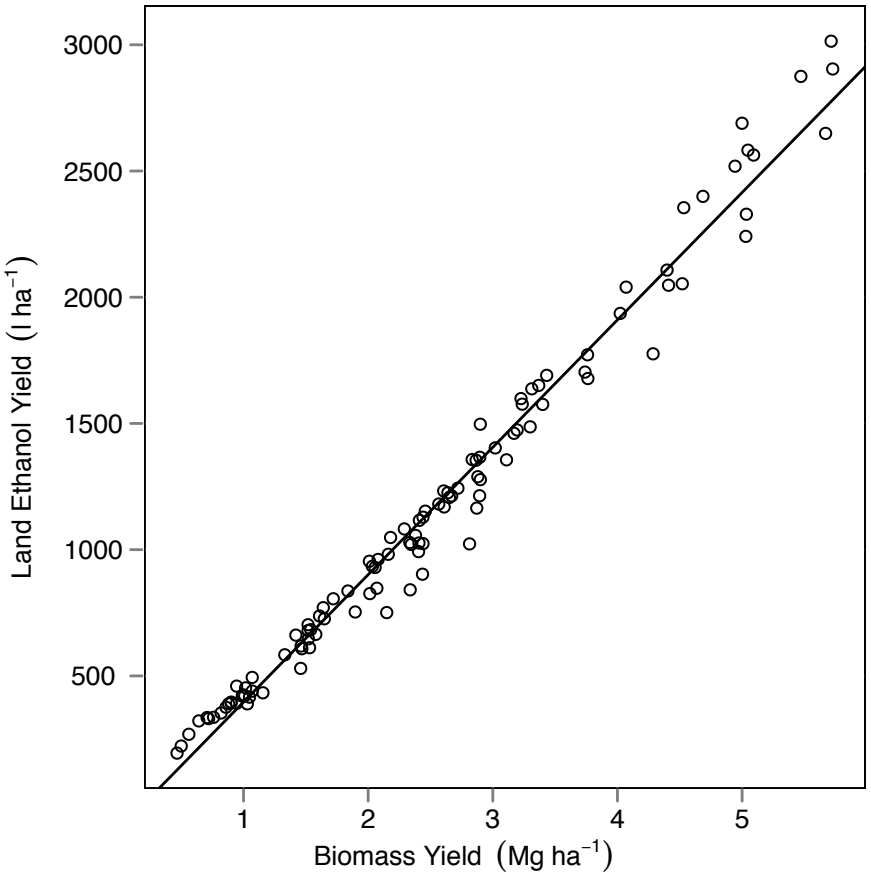

Figure 1.4. Correlation between land ethanol yield (l ha^{-1}) and biomass yield (Mg ha^{-1}). Points represent values from conservation grasslands harvested in the autumn of 2009, 2010, and 2011. Regression line from linear model with R^2 value = 0.98.

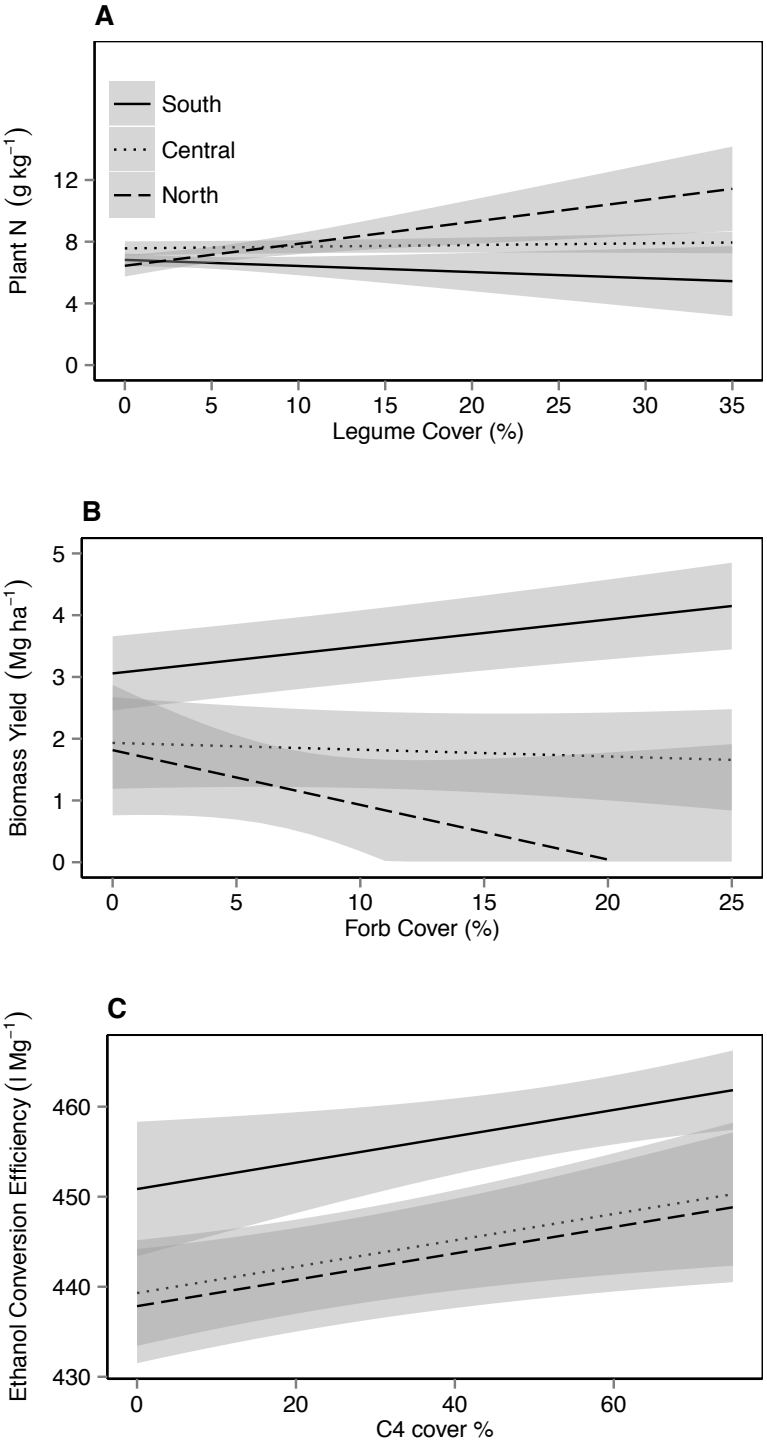

Figure 1.5. Estimated effect of plant functional group composition on bioenergy potential. Regression line estimates (± 90% CI) of the effect of legume cover on the concentration of N in biomass after harvest (A), the effect of forb cover on biomass yield (B), and the effect of C4 cover on ethanol conversion efficiency (C). Estimates are from the best fitting models with all other covariates held constant at their average values.

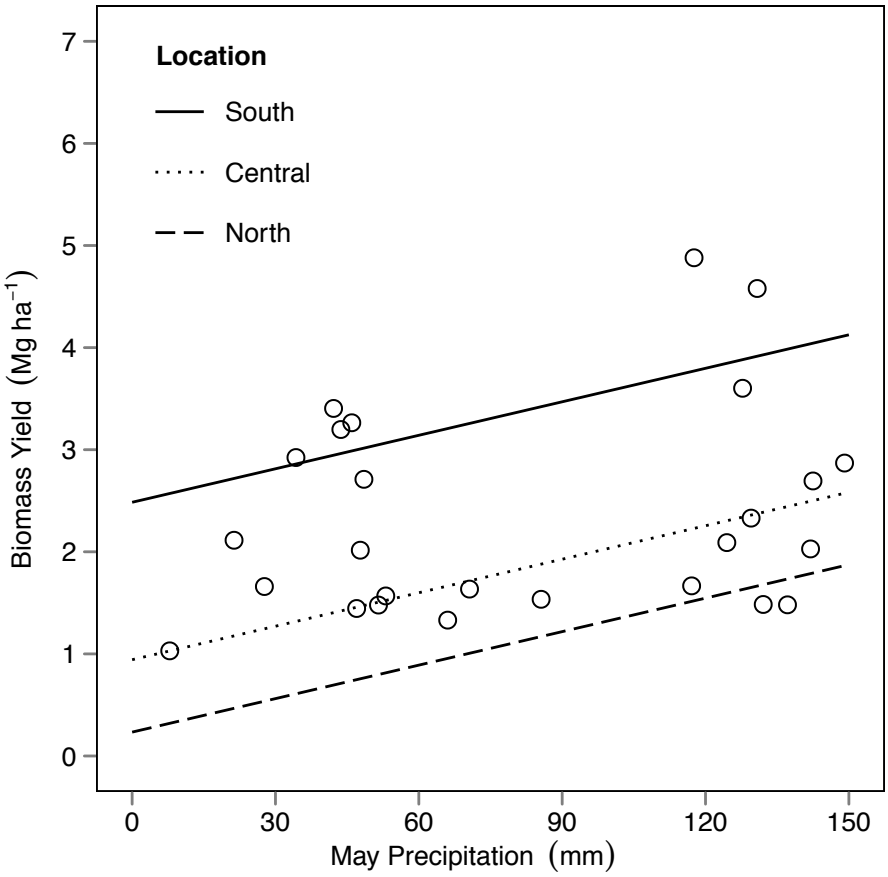

Figure 1.6. Estimated effect of May precipitation on biomass yield. Dots represent average measured biomass yield and May precipitation values by block. Regression lines are model estimates for bioenergy yield across the precipitation gradient for each location, with all other covariates held constant 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

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:

$$\begin{aligned} &Theoretical\ Ethanol\ Yield\ (L\ Mg^{-1}) \\ &\quad = [(\%\ Arabinose + \%\ Xylose)\times 737.55] \\ &\quad + [(\%\ Glucose + \%\ Galactose + \%\ Mannose)\times 720.66] \end{aligned}$$

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

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).

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

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.

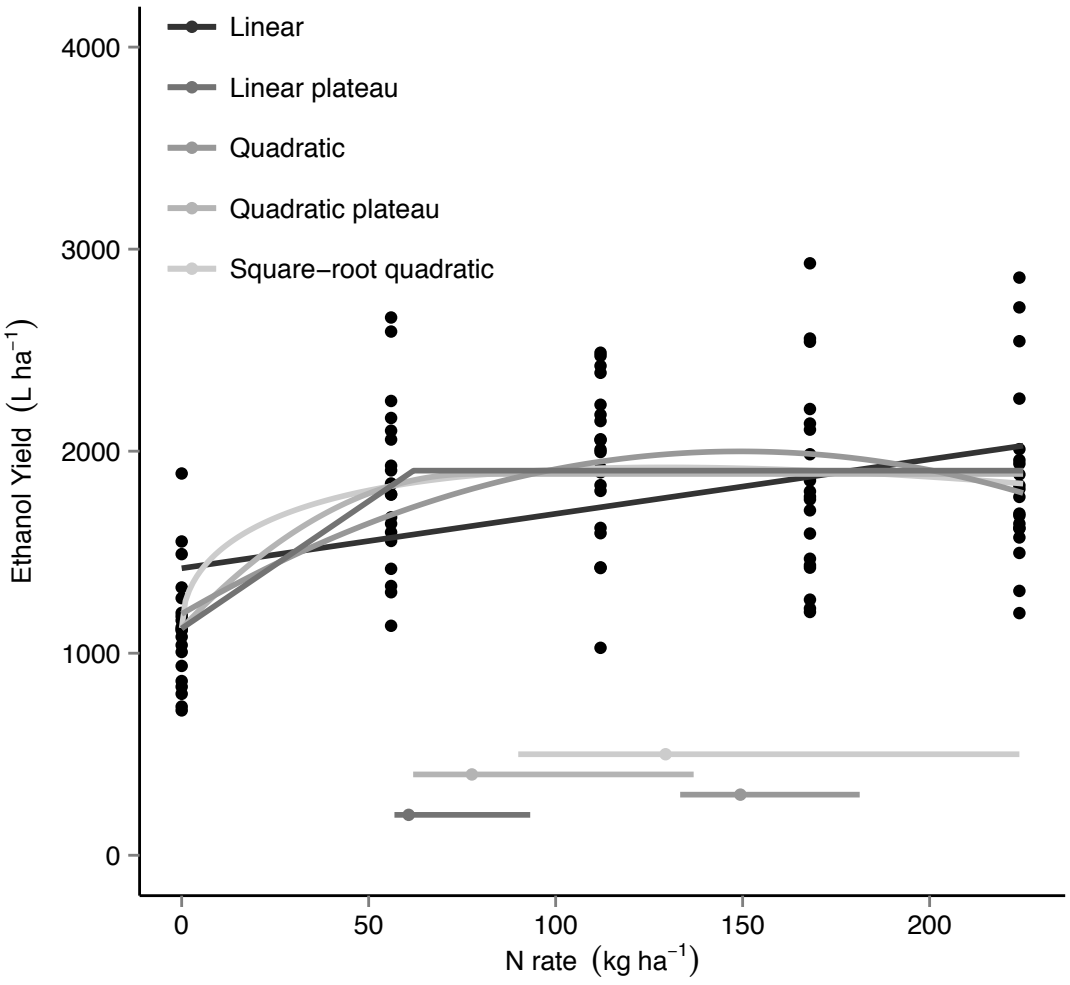

Figure 2.1. Measured land ethanol yield at five nitrogen fertilization rates (0, 56, 112, 168, 224 kg N ha^{-1}) at Rosemount in 2009. Also shown are model fit curves from five response functions along with the agronomically optimum nitrogen rate and 95% confidence intervals for each model.

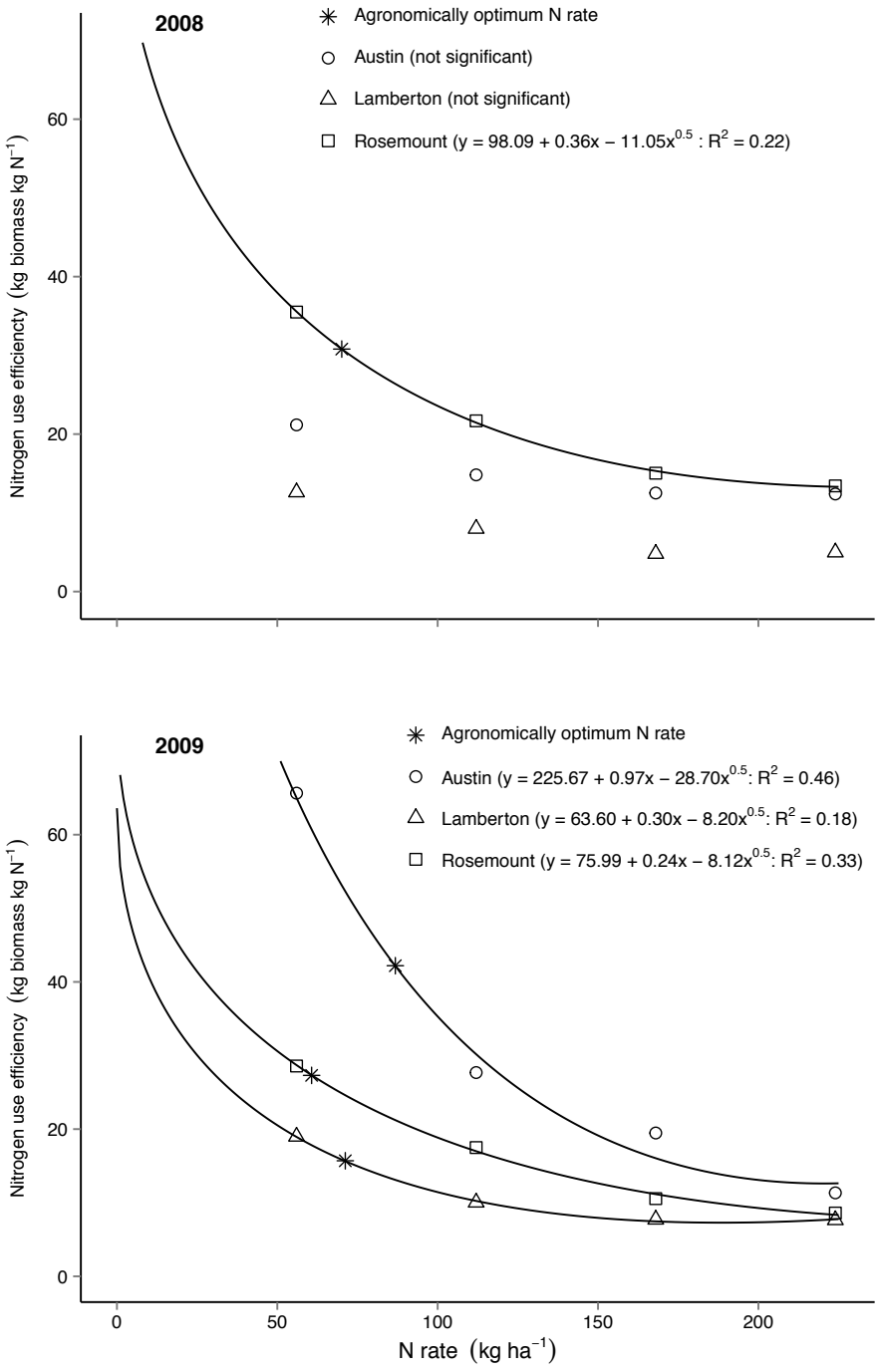

Figure 2.2. Average nitrogen use efficiency (NUE) at four N fertilization rates (56, 112, 168, 224 kg N ha^{-1}) for three locations in 2008 and 2009. Also shown is the best-supported model fit for NUE at each site-year environment, with the agronomically optimum nitrogen rate based on land ethanol yield 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.

	Austin			Lamberton			Rosemount		
Month	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					
Location	Year	0	56	112	168	224	Mean	Model[a]	β_0 (intercept)	β_1	β_2 (maximum)	AONR[b] (kg N ha^{-1})	Biomass 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})								
Location	Year	0	56	112	168	224	Mean	Model[a]	AONR[b] (kg N ha^{-1})	Removal at AONR
		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								

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

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

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.

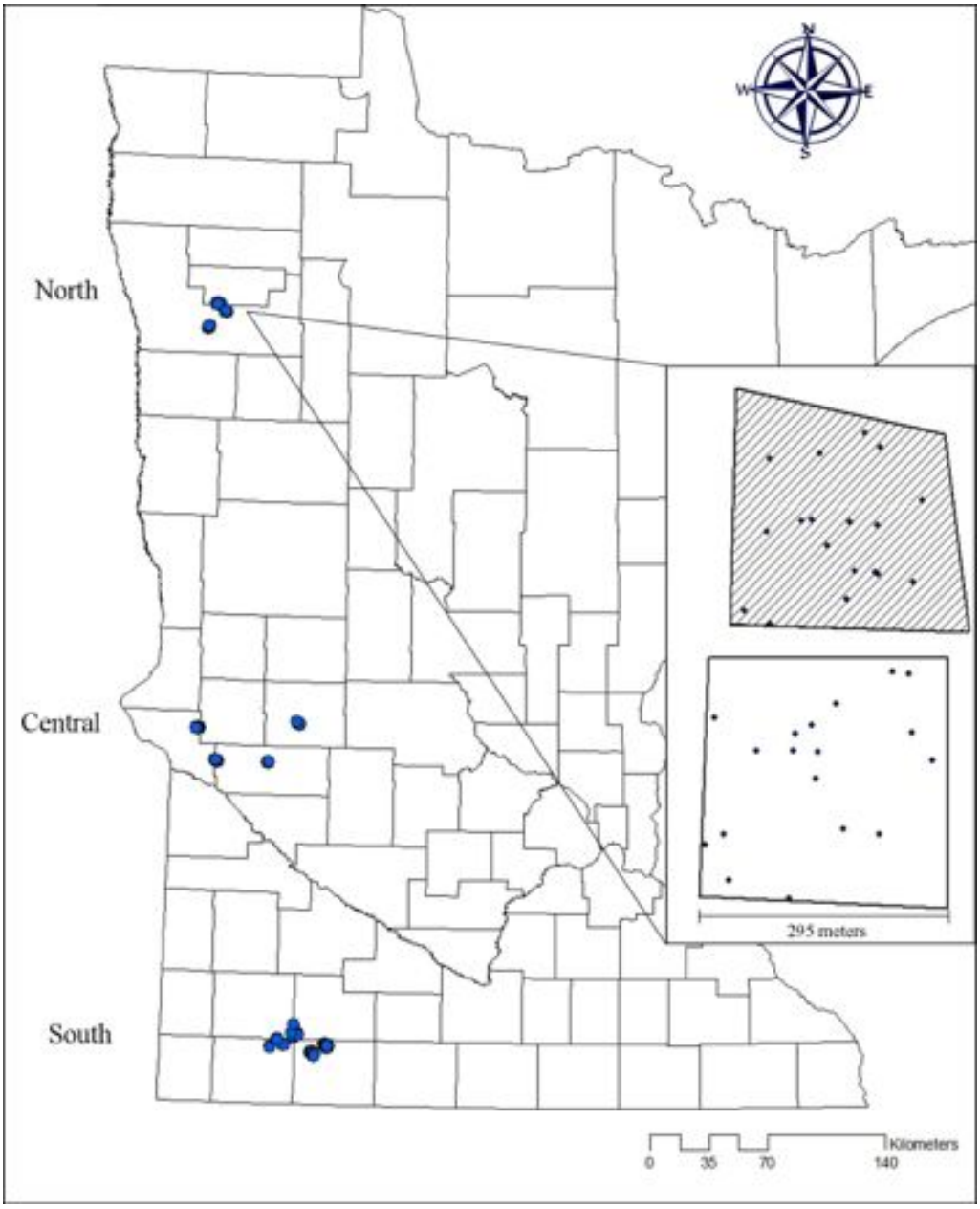

Figure 3.1. Map of the study area in Minnesota, USA. Inset shows 100% harvest plot and an unharvested control plot with randomly distributed sample quadrats where plant community composition was measured in 2011.

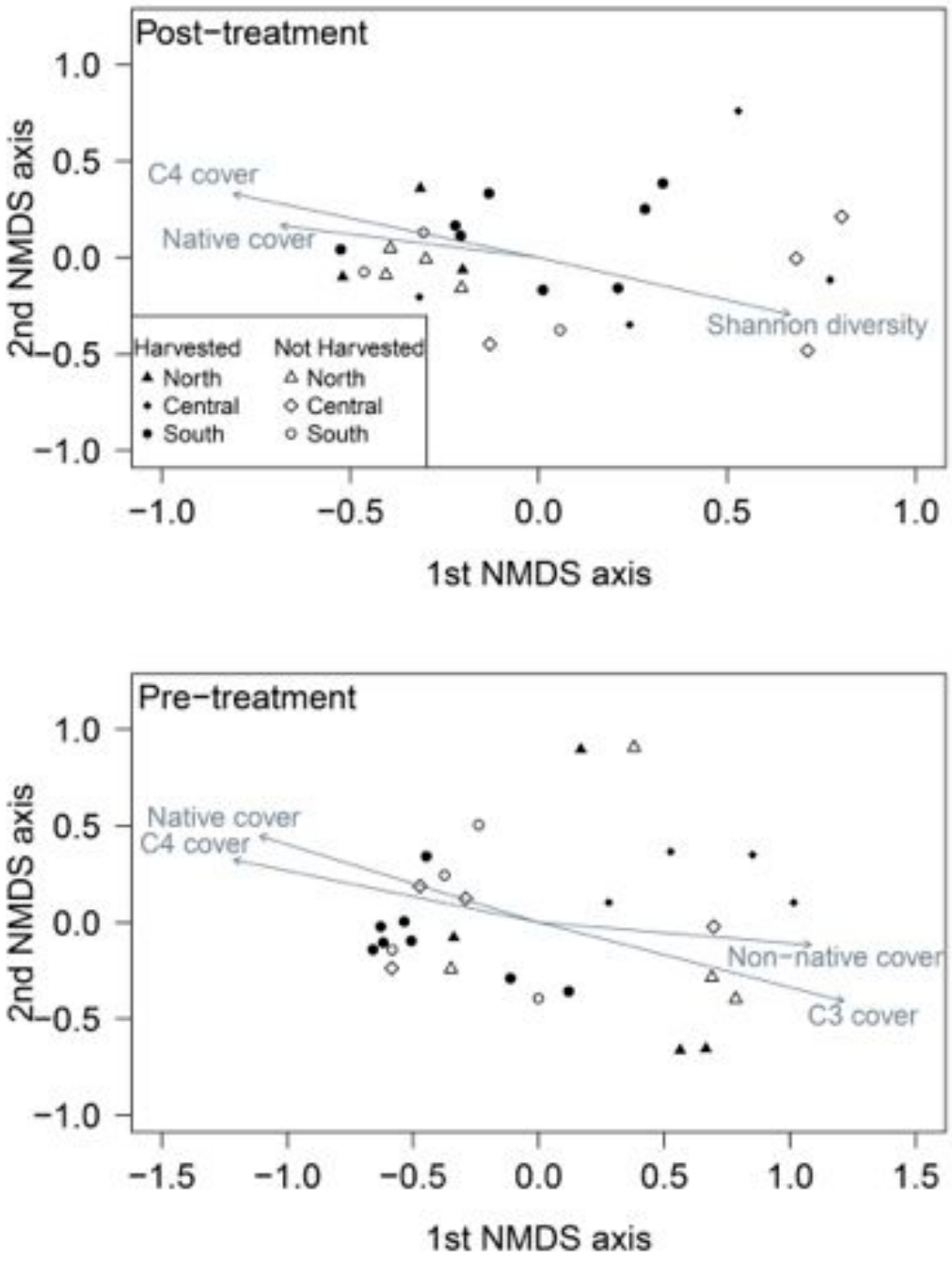

Figure 3.2. Non-metric multidimensional scaling ordination of plant communities in grasslands prior to biomass harvest (Pre-treatment) and following two (North) and three (Central and South) year of biomass harvest (Post-treatment). Lines represent gradients for metrics of plant community composition, with the length of the line representing strength of correlation to axes.

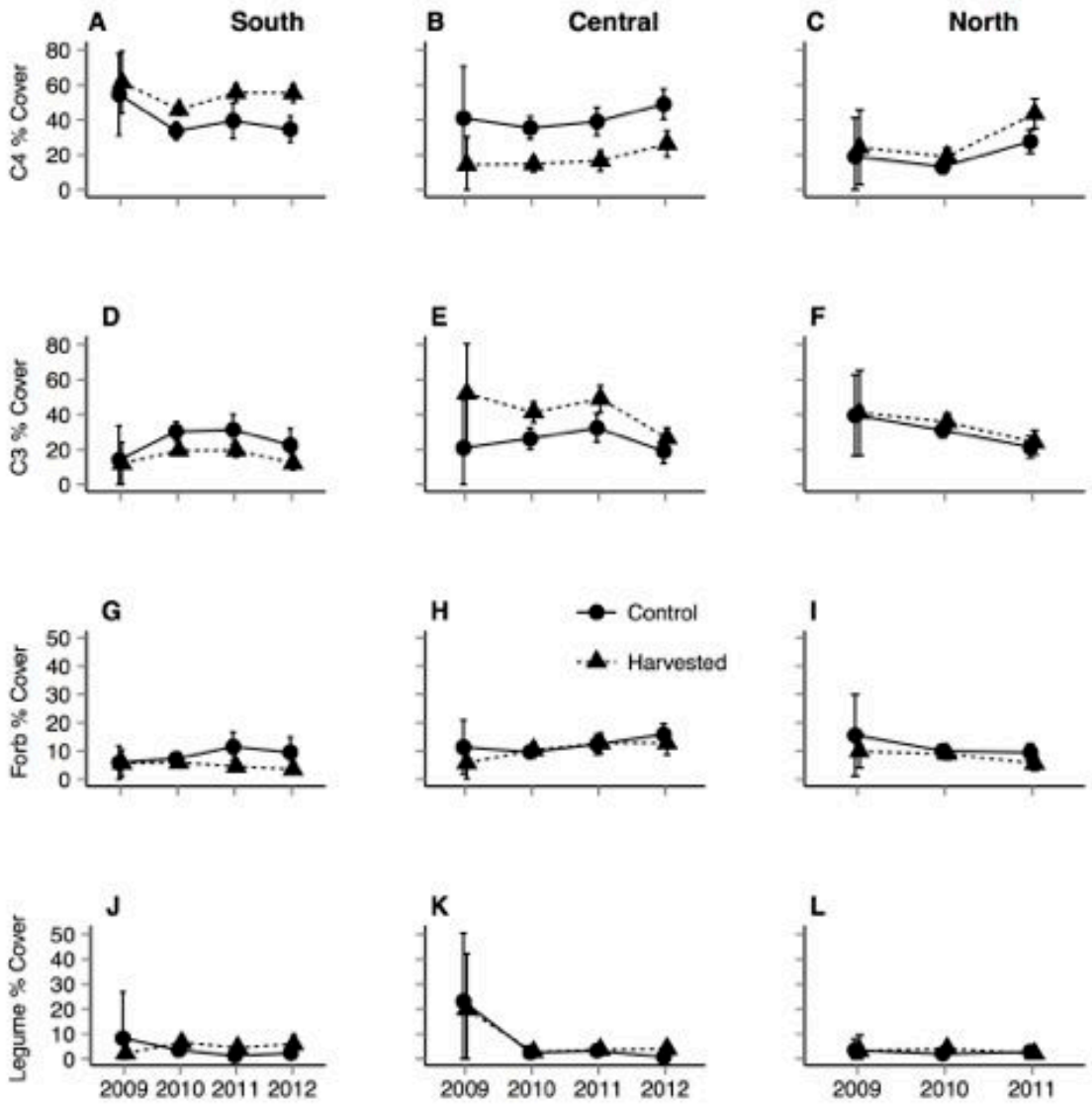

Figure 3.3. Average percent cover of the four major plant functional groups in harvested and unharvested plots located in the south, central and north locations from 2009 (pre-treatment) to 2012.

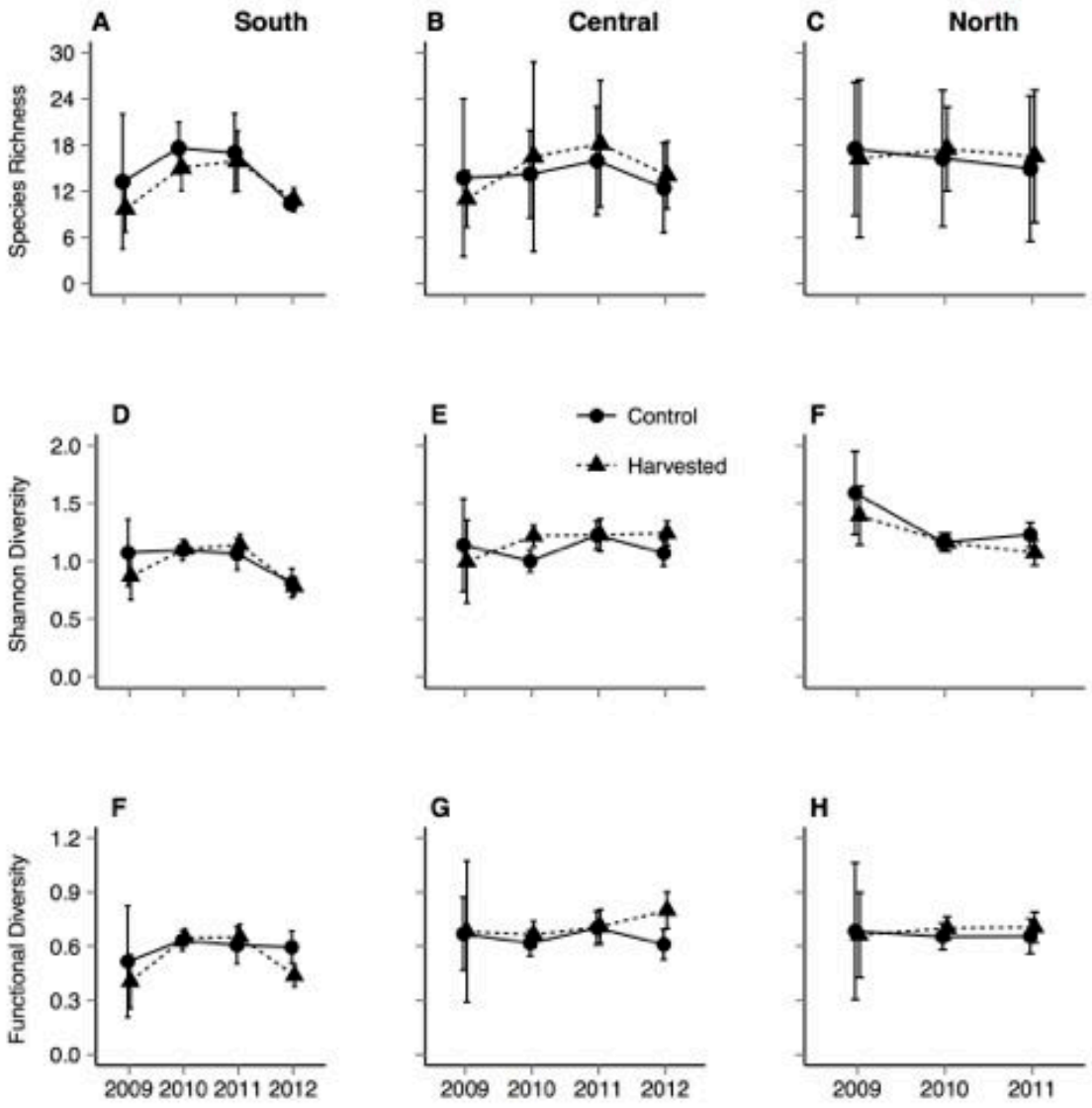

Figure 3.4. Average species richness, species, and functional group diversity in harvested and unharvested plots located in the south, central and north locations from 2009 (pre-treatment) to 2012.

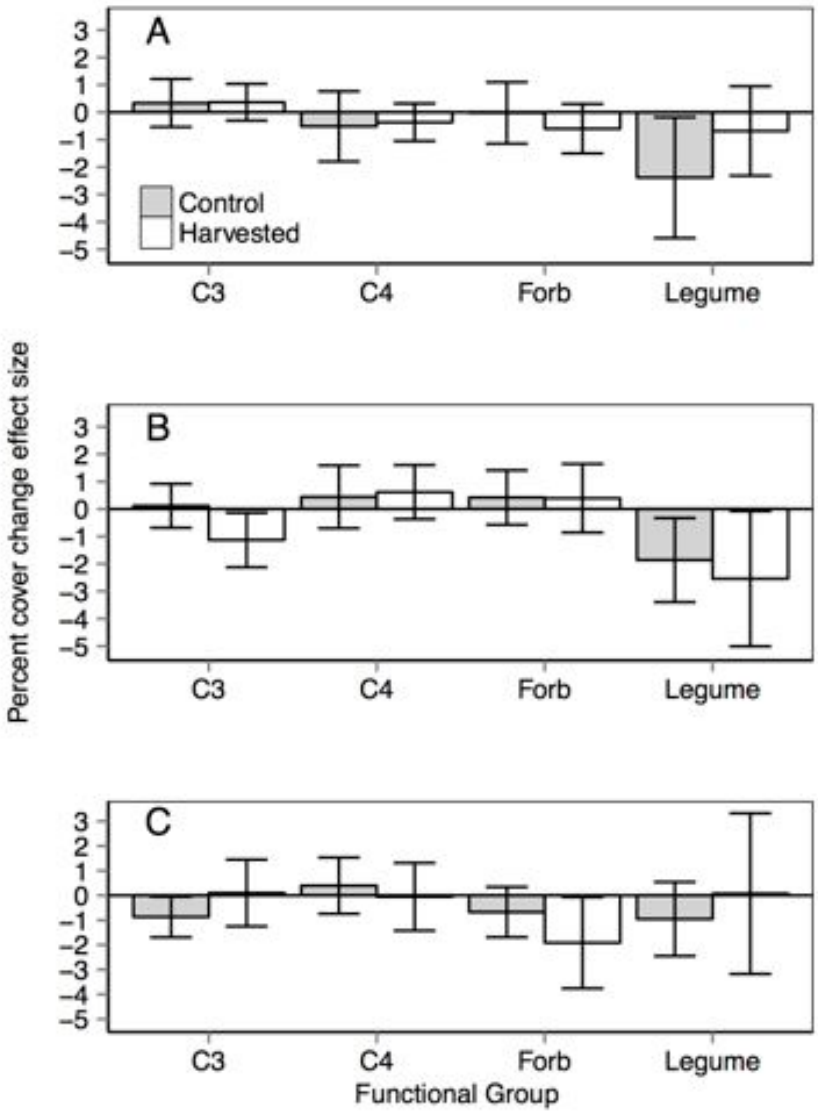

Figure 3.5. Effect sizes (Hedges' *g*) and associated 95% confidence intervals for the change in functional group cover from pre-treatment to final year post-treatment conditions in the south (A), central (B), and north (C) locations in Minnesota, USA.

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	W*i*
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

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

"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.

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.

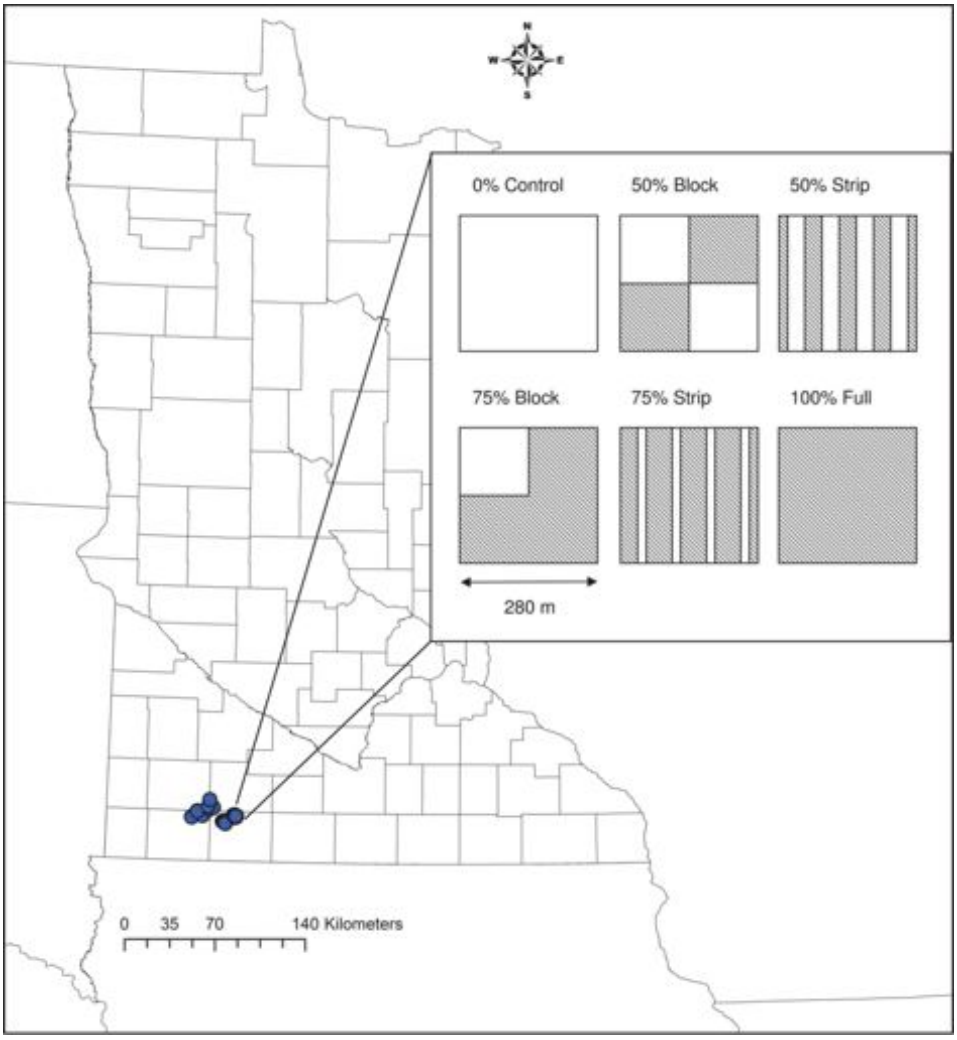

Figure 4.1. Distribution of 28 conservation grassland plots on Wildlife Management Areas in southwest Minnesota. Inset is a graphical depiction of the six biomass harvest treatments randomly assigned to each 8 ha plot (stippled regions indicate harvest).

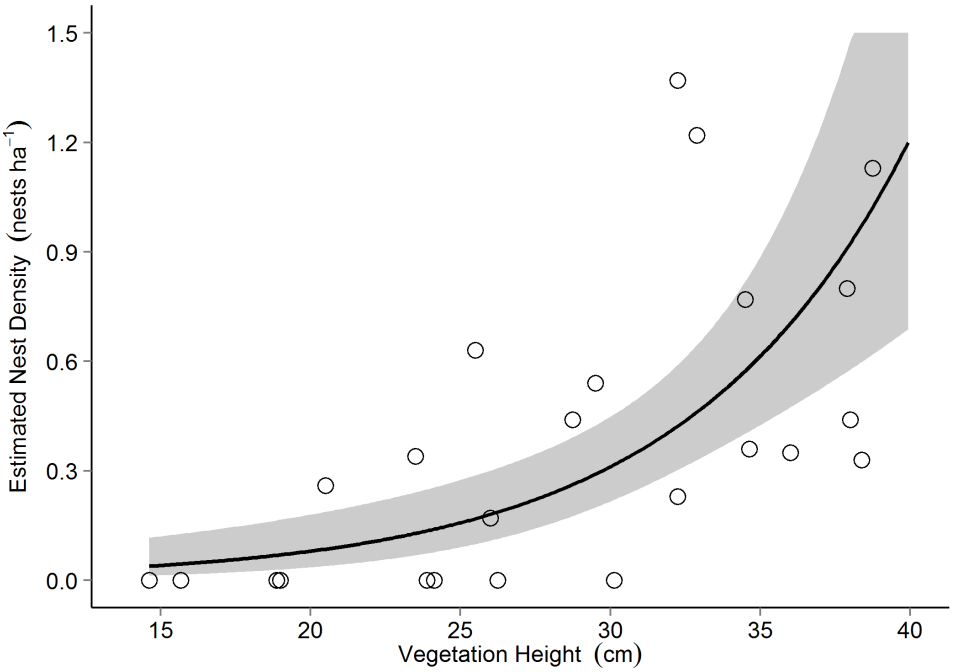

Figure 4.2. Model-based estimates of post-harvest nest density in relation to vegetation height (regression line; ±95% prediction intervals), with percent grassland and wetland held constant at their mean values of 26% and 2%, respectively. Data points are observed values from each nesting field, corrected 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 (ΔAIC_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	ΔAIC_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.

		N fertilizer rate (kg N ha^{-1})						
Location	Year	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	

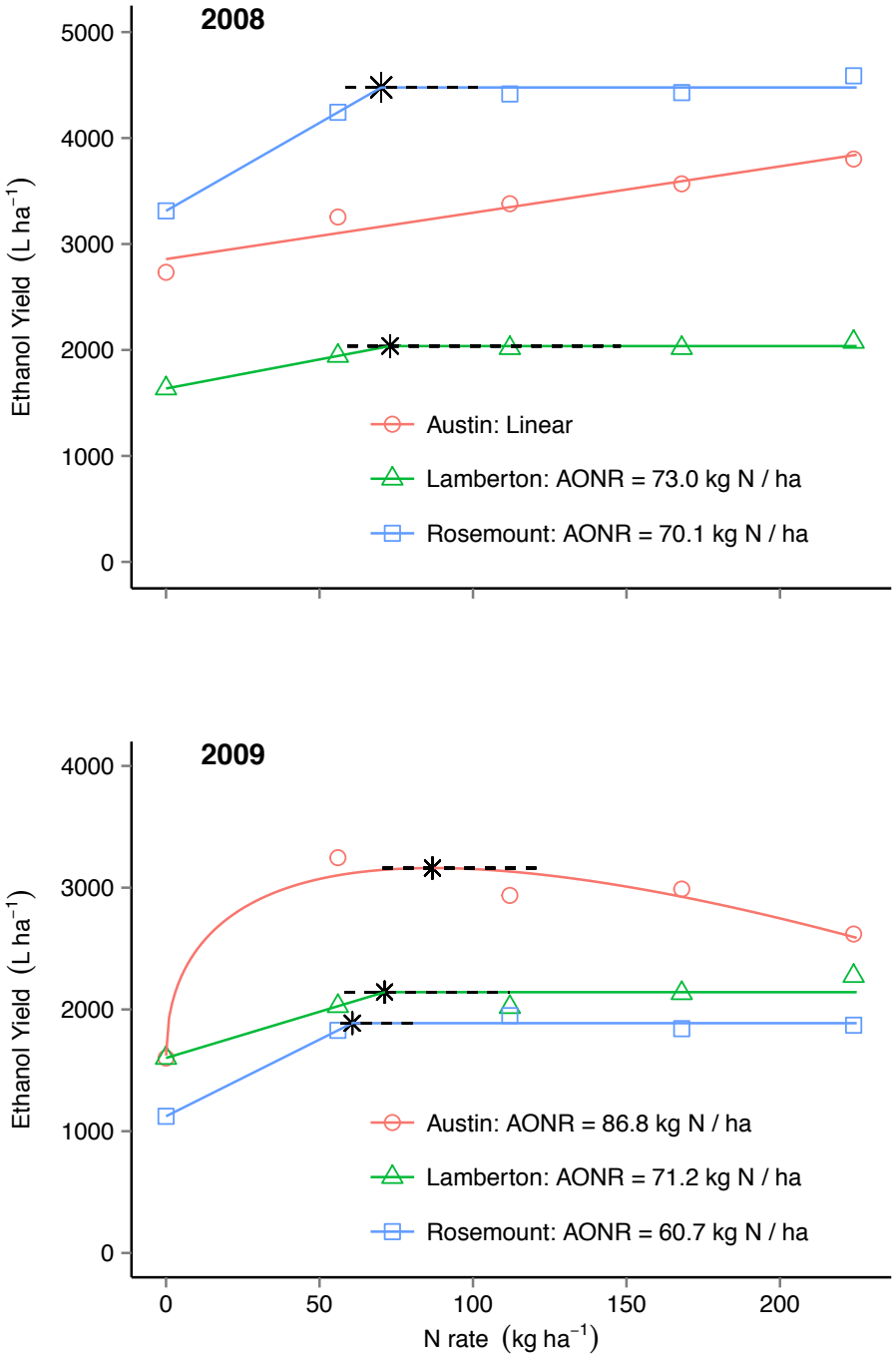

Figure B.1. Average land ethanol yield for five nitrogen fertilization rates at three locations in 2008 and 2009. Regression lines for each site:year combination are from best-supported models. Asterisks indicate agronomically optimum nitrogen rates (AONR) and 95% confidence interval (dashed lines) based on model estimates.

Appendix C

Chapter 4 Supporting Information

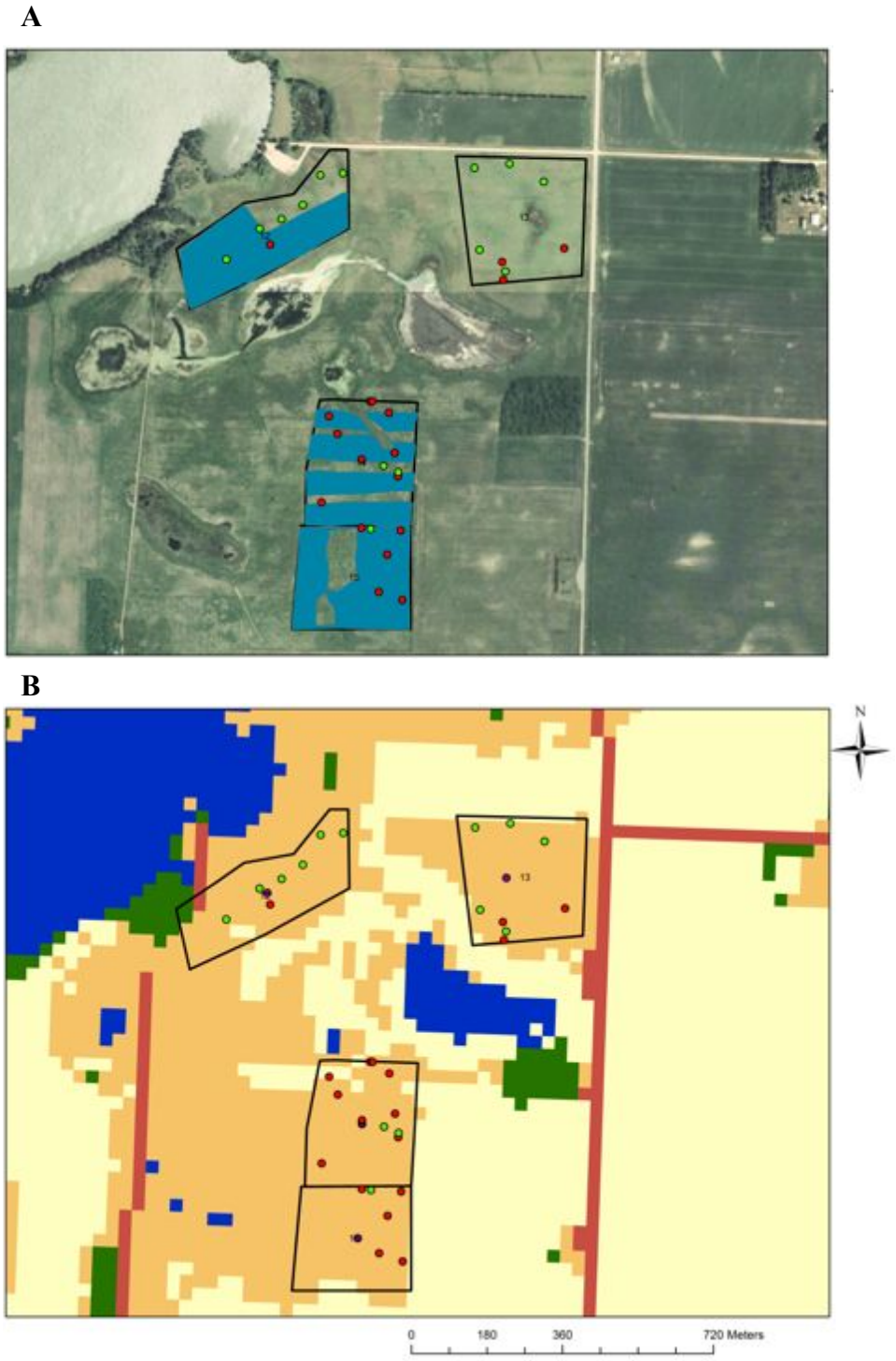

Figure C.1. Plot outlines, harvested areas (blue shading), and nest locations in 2009 (pre-harvest; red) and 2010 (post-harvest; green) transposed to an aerial photograph of the south research location (A) and a digitized land cover map characterizing grassland (light brown) and wetland regions (blue) (B).