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Tradeoffs in ecosystem services of prairies managed for bioenergy production

by

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A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Co-majors: Sustainable Agriculture; Ecology and Evolutionary Biology

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Iowa State University

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TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Agriculture is the leading cause of land-use change globally and in the United States (Turner et al. 2007, Broussard and Turner 2009, Foley et al. 2011), and it is projected to be the leading cause of land-use change globally in the future (Lambin and Meyfroidt 2011). Within in the U.S., Iowa leads the nation in the largest percentage of land converted to agriculture and the least amount of native vegetation remaining (Samson and Knopf 1994, Nickerson et al. 2011). Because agriculture is the dominant land use, it is important to understand the impacts that agriculture has on the landscape.

The physical structure and temporal dynamics of agricultural systems are almost always simpler than native ecosystems (Altieri 1995, Swift et al. 2004). In Iowa, for example, more than 85% of the landscape was historically tallgrass prairie (Samson and Knopf 1994), whereas now more than 65% of the landscape is used to grow monocultures of either corn or soybean (NASS 2012) (Figure 1). Prairies are comprised of a diverse array of mostly perennial species that have differing heights, structures, and phenologies. Both corn and soybean, on the other hand, are summer annual plants that are planted in the spring and senesce and dry down in the field in the fall. Because they are grown in monocultures, all of the plants grown in one field have the same physical structure.

The simplification and homogenization of the landscape that has occurred in agricultural regions such as Iowa has resulted in a wide array of environmental problems. Because corn and soybean are summer annuals, there is very little active plant growth on the landscape

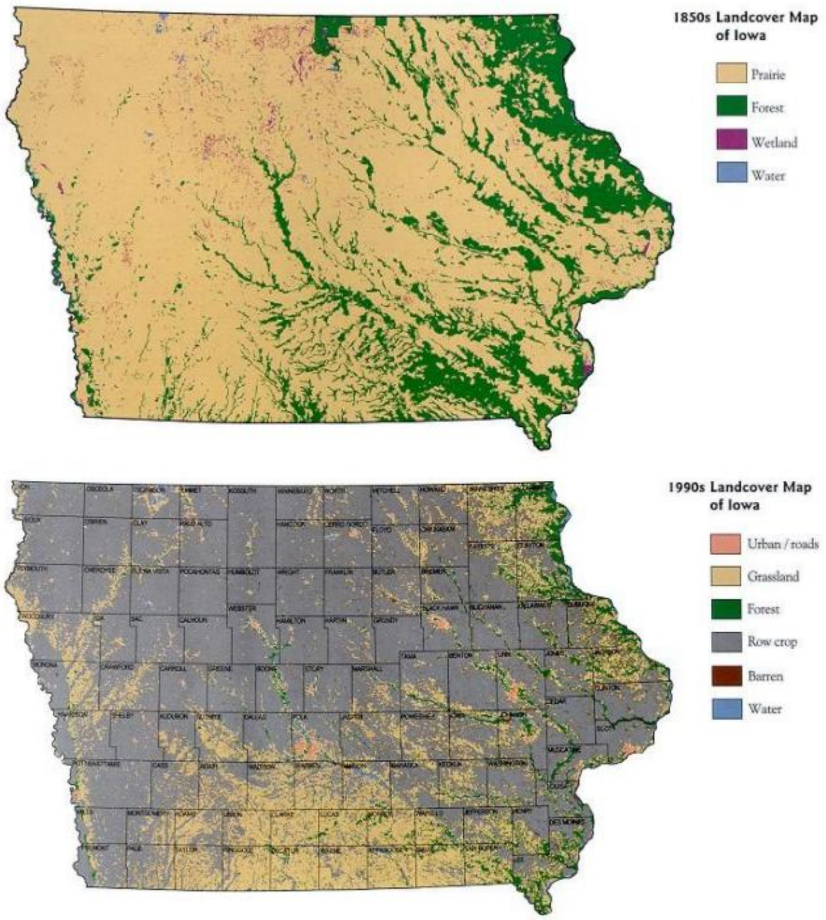

Figure 1. Landcover maps of Iowa from the 1850s (top) and 1990s (bottom). Images courtesy of the Iowa Department of Natural Resources.

from early fall through late spring, which increases the potential for nitrogen pollution and soil erosion including the associated phosphorus pollution (Larson et al. 1983, Schilling and Spooner 2006, Heggenstaller et al. 2009a). The extensive use of subsurface drainage systems to improve crop growth further increases nitrogen pollution (Sugg 2007). Annual plants also produce fewer roots than perennial plants (Glover et al. 2010) and soil tillage is often used when growing annual crops. Both of these factors decrease soil quality by reducing the amount of organic matter in the soil (McLauchlan 2006, Piñiero et al. 2009, but see Dolan et al. 2006). The absence of plant cover over winter and the homogeneous structure of the plant canopies reduces the amount of animal habitat compared to more diverse land covers (Best et al. 2001, Swift et al. 2004, Berges et al. 2010). Furthermore, maintaining monocultures is difficult and requires the use of biocides (i.e. herbicides and insecticides) to eliminate unwanted species, which impacts species beyond the target weeds and pests (Lewis et al. 1997).

Prairies can provide a wide range of ecosystem services and can help to ameliorate some of the environmental damage resulting from annual crop production. The deep, living roots of prairie plants and the continuous soil cover that they provide reduce soil erosion and nitrogen and phosphorus pollution (Lee et al. 1999, Tomer et al. 2012, Helmers et al. 2012). Prairies produce a large quantity of roots that are more recalcitrant to decomposition than corn and soybean which increases soil organic carbon concentrations and the potential of prairies to sequester carbon (Johnson et al. 2007, Fornara et al. 2009, Piñiero et al. 2009). Prairies provide habitat to a variety of animals including game animals, songbirds, and beneficial insects (Best et al. 1995, Fargione et al. 2009, Gardiner et al. 2010).

The whole landscape does not need to be returned to prairie vegetation, however, in order to reap the environmental benefits provided by prairies. Strategic placement of small areas of prairie into the landscape can have disproportionate benefits (Schulte et al. 2008, Liebman et al. *in press*). For example, converting 10-20% of a watershed used for corn-soybean production to prairie vegetation has been found to reduce sediment losses due to water erosion by 95% compared to similar watersheds with 100% corn-soybean production (Helmers et al. 2012).

Growing Prairies for Bioenergy Production

There is great interest in the U.S. in expanding renewable energy production, including producing energy from plant biomass. In 2007 the U.S. Congress passed the Renewable Fuels Standard in the Energy Independence and Security Act, which mandates that the U.S. will produce 136 billion liters of biofuels per year by 2022, of which 58% must be "advanced biofuels" including cellulosic biofuels (Figure 2) (U.S. Congress 2007). Direct combustion and electricity generation from biomass is also being studied and implemented on local scales. At the edge of the Minneapolis-St. Paul metropolitan area, for example, there is a

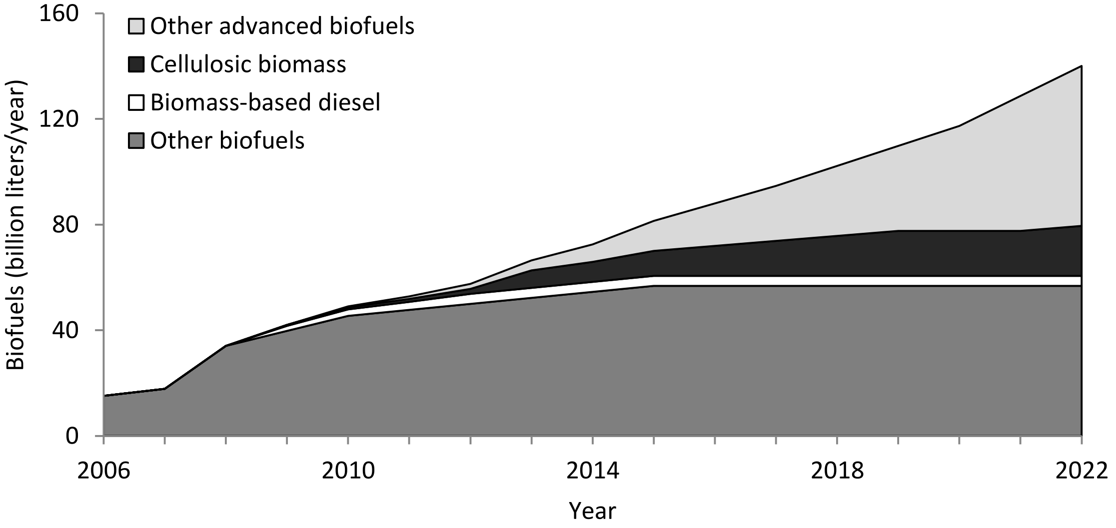

Figure 2. Biofuel production mandates from 2006 to 2022 as established by the Energy Independence and Security Act of 2007. Only the "other biofuels" category can include ethanol from corn grain.

pilot project that is attempting to generate 30% of the energy from a power plant from perennial plant biomass (Jordan and Warner 2010).

Bioenergy production from plant biomass may either exacerbate current environmental degradation caused by agricultural production or may be a means of enhancing environmental quality. Bioenergy production is likely to lead to increased environmental degradation if it results in more land being put into agricultural production either through removal of land from conservation programs (Secchi et al. 2009) or indirect land use changes as existing cropland is used to produce bioenergy rather than food or feed (Fargione et al. 2008, Searchinger et al. 2008). Intensifying production activities on existing land, such as increasing nutrient inputs or removing all crop residues, may also exacerbate current environmental damage (Tilman et al. 2009). Alternatively, environmental quality might be enhanced by bioenergy production if perennial plants such as prairies are used.

Managing prairies for the production of any particular ecosystem service, such as biomass production, is likely to affect the ecology of the systems. Two important management practices that are likely to occur in prairies managed for bioenergy production are an annual harvest at the end of the growing season and fertilization (Mitchell et al. 2008, U.S. DOE 2011). Harvesting biomass after plants have senesced results in the harvest of relatively dry biomass that is nutrient poor due to translocation and leaching of nutrients to the roots and soil, respectively (Parrish and Fike 2005, Adler et al. 2006). The effects of harvesting prairie biomass for bioenergy has not been studied as extensively as other forms of prairie disturbance such as burning, haying, and grazing, all of which are known to affect prairie composition (Howe 1994, Collins et al. 1998). Fertilization, particularly nitrogen fertilization, has been recommended in prairies managed for bioenergy production in order to increase the aboveground biomass production (Parrish and Fike 2005, Heggenstaller et al. 2009b). Nitrogen fertilization, however, often reduces species diversity (Suding et al. 2005, Michalet et al. 2006) and shifts species compositions to those species that are better competitors for light rather than soil resources (Tilman 1988). If prairies are used for bioenergy production, research is needed to examine the effects of these management regimes on the ecology of the prairies and the suitability of the prairie biomass to be used as a bioenergy feedstock.

Because reincorporating prairies back onto the landscape in states such as Iowa is likely to have significant environmental benefits and there currently is political and social willpower to increase bioenergy – particularly biofuel – production, there is a need to examine the feasibility of growing prairies for bioenergy production so that environmentally-beneficial bioenergy feedstocks are considered in addition to other feedstocks, such as corn, that are likely to further damage the environment (Donner and Kucharik 2008). The overarching goal of my research, therefore, was to examine how managing prairies for bioenergy production affected the ecology and agronomic production of the prairies. I specifically focused on the feedbacks between prairie diversity and nitrogen fertilization and their effects on productivity. I examined two forms of prairie diversity: species diversity and functional-group diversity. Functional-groups in prairies are groups of plants that differ in their phenology, nutrient-use strategies and sources, and growth forms (Craine et al. 2002).

There are four broadly-defined prairie functional groups: cool-season (C_3) grasses, warm-season (C_4) grasses, leguminous forbs, and non-leguminous forbs (Kindscher and Wells, 1995; Craine et al., 2002). In addition to studying managed prairie systems, I studied corn systems so that I could directly compare prairie and corn systems.

Dissertation Organization

My research is presented in four chapters that are based on the work from two field experiments: the Comparison of Biofuel Systems (COBS) experiment and the Diversity and Nitrogen (DIVN) experiment. The COBS experiment compares the agronomic performance and ecosystem services provided by corn and prairie biomass cropping systems. There are five cropping systems being examined at COBS: (1) corn-soybean rotation with grain removal, (2) continuous corn with grain and stover removal, (3) continuous corn with a rye cover crop with grain and stover removal, (4) prairie with annual nitrogen fertilization with biomass removal, and (5) unfertilized prairie with biomass removal. The DIVN experiment examines the relationships among prairie functional-group identity, nitrogen fertilization, and above- and belowground growth and productivity. The DIVN experiment contains prairie species representing different functional groups – C_3 grasses, C_4 grasses, legumes, and multi-functional group mixtures – grown with and without nitrogen fertilizer, as well as fertilized corn.

In Chapter 2, I describe a study from the COBS experiment titled “Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy” in which we tested the hypothesis that nitrogen fertilization would increase the aboveground productivity of prairies managed for bioenergy production but would decrease diversity. We tested our hypothesis by measuring aboveground biomass production and characterizing plant community composition, including species and functional-group diversity, in fertilized and unfertilized prairies that were harvested annually late in the growing season.

In Chapter 3, I describe a study from the DIVN experiment titled “Nutrient enrichment reduces complementarity and increases priority effects in prairies managed for bioenergy” in which we sought to answer two questions about the growth and resource capture of prairies managed for bioenergy production and other ecosystem services. First, how do the resource capture and growth dynamics of prairie functional groups differ over the growing season, and are those dynamics altered by spring nitrogen fertilization? Second, do complementarity and/or priority effects occur in fertilized and unfertilized functionally-diverse prairies?

In Chapter 4, I describe a second study from the DIVN experiment titled “Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production” in which we compared contrasting prairie functional groups, grown with and without nitrogen fertilization, with corn with respect to harvestable biofuel feedstock production, nutrient allocation, and root production. We hypothesized (1) that nitrogen fertilization would increase prairie productivity and nutrient content, and (2) that fertilized prairie species would produce similar amounts of total biomass as corn but would allocate more biomass and nutrients to roots.

In Chapter 5, I describe a third study from the DIVN experiment titled "Functional group and fertilization affect the composition and bioenergy yields of prairie plants." The primary objective of this study was to determine whether functional-group identity and/or nitrogen fertilization affected the feedstock characteristics and bioenergy yields of prairie plants. Our secondary objective was to compare the prairie feedstock characteristics and bioenergy yields to those of corn stover.

In Chapter 6, I highlight some of the main results from the studies and present considerations for future research.

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CHAPTER 2. NITROGEN FERTILIZATION INCREASES DIVERSITY AND PRODUCTIVITY OF PRAIRIE COMMUNITIES USED FOR BIOENERGY

A paper published in *Global Change Biology Bioenergy*

Meghann E. Jarchow[1] and Matt Liebman[2]

Abstract

The use of prairie biomass as a renewable source of energy may constitute an important opportunity to improve the environmental sustainability of managed land. To date, assessments of the feasibility of using prairies for bioenergy production have focused on marginal areas with low yield potential. Growing prairies on more fertile soil or with moderate levels of fertilization may be an effective means of increasing yields, but increased fertility often reduces plant community diversity. At a fertile site in central Iowa with high production potential, we tested the hypothesis that nitrogen fertilization would increase aboveground biomass production but would decrease diversity of prairies managed for bioenergy production. Over a three-year period, we measured aboveground biomass production after plant senescence and species and functional-group diversity in June and August for multispecies mixtures of prairie plants that received no fertilizer or 84 kg N ha^{-1} year^{-1}. We found that nitrogen fertilization increased aboveground biomass production, but with or without fertilization, the prairies produced a substantial amount of biomass: averaging 12.2 and 9.1 Mg ha^{-1} in fertilized and unfertilized prairies, respectively.

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[1] Primary researcher and author
[2] Provided input at all stages and supervised the work

The unfertilized prairie had higher species diversity in June, whereas the fertilized prairie had higher species diversity in August at the end of the study period. Functional-group diversity was almost always higher in the fertilized prairie. Composition of the unfertilized prairie was characterized by C_4 grasses and legumes, whereas the fertilized prairie was characterized by C_3 grasses and forbs. Although most research has found that nitrogen fertilization reduces prairie diversity, our results indicate that early-spring nitrogen fertilization, when used in combination with a post-senescence annual harvest, may increase prairie diversity. Managing prairies for bioenergy production, including the judicious use of nitrogen fertilization, may be an effective means of increasing the amount of saleable products from managed lands while also increasing plant diversity.

Introduction

The use of perennial plant materials as a renewable source of energy may constitute an important opportunity to improve the environmental sustainability of managed land (Tilman *et al.* 2009). Currently, the production of energy from agricultural products is primarily in the form of ethanol from corn grain in the United States, which used more than 45% of the domestic corn crop in 2011 (USDA ERS 2012). Concomitantly, using corn grain to produce ethanol has promoted landscape simplification and homogenization through conversion of Conservation Reserve Program grasslands to annual row crops (Secchi *et al.* 2009), and has been implicated in increasing environmental damage, such as increased nitrate leaching into water bodies (Donner & Kucharik 2008) and increased rates of soil erosion (Cox *et al.* 2011). Collection and conversion of corn stalks and leaves (i.e. stover) and other grain-crop residues could foster the production of lignocellulosic-based biofuels without displacing food and feed crop production (U.S.DOE 2011), but this practice may be undesirable in many situations because of its potential to degrade soil carbon stocks, fertility, and long-term productivity (Linden *et al.* 2000, Wilhelm *et al.* 2007, Blanco-Canqui & Lal 2009, Blanco-Canqui 2010).

In contrast, perennial prairie vegetation has the potential to be used as a bioenergy feedstock that produces a substantial amount of biomass as well as numerous ecosystem services. Although creating and managing prairies for bioenergy production is likely to result in prairie systems that differ from native or restored prairies in terms of plant community composition, these created prairies would provide numerous ecosystem services including more habitat for wildlife (Fargione *et al.* 2009) and beneficial insects (Gardiner *et al.* 2010) and decreased nitrogen, phosphorus, and sediment movement into water bodies (Helmers *et al.* 2012). Placement of small areas of these prairie systems at strategic locations in landscapes dominated by annual crops may be especially important for maintaining agricultural productivity in the face of emerging soil conservation challenges related to a higher frequency of high intensity precipitation events (National Assessment Synthesis Team 2001, Angel *et al.* 2005, Helmers *et al. in review*) and from new crop pests, such as the soybean aphid (*Aphis glycines* Matsumura) (Gardiner *et al.* 2009).

Assessments of the feasibility of using prairie plant biomass as a bioenergy feedstock, such as the U.S. Department of Energy’s “U.S. Billion-Ton Update,” have focused on relatively low-yielding unfertilized prairie systems on marginal cropland, including the sand prairies at

Cedar Creek Ecosystem Science Reserve (U.S. DOE 2011). Due to the low yields of the prairie systems analyzed, the authors of the "U.S. Billion-Ton Update" suggested that fertilized monocultures of switchgrass (*Panicum virgatum* L.) might be a more suitable bioenergy feedstock due to greater yields in the central Great Plains (U.S. DOE 2011). Using switchgrass as a bioenergy feedstock could provide a variety of ecosystem services not provided by annual row crops, including reduced nutrient pollution and soil erosion (Kort *et al.* 1998, Parrish & Fike 2005), but would not provide the increase in native plant and animal diversity that may be desirable for more sustainable bioenergy production (Robertson *et al.* 2008).

Diversity can foster increases in the productivity of prairie communities. In a meta-analysis examining diversity-productivity relationships, diversity increased plant productivity in 79% of the studies reviewed, had no effect in 21% of the studies, and was never found to decrease productivity (Cardinale *et al.* 2007). However, in managed systems, including those used for bioenergy feedstock production, it is desirable for diversity to increase productivity above the yield of the most productive species (i.e. transgressive overyielding), which occurred in 12% of the cases examined by Cardinale *et al.* (2007). In the remaining studies reviewed by Cardinale *et al.* (2007), yield of the most diverse systems did not differ from the most productive monoculture in 63% of cases and more diverse systems produced less biomass than the most productive monoculture in 25% of cases.

Fertilization, particularly nitrogen fertilization, is generally effective at increasing aboveground biomass production in most ecosystems, including prairies (Reich *et al.* 2003, Suding *et al.* 2005, LeBauer & Treseder 2008), but often reduces diversity (Suding *et al.* 2005, Michalet *et al.* 2006) and shifts species compositions to those species that are better competitors for light rather than soil resources (Tilman 1988). When grown in monocultures in nutrient-poor soil, relative growth rates of non-leguminous prairie seedlings were stimulated by nitrogen fertilization, with forbs having the strongest response followed by C_3 grasses then C_4 grasses, and legumes not consistently stimulated by nitrogen fertilization (Reich *et al.* 2003). It may be expected, therefore, that forbs and C_3 grasses would become more abundant in prairies with nitrogen fertilization. C_3 grasses, particularly exotic C_3 grasses, often become dominant in fertilized prairies and grasslands (Wedin & Tilman 1996, Suding *et al.* 2005, Foster *et al.* 2009, Pan *et al.* 2010). Forbs are often a more minor component of reconstructed prairies whether fertilized or not (Baer *et al.* 2002, Polley *et al.* 2005, Foster *et al.* 2009, Pan *et al.* 2010), and legumes become less abundant with nitrogen fertilization (Jarchow & Liebman 2012).

Shifts in the species composition of prairie communities can affect both bioenergy feedstock suitability and ecosystem services that are provided. The stiff and nutrient-poor stems of C_4 grasses have been found to be effective at reducing sediment, nitrogen, and phosphorus losses through surface runoff when incorporated into riparian buffers (Lee *et al.* 1999) and have high theoretical maximum ethanol yields (Jarchow *et al. in press*). C_3 grasses begin growth early in the spring (Jarchow & Liebman 2012) when the potential for nitrate leaching is highest (Heggenstaller *et al.* 2009), but have relatively low higher heating values and high ash concentrations (Jarchow *et al. in press*), making them less desirable as bioenergy feedstocks. Forbs, including legumes, are important sources of food for wildlife and beneficial insects (Fiedler *et al.* 2008, Fargione *et al.* 2009).

In this study we tested the hypothesis that nitrogen fertilization would increase the aboveground productivity of prairie systems managed for bioenergy production but would decrease diversity. We tested our hypothesis by measuring aboveground biomass production and characterizing plant community composition, including species and functional-group diversity, in fertilized and unfertilized prairie systems that were harvested annually late in the growing season.

Methods

Study site and experimental design

We conducted the study in Boone County, IA, on the Iowa State University Agronomy and Agricultural Engineering Research Farm (41°55'N, 93°45'W). Soils at the site were primarily Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). The 60-year mean growing season (April through November) precipitation near the site was 72 cm. Growing season precipitation in 2008, 2009, 2010, and 2011 was 115 cm, 75 cm, 116 cm, and 61 cm, respectively. Late-spring to early-summer flooding occurred in 2008, and summer flooding occurred in 2010.

Prior to initiation of the field experiment in 2008 the site was used for corn and soybean production and was planted with soybean in 2007. Soil sampling to 15 cm in November 2007 indicated mean soil pH was 6.7, mean organic matter concentration (via dry combustion analysis with a conversion factor of 1.724 from total carbon to organic matter [Schumacher 2002]) was 51 g kg^{-1}, mean extractable phosphorus concentration (via Bray-1 procedure) was 11 mg kg^{-1}, and mean extractable potassium (via Mehlich-3 procedure) was 141 mg kg^{-1}. Because the prairie treatments discussed here were components of a larger bioenergy cropping-systems experiment, phosphorus and potassium were added in May 2008 to all treatments including the prairie treatments to ensure that sufficient phosphorus and potassium were available for annual-crop growth. Phosphorus was added at a rate of 78 kg P_2O_5 ha^{-1} (34 kg P ha^{-1}). Potassium was added at a rate of 146 kg K_2O ha^{-1} (122 kg K ha^{-1}).

Plots were 27 m x 61 m and were arranged as a randomized complete block design with four replicates. Prairie treatments comprised a multispecies mixture that received no fertilizer or the same mixture fertilized with 84 kg N ha^{-1} $year^{-1}$. Both prairie treatments were seeded with the same custom seed mix obtained from Prairie Moon Nursery (Table 1) (Winona, MN), which contained 31 species, including C_3 and C_4 grasses and leguminous and non-leguminous forbs (hereafter legumes and forbs, respectively). All species were perennial and were sourced from within 240 km of Boone County, IA. The composition of the seed mix by weight was 12% C_3 grasses, 56% C_4 grasses, 8% legumes, and 24% forbs. The prairies were seeded on 19 May 2008 with a Truax® drill (FLEXII Series Grass Drill, Truax

Company, Inc., New Hope, MN). The fertilized prairie treatment received no fertilizer in the first year because we expected weeds to respond more strongly than prairie plants to fertilization during the establishment phase. Both prairie treatments were mowed on 14 July and 7 August 2008 for weed control and were not harvested for biomass in 2008. Nitrogen fertilizer was applied to the fertilized prairie treatment as ammonium nitrate (34% N) on 17 April 2009 and 29 March 2010, and as urea-ammonium nitrate (32% N) on 11 April 2011.

Data collection and analyses

Aboveground biomass was harvested after plant senescence in mid-October in 2009-2011. Biomass was first harvested with a self-propelled forage harvester (John Deere model 5830, John Deere Co., Moline, IL) to a cut height of between 8 cm and 20 cm. Fresh weights of the biomass removed from the plots were determined in the field. A subsample of biomass from each plot was then taken for moisture determination, dried at 60°C for at least 2 days, and weighed. The remaining aboveground biomass was determined by harvesting 4 - 0.28 m^2 samples to ground height, drying the samples at 60°C for at least 4 days, and weighing the samples. Biomass values for each experimental unit were calculated on the basis of summing machine- and hand-harvested samples.

To determine the composition of the fertilized and unfertilized prairies, the point intercept method (Jonasson 1988) was used in mid-June and mid-August of 2009, 2010, and 2011. Eight 1-m^2 areas were sampled per plot at each sampling period. Two samples, with randomly determined locations that excluded the exterior 2 m of the plot, were taken in each of the four quadrants of the plot. At each sampling location, a pin was dropped 12 times for a total of 96 pin drops per plot. With each pin drop, we recorded the species identity and number of hits of all living plant tissue. Senesced plant tissue was not recorded. Plant cover was determined by dividing the number of hits by the total number of pin drops.

We used Simpson's diversity index, Simpson's evenness index, and species number per plot to characterize prairie species diversity. We also used Simpson's diversity index to characterize prairie functional-group diversity, which we divided into C_3 grasses, C_4 grasses, legumes, and forbs. We used Simpson's diversity index because it is less sensitive to rare species than other indices such as Shannon's diversity index. Of the 58 species found in the plots, only 15 species had percent covers greater than 1% at any of the sampling periods (Table 2), indicating that the remaining 48 species had relatively small effects on the functioning of the prairie treatments. Trees were excluded from our functional-group analysis, even though we found cottonwood (*Populus deltoides* Bartram ex Marsh.) seedlings in both the unfertilized and fertilized prairie plots, because they represented less than 0.1% plant cover on all sampling dates. Simpson's diversity (1/D) was calculated as the inverse of $D = \sum p_i^2$ where p_i is the proportional abundance of the i^{th} species or functional group. Simpson's evenness was calculated by dividing Simpson's species diversity index by the species number.

We analyzed the aboveground biomass using repeated-measures analysis of variance that assessed differences among years with a compound symmetry covariance model (SAS Institute 2011). Species and functional-group diversity, species evenness, and species number were analyzed with repeated-measures ANOVA using an unstructured covariance model that accounted for the two levels of repeated measures (i.e. sampling twice per year over three years). Pairwise comparisons between unfertilized and fertilized prairie treatments were performed using contrasts. All values were considered significant at $P \leq 0.05$. Change in species diversity, evenness, and richness within the unfertilized and fertilized prairies from June 2009 to August 2011 were assessed using regression analyses that included a first-order autoregressive (AR(1)) covariance model (SAS Institute 2011). We used non-metric multidimensional scaling (NMS) to visualize the species compositions with Bray-Curtis distance measures in PC-ORD (McCune & Mefford 2011). Data were square root transformed before NMS analysis to reduce the effects of the dominant species, which can have disproportionate effects without transformation (Faith *et al.* 1987).

Results

The unfertilized prairie treatment produced 10.5, 9.6, and 7.2 Mg ha^{-1} of aboveground biomass in 2009, 2010, and 2011, respectively, whereas the fertilized prairie treatment produced 13.0, 13.9, and 9.7 Mg ha^{-1} of aboveground biomass in those years (Figure 1). Thus, the fertilized prairie produced 24%, 44%, and 34% more aboveground biomass than the unfertilized prairie in 2009, 2010, and 2011, respectively ($P_{treatment} < 0.0001$, $P_{year} < 0.0001$, $P_{treatment*year} = 0.008$).

Nitrogen fertilization had more complex effects on prairie diversity than on aboveground biomass production. Species richness was generally higher in the unfertilized prairie than the fertilized prairie ($P_{treatment} = 0.0005$), but the differences between treatments varied over the sampling period ($P_{date} = 0.061$, $P_{year} = 0.02$, $P_{treatment*date} = 0.0009$, $P_{treatment*year} = 0.18$, $P_{treatment*date*year} = 0.36$) (Figure 2a). Species richness was always higher in the unfertilized prairie than fertilized prairie in August of all years, and was also higher in June 2011. Regressions of species richness from June 2009 to August 2011 indicated that there was no increase in richness for either the unfertilized (P = 0.14) or fertilized (P = 0.36) prairies over the course of the study. Simpson's evenness differed between treatments ($P_{treatment} = 0.03$), between the June and August sampling dates ($P_{date} = 0.001$, $P_{treatment*date} < 0.0001$), and among the three years of the study ($P_{year} = 0.03$, $P_{treatment*year} = 0.14$) (Figure 2b). There was no difference in species evenness between treatments in June (P = 0.20). In August, however, species evenness was higher in the fertilized prairie (P = 0.03). Species evenness decreased from June 2009 to August 2011 in the unfertilized prairie (P < 0.0001), but showed no trend in the fertilized prairies (P = 0.96).

Because species diversity is a combination of species richness and evenness, diversity differed among the treatments (P = 0.007), but there were also strong interaction effects over the course of the study ($P_{date} = 0.01$, $P_{year} = 0.006$, $P_{treatment*date} = 0.0006$, $P_{treatment*year} = 0.002$, $P_{treatment*date*year} = 0.006$). Species diversity was always higher in the unfertilized prairie than the fertilized prairie in June (Figure 2c). In August, however, species diversity was higher in the unfertilized prairie in 2009 but became higher in the fertilized prairie by

2011 (Figure 2c). Species diversity in the unfertilized prairies decreased over the study period ($P < 0.0001$), whereas species diversity did not exhibit consistent trend in the fertilized prairies ($P = 0.58$). Only fifteen species had percent covers greater than 1% at any point during the study period, and therefore were most important in affecting prairie diversity (Table 2).

Prairie community composition was strongly affected by nitrogen fertilization (Figure 3). The three axes of the NMS ordination, which had a stress value of 6.3, separated out important changes in the prairie communities due to the effects of nitrogen fertilization, sampling date, and year. Axis 1 separated the data primarily based on the abundance of forbs (to the left) and some C_4 grasses and legumes (to the right) (Figure 3a). The unfertilized prairie samples were clustered in the center and right on Axis 1 due to the greater abundance of C_4 grasses and legumes. The fertilized prairie samples spanned Axis 1, and moved from right to left from 2009 to 2011 as the prairie became more dominated by forbs such as oxyeye sunflower (*Heliopsis helianthoides* (L.) Sweet) and bergamot (*Monarda fistulosa* L.).

The abundance of Canada wildrye (*Elymus canadensis* L.), a C_3 grass, was the vector most important for separating samples on Axis 2, and was negatively associated with Canadian milkvetch (*Astragalus canadensis* L.), a legume. The unfertilized prairie generally had lower abundances of Canada wildrye than the fertilized prairie and higher abundances of Canadian milkvetch. The abundance of Canada wildrye was generally higher in June than August and generally decreased from 2009 to 2011 for both the unfertilized and fertilized prairies (Figure 3a). The presence of legumes, including roundhead lespedeza (*Lespedeza capitata* Michx.), purple prairie clover (*Dalea purpurea* Vent.), and showy ticktrefoil (*Desmodium canadense* (L.) DC.), had a strong effect between Axes 1 and 2 (to the lower left), and the unfertilized prairie samples were clustered in this region (Figure 3a).

Axis 3 separated the June from the August samples based on plants that were most abundant early (to the top) or late (to the bottom) in the growing season (Figure 3a,b). The presence of big bluestem (*Andropogon gerardii* Vitman), a C_4 grass, was most strongly associated with the samples in August.

As demonstrated by the NMS ordination, the functional-group composition of the unfertilized and fertilized prairies differed greatly ($P_{treatment} = 0.0002$), and there were strong interactive effects over the course of the study ($P_{date} = 0.07$, $P_{year} = 0.01$, $P_{treatment*date} = 0.003$, $P_{treatment*year} < 0.0001$, $P_{treatment*date*year} = 0.001$) (Figure 4). Functional-group diversity, in contrast to species diversity, was higher in the fertilized prairie than unfertilized prairie for all dates except June 2010 (Figure 5). Functional-group evenness was lower in the unfertilized prairie because they consistently were dominated by C_4 grasses (Figure 4). In the unfertilized prairie, C_4 grasses comprised 62%, 57%, and 69% of the vegetation cover in June 2009, 2010, and 2011, respectively, and 70%, 77%, and 77% of the cover in August of those years. The fertilized prairie had more equal abundances among C_3 grasses, C_4 grasses, and forbs, although legumes comprised less than 1% of the fertilized prairie throughout the study period (Figure 4). By 2011 the composition of the fertilized prairie treatment was 51%

C_3 grasses, 24% C_4 grasses, and 25% forbs in June, and 31% C_3 grasses, 33% C_4 grasses, and 35% forbs in August.

Discussion

As we expected, aboveground biomass production was higher in fertilized prairies. Nitrogen fertilization increased aboveground biomass production by 24% to 44%, which is within the range of stimulation due to fertilization found in other studies. Turner *et al.* (1997) reported that in native tallgrass prairie in eastern Kansas, fertilization at a rate of 100 kg N ha^{-1} $year^{-1}$ increased aboveground biomass 57% in annually burned sites, which were more nitrogen limited, and 15% in unburned sites, which were less nitrogen limited. Nitrogen fertilization at 100 kg N ha^{-1} $year^{-1}$ increased aboveground biomass production by >50% in 1 to 3 year-old reconstructed prairies in southern Minnesota, and by ~40% in prairies older than 3 years (Camill *et al.* 2004).

With or without nitrogen fertilization, the prairies grown in the present study produced a substantial amount of aboveground biomass, with a mean of 12.2 Mg ha^{-1} for the fertilized treatment and a mean of 9.1 Mg ha^{-1} for the unfertilized treatment. Therefore, the fertilized prairie observed in the present study produced substantially more aboveground biomass than the perennial systems referred to in the “U.S. Billion-Ton Update”: more than three times that of sand prairies (3.9 Mg ha^{-1}), more than double that of ‘managed native prairie’ (5.6 Mg ha^{-1}), and 9% more than the upland switchgrass forage variety ‘Shawnee’ (11.2 Mg ha^{-1}) (U.S. DOE 2011). The unfertilized prairie observed in our study, while producing less aboveground biomass than the fertilized prairie, still produced considerably more than the estimates of prairie biomass used in the "U.S. Billion-Ton Update". Our results suggest that prairies have the potential to be a more productive biofuel feedstock than previously stated.

Nitrogen fertilization, in contrast to our hypothesis and the findings of other researchers, did not consistently reduce prairie diversity. The unfertilized prairie had higher species diversity than the fertilized prairie in June due to small increases in both species richness and evenness. Species diversity was higher in the fertilized prairie in August 2011, however, due to greater evenness. Functional-group diversity was also almost always higher in the fertilized prairie than the unfertilized prairie in both June and August due to increased functional-group evenness.

A decline in species diversity with nitrogen fertilization is often associated with increased dominance by C_3 grass species, often exotics, under a variety of management regimes, including annual burning, annual mowing, and no aboveground biomass removal (Wedin & Tilman 1996, Suding *et al.* 2005, Pan *et al.* 2010). We observed very low (< 0.1%) abundance of exotic C_3 grasses, and although native C_3 grasses were more dominant in the fertilized than unfertilized prairie in our study, C_3 grass abundance did not increase and generally decreased as the study progressed. Forbs, such as oxeye sunflower and bergamot, became more abundant in the fertilized prairie, which is also in contrast to other studies, which have found forb abundance to either remain constant or decrease with nitrogen fertilization (Patrick *et al.* 2008, Pan *et al.* 2010).

The timing of the application of nitrogen fertilizer is likely to influence its effects on species and functional-group diversity. In managing our prairies for bioenergy production, we applied nitrogen in a single dose in early spring. The timing of nitrogen fertilization varied among other studies, including multiple applications during the growing season (Wedin & Tilman 1996) and application as a slow-release fertilizer (Patrick *et al.* 2008, Pan *et al.* 2010), but consistently resulted in decreased diversity.

The marked increase in forb abundance with nitrogen fertilization in prairies managed with an annual, post-senescence harvest is a unique aspect of our results relative to other studies. We hypothesize that the combination of management strategies used here resulted in increased forb abundance. Spring nitrogen fertilization stimulated C_3 grass growth, which accelerated its phenology and also reduced the growth of C_4 grasses (Jarchow & Liebman 2012). Because the prairies were not harvested within the growing season, the fertilized C_3 grasses flowered and began senescing earlier in the growing season than unfertilized C_3 grasses, as evidenced by their decreased abundance in August compared to June (Figure 4). The forbs may have had sufficient light resources later in the growing season to become better established in the fertilized prairie and therefore become more abundant over the course of the study, which has been found to be an important factor in enhancing forb abundance in other prairies (Williams *et al.* 2007). Alternatively, the native C_3 grasses used in this study may be less aggressive than the exotic C_3 grasses that become dominant in other prairies (Martin *et al.* 2005). Additional research examining the probable interactive effects of nitrogen fertilization and the timing of biomass harvest on prairie composition is necessary.

Prairie community composition is not static. Compositional changes have been found to be greatest in the first year of prairie establishment, however, and become much smaller after 3 years (Camill *et al.* 2004). We have presented data from years 2 through 4 after prairie establishment. We recognize that long-monitoring of the effects of nitrogen fertilization and annual harvest on the prairie systems described here is necessary to more completely assess community dynamics. Furthermore, additional research is needed to determine if similar results are found for prairies that are seeded into sites, such as old fields, that have greater perennial weed seed and bud banks; for prairies that are seeded with a different seed mix; and for established prairies that are subsequently fertilized.

Nonetheless, most research has found that nitrogen fertilization reduces prairie diversity, but we observed that early-spring nitrogen fertilization, when used in combination with a post-senescence annual harvest, may increase prairie diversity by increasing species and functional-group evenness. Furthermore, we have found that fertilized prairie can produce a substantial amount of aboveground biomass, similar to the productivity of monocultures of fertilized switchgrass. Consequently, we believe that managing prairies for bioenergy production, including the judicious use of nitrogen fertilization, may be an effective means of increasing the amount of saleable biomass from managed lands while also increasing plant diversity.

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Table 1. Species list, functional group identity, Iowa coefficient of conservatism, and seeding rates of prairie seeding mix. The same mix was used for the unfertilized and fertilized prairies.

Species		Functional group	Iowa coefficient of conservatism	Seeding rate^{1,2}	
Latin name	**Common name**			Seeds m^{-2}	% by weight
Amorpha canescens	Lead plant	Legume	8	8	1.0
Andropogon gerardii	Big bluestem	C$_4$ grass	4	99	20.0
Asclepias tuberosa	Butterfly milkweed	Forb	6	5	2.5
Aster novae-angliae	New England aster	Forb	3	16	0.5
Astragalus canadensis	Canadian milkvetch	Legume	4	4	0.5
Baptisia leucantha	White wild indigo	Legume	6	2	2.0
Dalea purpurea	Purple prairie clover	Legume	8	19	2.5
Desmanthus illinoensis	Illinois bundleflower	Legume	4	3	1.5
Desmodium canadense	Showy ticktrefoil	Legume	6	4	1.5
Echinacea pallida	Purple prairie coneflower	Forb	7	8	3.0
Elymus canadensis	Canada wildrye	C$_3$ grass	5	31	12.0
Eryngium yuccifolium	Rattlesnake master	Forb	8	6	1.5
Helianthus laetiflorus	Showy sunflower	Forb	na	5	0.7
Helianthus maximilliani	Maxmillian’s sunflower	Forb	4	5	0.7
Heliopsis helianthoides	Early sunflower	Forb	4	6	2.0
Lespedeza capitata	Round-head lespedeza	Legume	3	8	2.0
Liatris pychostachya	Prairie blazing star	Forb	6	8	1.5
Monarda fistulosa	Wild bergamot	Forb	2	26	0.8
Panicum virgatum	Switchgrass	C$_4$ grass	5	55	8.0
Pycnanthemum virginianum	Common mountain mint	Forb	4	33	0.3
Ratibida pinnata	Yellow coneflower	Forb	4	22	1.5
Rudbeckia hirta	Black-eyed Susan	Forb	2	46	1.0
Schizachyrium scoparium	Little bluestem	C$_4$ grass	5	59	8.0
Silphium integrifolium	Rosin weed	Forb	4	0.3	0.5
Silphium lactiniatum	Compass plant	Forb	7	0.5	1.5
Solidago rigida	Stiff goldenrod	Forb	4	20	1.0
Sorghastrum nutans	Indiangrass	C$_4$ grass	4	119	20.0
Sporobolus heterolepis	Prairie dropseed	C$_4$ grass	9	4	0.5
Tradescantia ohiensis	Ohio spiderwort	Forb	4	6	1.5
Vernonia fasciculate	Common ironweed	Forb	1	9	0.8
Veronicastrum virginicum	Culver’s root	Forb	5	8	0.2

[1] Estimated seeding rate

[2] Grass seed weights are presented as pure live seed (PLS) amounts

Table 2. Mean percent cover (SE) of all species with percent covers ≥ 1% on any sampling date from June 2009 through August 2011 for the unfertilized and fertilized prairies. Species codes used are: ANGE *Andropogon gerardii*, DAPU *Dalea purpurea*, DEIL *Desmanthus illinoensis*, DECA *Desmodium canadense*, ELCA *Elymus canadensis*, HELA *Helianthus laetiflorus*, HEMA *Helianthus maximiliani*, HEHE *Heliopsis helianthoides*, MOFI *Monarda fistulosa*, PAVI *Panicum virgatum*, POPR *Poa pratensis*, RAPI *Ratibida pinnata*, RUHI *Rudbeckia hirta*, SONU *Sorghastrum nutans*, and VEFA *Vernonia fasciculata*.

	Species														
	ANGE	DAPU	DEIL	DECA	ELCA	HELA	HEMA	HEHE	MOFI	PAVI	POPR	RAPI	RUHI	SONU	VEFA
June 2009															
Unfertilized	20.4(4.6)	1.5(0.5)	0.4(0.4)	0.6(0.2)	23.8(1.2)	1.5(0.3)	0.0(0.0)	0.4(0.1)	0.3(0.1)	25.7(1.8)	0.0(0.0)	3.4(1.4)	4.8(0.7)	14.6(2.9)	0.1(0.1)
Fertilized	14.1(0.9)	0.3(0.1)	0.0(0.0)	0.6(0.2)	40.6(1.8)	1.1(0.5)	0.0(0.0)	0.7(0.2)	1.4(0.5)	17.0(1.6)	0.0(0.0)	3.4(0.5)	8.6(1.1)	10.9(1.6)	0.0(0.0)
August 2009															
Unfertilized	26.2(2.0)	0.3(0.1)	0.1(0.0)	0.6(0.2)	21.9(1.2)	0.2(0.1)	0.7(0.3)	0.5(0.2)	0.4(0.1)	22.4(0.6)	0.0(0.0)	3.4(1.1)	0.8(0.1)	21.2(2.0)	0.1(0.1)
Fertilized	22.4(1.6)	0.0(0.0)	0.0(0.0)	0.2(0.1)	37.1(0.8)	0.0(0.0)	0.7(0.2)	0.7(0.3)	0.6(0.2)	16.9(1.6)	0.0(0.0)	2.0(0.5)	1.5(0.2)	17.3(0.5)	0.1(0.1)
June 2010															
Unfertilized	23.7(1.6)	0.8(0.4)	0.1(0.0)	0.8(0.2)	34.1(1.8)	1.4(0.6)	0.1(0.1)	0.3(0.2)	0.9(0.4)	13.5(0.9)	0.0(0.0)	2.8(0.5)	0.4(0.1)	19.6(1.4)	0.2(0.1)
Fertilized	12.0(2.3)	0.0(0.0)	0.2(0.2)	0.3(0.1)	66.6(5.7)	0.6(0.1)	0.0(0.0)	2.3(0.8)	2.3(0.9)	3.9(0.7)	0.0(0.0)	5.0(1.5)	0.1(0.0)	5.9(1.5)	0.0(0.0)
August 2010															
Unfertilized	35.8(1.4)	0.5(0.2)	1.0(0.8)	1.2(0.5)	13.2(2.0)	0.6(0.3)	0.1(0.1)	0.8(0.3)	0.9(0.6)	13.4(2.0)	0.0(0.0)	2.6(0.4)	0.3(0.1)	27.6(1.9)	0.3(0.2)
Fertilized	19.2(1.6)	0.0(0.0)	0.0(0.0)	0.2(0.1)	45.4(4.0)	0.8(0.3)	1.7(0.4)	3.3(1.8)	3.8(0.1)	8.6(1.7)	0.0(0.0)	4.7(1.2)	0.1(0.1)	11.6(2.3)	0.0(0.0)
June 2011															
Unfertilized	32.6(2.8)	2.7(1.6)	0.0(0.0)	1.2(0.5)	17.9(1.4)	0.7(0.1)	0.1(0.0)	1.4(0.3)	1.1(0.4)	6.2(1.0)	0.1(0.1)	3.3(0.9)	0.3(0.0)	30.6(3.1)	0.2(0.1)
Fertilized	17.5(1.8)	0.0(0.0)	0.0(0.0)	0.1(0.1)	50.3(2.6)	0.7(0.4)	4.2(1.8)	5.2(1.1)	4.8(0.4)	2.0(0.2)	0.2(0.2)	9.7(1.7)	0.1(0.0)	3.8(1.1)	0.2(0.1)
August 2011															
Unfertilized	40.0(2.1)	0.4(0.2)	2.1(1.4)	1.5(0.3)	10.0(0.8)	0.9(0.2)	0.4(0.4)	1.1(0.2)	1.2(0.1)	6.9(0.7)	0.0(0.0)	3.4(0.5)	0.5(0.1)	29.6(2.6)	0.1(0.1)
Fertilized	25.5(1.8)	0.0(0.0)	0.2(0.2)	0.1(0.0)	29.6(1.9)	2.6(0.8)	6.4(2.3)	9.7(1.5)	8.1(2.5)	2.6(1.2)	1.2(0.8)	5.4(1.8)	0.2(0.1)	5.0(0.9)	1.1(0.5)

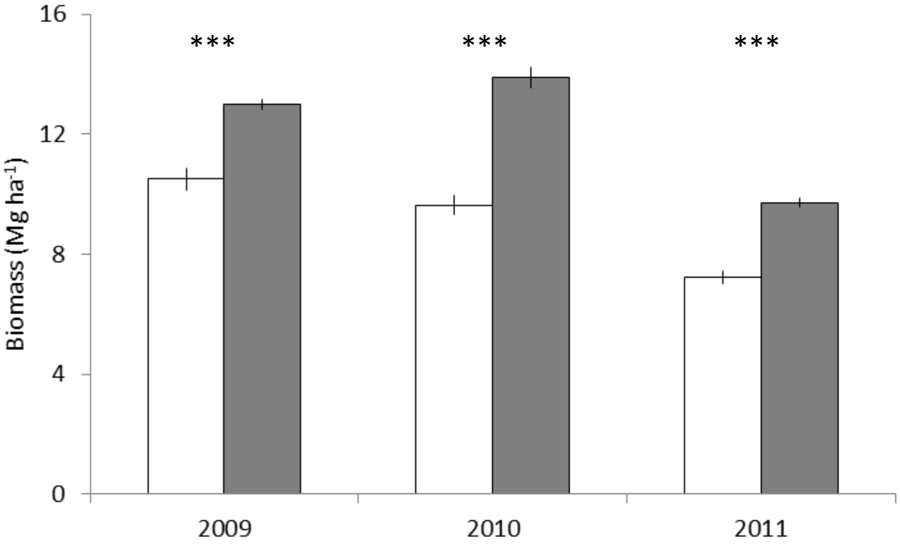

Figure 1. Aboveground biomass (± SE) of unfertilized (white) and fertilized (gray) prairies in 2009, 2010, and 2011. Asterisks above treatments indicate differences between treatments within years (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

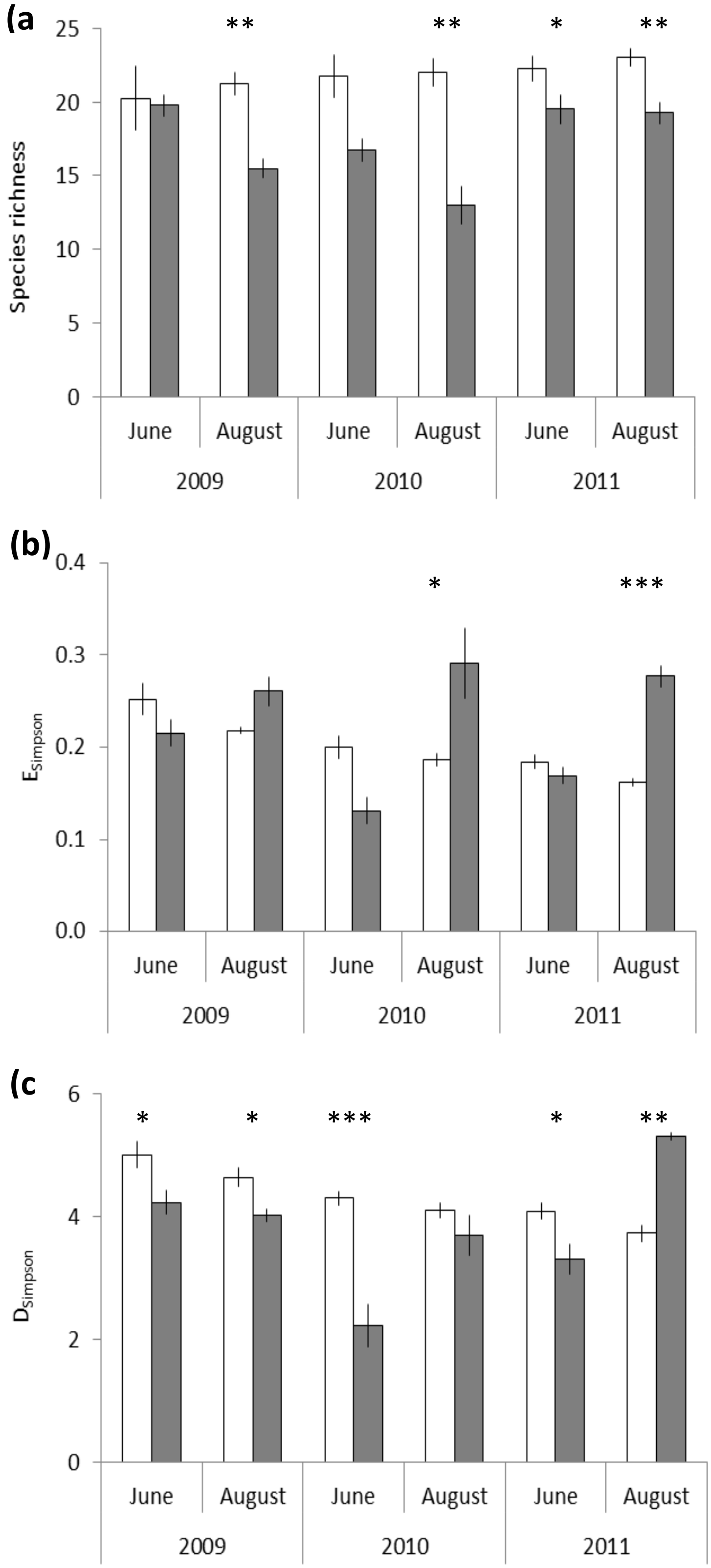

Figure 2. Species diversity parameters (± SE) including species richness (a), Simpson's species evenness (b), and Simpson's species diversity (1/D) (c) of unfertilized (white) and fertilized (gray) prairies in June and August of 2009, 2010, and 2011. Asterisks above treatments indicate differences between treatments within years and sampling date (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

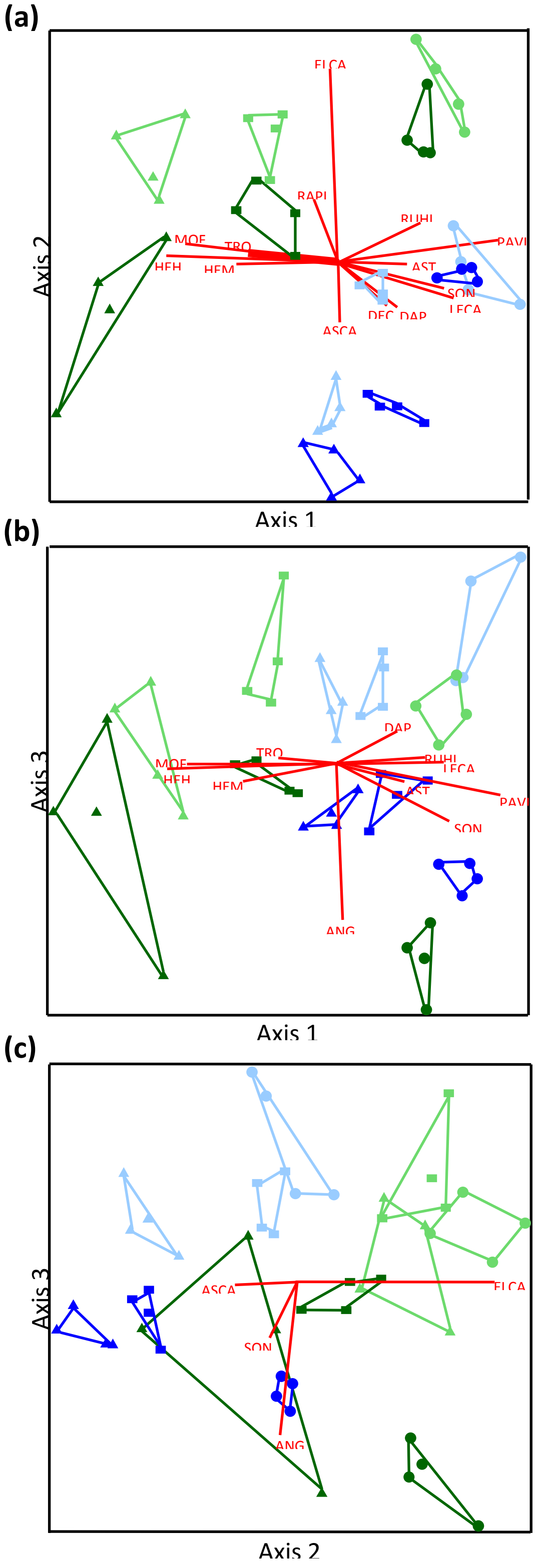

Figure 3. Non-metric multidimensional scaling (NMS) ordination of unfertilized (blue hues) and fertilized (green hues) prairie communities in June (less saturated colors) and August (more saturated colors) in 2009 (circles), 2010 (squares), and 2011 (triangles). The ordination is three dimensional and is presented as Axes 1 and 2 (a), Axes 1 and 3 (b), and Axes 2 and 3 (c). Biplot vectors shown have R^2 values of at least 0.25. Species codes used are: ANGE *Andropogon gerardii*, ASCA *Astragalus canadensis*, ASTU *Asclepias tuberosa*, DAPU *Dalea purpurea*, DECA *Desmodium canadense*, ELCA *Elymus canadensis*, HEMA *Helianthus maximiliani*, HEHE *Heliopsis helianthoides*, LECA *Lespedeza capitata*, MOFI *Monarda fistulosa*, PAVI *Panicum virgatum*, RAPI *Ratibida pinnata*, RUHI *Rudbeckia hirta*, SONU *Sorghastrum nutans*, and TROH *Tradescantia ohioensis*.

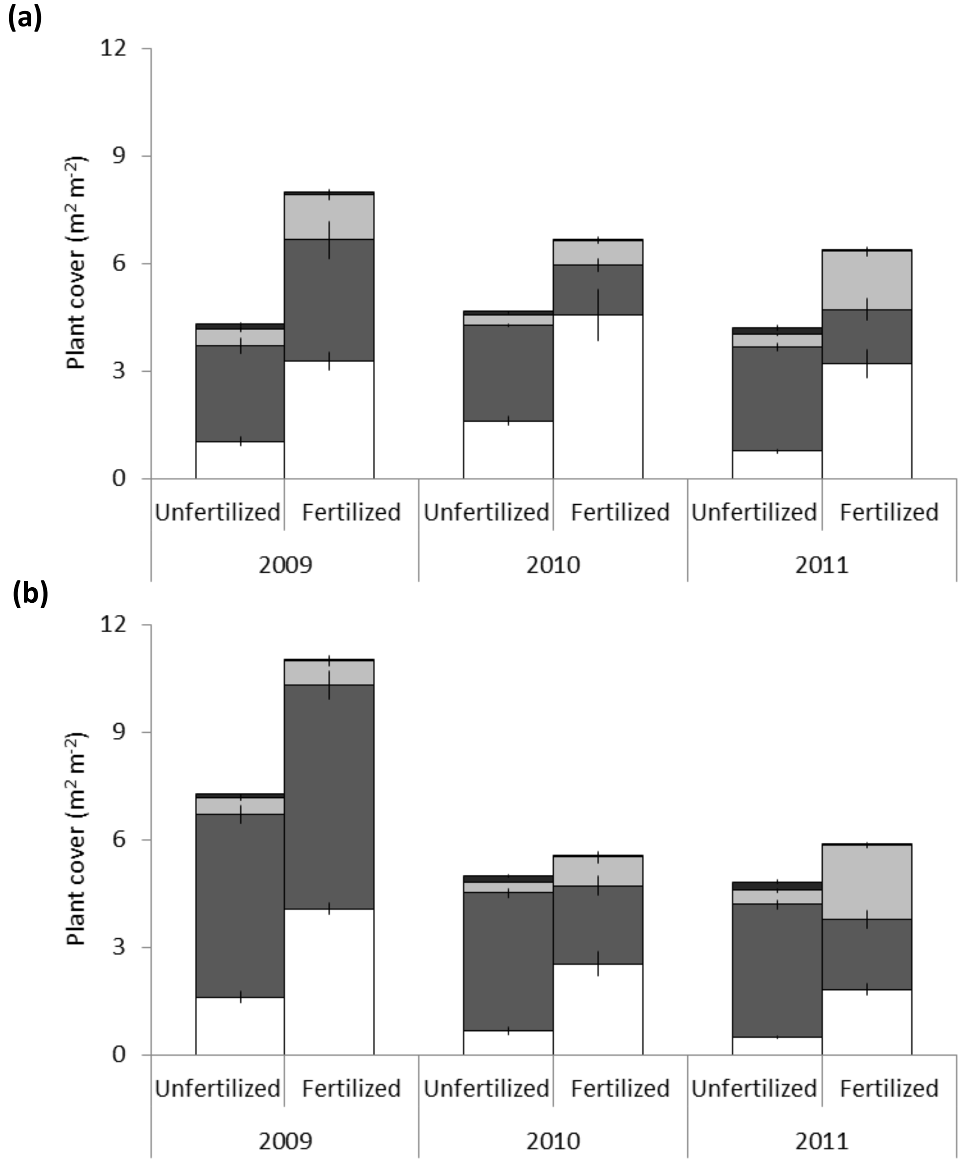

Figure 4. Plant cover (± SE) of C_3 grasses (white), C_4 grasses (dark gray), forbs (light gray), and legumes (black) in the unfertilized and fertilized prairies in June (a) and August (b) of 2009, 2010, and 2011.

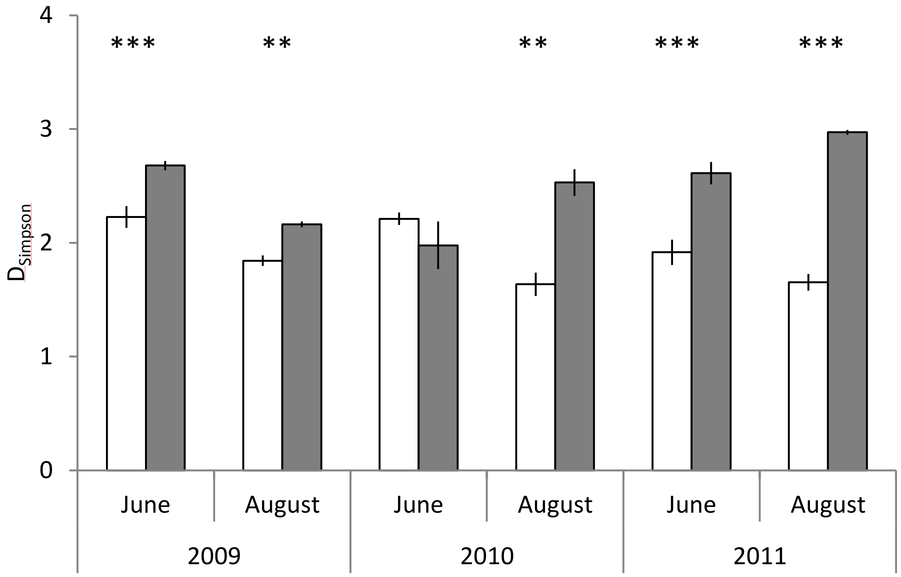

Figure 5. Simpson's functional-group diversity (1/D) of unfertilized (white) and fertilized (gray) prairies in June and August of 2009, 2010, and 2011. Asterisks above treatments indicate differences between treatments within years and sampling date (* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).

CHAPTER 3. NUTRIENT ENRICHMENT REDUCES COMPLEMENTARITY AND INCREASES PRIORITY EFFECTS IN PRAIRIES MANAGED FOR BIOENERGY

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Abstract

Prairies are a potential bioenergy feedstock that could benefit the environment while providing biomass for bioenergy. Increased diversity and nitrogen fertilization are two methods of increasing prairie productivity. Diversity may enhance complementarity of resource use within prairies, whereas the application of nitrogen fertilizer may cause priority effects whereby species with early phenologies or high responsiveness to fertilization are stimulated. We tested the effects of functional-group identity and nitrogen fertilization on resource capture and growth of prairie plants. To determine whether functionally-diverse mixtures of prairie plants exhibited complementarity and/or priority effects, we measured light interception, canopy duration, and aboveground biomass production of C_3 grasses, C_4 grasses, legumes, and multi-functional group mixtures grown without fertilization and with 150 kg ha^{-1} spring-applied nitrogen fertilizer. Under the conditions of our experiment, nitrogen consistently stimulated C_3 grasses, whereas it stimulated C_4 grasses only in the middle and late parts of the growing season and had no effect on legumes. In functionally-diverse mixtures, priority effects of C_3 grasses in fertilized

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[1] Primary researcher and author

[2] Provided input at all stages and supervised the work

mixtures reduced overall resource capture and growth by suppressing growth of the more productive C_4 grasses in the middle and late parts of the growing season. Complementarity occurred in unfertilized mixtures in which C_3 and C_4 grasses were dominant at different parts of the growing season. Prairies receiving more modest nutrient inputs than those used in this study or nutrient inputs later in the growing season may maximize the production of multiple ecosystem services by producing large amount of biomass while still capturing resources throughout the whole growing season.

1. Introduction

There are increasing challenges to agricultural production in the United States. In addition to providing food, feed, and fiber, bioenergy production is also being added to the portfolio of desired agricultural goods. With the passage of the Energy Independence and Security Act of 2007, the US committed to producing 136 hm^3 of biofuels annually by 2022 [1]. There is also increased emphasis being placed on designing multifunctional agricultural systems that provide many ecosystem services including provisioning services, such as biofuel feedstocks, and regulating services, such as water quality protection [2]. Climate change is expected to make managing agricultural systems more challenging as weather becomes less predictable and more extreme in its impacts [3], and increasing volatility and price of fossil-fuel derived inputs is expected to reduce crop profitability [4]. These impending changes may be particularly challenging for the production of annual row crops, which cover more than one third of the Midwest US [5], because these systems have been designed to maximize productivity at the expense of providing other ecosystem services [6], have been bred to thrive under a narrow range of climactic conditions [7], and require large amounts of fossil-fuel derived inputs [8].

The production of bioenergy could lead to increased environmental damage if, for example, corn acreage is expanded to produce ethanol [9]. Alternatively, it could also be an opportunity to diversify the landscape and introduce new cropping systems that optimize the production of both provisioning and other ecosystem services. Prairies are one potential bioenergy cropping system that could benefit the environment while providing biomass for bioenergy [10,11]. Prairies markedly reduce nitrogen and phosphorus pollution and sediment loss because they provide living plant cover throughout the growing season [12]. Diverse native ecosystems, such as prairies, have the capacity to be more resilient to perturbations, such as weather extremes, due to high functional diversity and redundancy [13,14]. Prairies also require fewer purchased inputs because the diversity in prairies suppresses weeds [15] and the perenniality of prairies reduces annual management requirements compared to annual crops [16].

In prairies managed for bioenergy, increasing aboveground biomass production could increase the profitability of the system. Diversity can be a mechanism to enhance prairie productivity [17,18] due to complementarity in the location, timing, or forms of resources used, allowing communities of diverse plant species or functional groups to more completely utilize available resources [19]. In some instances, however, increased species diversity has not increased the productivity of prairie species managed for bioenergy production [20,21]. Increased functional-group diversity, on the other hand, has been found to have larger effects on prairie productivity than increased species diversity [22,23]. Prairies have four broadly-defined functional groups: cool-season (C_3) grasses, warm-season (C_4) grasses, non-leguminous forbs, and legumes [24,25]. Increased diversity may also result in increased productivity due to selection effects whereby more diverse prairies are more likely to have the most productive species which become dominant, but selection effects can also result in reduced productivity if less-productive species become dominant [26,27]. Priority effects are a form of selection effects whereby plants with early phenologies or those that are fast growing are able to outcompete other plants with later phenologies or slower growth rates [28,29]. Priority effects can either increase or decrease productivity [30].

Nitrogen fertilization is another approach through which prairie aboveground productivity can be increased [31-34]. Nitrogen fertilization is commonly recommended when growing prairie grasses for bioenergy [35, but see 36]. Yields of four C_4 grasses managed for bioenergy production increased by more than 50% with annual nitrogen fertilization of 140 kg ha^{-1} [37]. Nitrogen fertilization can alter priority effects by stimulating species whose phenologies or physiologies are especially responsive to fertilization.

If prairies are managed to provide biomass for bioenergy in addition to multiple other ecosystem services, prairie functional-group diversity and nutrient status may have strong interactive effects on the resource capture and growth of the prairies. We sought to answer two primary questions:

1. How do the resource capture and growth dynamics of prairie functional groups differ over the growing season, and are those dynamics altered by spring nitrogen fertilization?
2. Do complementarity and/or priority effects occur in fertilized and unfertilized functionally-diverse prairies?

2. Methods

2.1. Study site and experimental design

We conducted a field experiment at the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone County, IA, USA (42°00’N, 93°43’W). Soils at the site were Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). The site had previously been used for corn, soybean, and oat production, and soybean was grown the year preceding initiation of our experiment. We began the experiment in 2008, but allowed the plants to establish for one year before data collection began. Here, we present data from 2009 and 2010. Average precipitation for the past 60 years at the site from April through October was 683 mm; precipitation at the site from April through October in 2009 and 2010 was 720 mm and 1158 mm, respectively.

The experiment was conducted in a randomized complete block design with four replicates. Treatments comprised a 4 x 2 factorial design with four classes of functional diversity and two levels of nitrogen fertilization. The four classes of functional diversity were: (1) a three-species mix of C_3 grasses (hereafter C_3 grasses); (2) a three-species mix of C_4 grasses (hereafter C_4 grasses); (3) a three-species mix of legumes (hereafter legumes); and (4) a nine-species mix of C_3 grasses, C_4 grasses, and legumes (hereafter mixture). We did not include non-leguminous forbs in this study because we were managing the prairies for bioenergy production, and prairies with high forb concencentrations have been found to be less productive than grass-dominated prairies [38]. The C_3 grasses used were prairie brome (*Bromus kalmii* A. Gray), Canada wildrye (*Elymus canadensis* L.), and slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners). The C_4 grasses used were big bluestem, switchgrass, and Indian grass. The legumes used were Illinois bundleflower (*Desmanthus illinoensis* (Michx.) MacMill. Ex B.L. Rob. & Fernald), showy ticktrefoil (*Desmodium canadense* (L.) DC.), and round-headed bush clover (*Lespedeza capitata* Michx.). In selecting the three species for each functional group, we chose species that were common to prairies in the area, native to Boone County, erect in growth habit (primarily for the legumes), and adapted to mesic conditions but which spanned a range of moisture preferences among the three species. The mixtures contained all nine species listed above. The levels of nitrogen fertilization were 0 kg ha^{-1} y^{-1} (hereafter unfertilized) and 150 kg ha^{-1} y^{-1} of nitrogen (hereafter fertilized) applied as ammonium nitrate in late March 2009 and early April 2010.

To control initial plant density and species composition, we transplanted prairie seedlings into the field. The prairie seedlings were started from seed in a greenhouse in February 2008 and transplanted into the field on 14-15 May 2008. The prairie seed was purchased from Prairie Moon Nursey (Winona, MN) and Allendan Seed (Winterset, IA). Field plots were 2 m x 2 m, and seedlings were transplanted at a density of 54 plants m^{-2}. In single functional group plots (C_3 grasses, C_4 grasses, and legumes), each species was transplanted at a density of 18 plants m^{-2}, and in mixtures, each of the nine species was transplanted at a density of 6 plants m^{-2}. Plant locations within plots were randomized. The plots were watered in May 2008 to encourage seedling establishment, and dead transplants were replaced through June 2008. The plots were weeded by hand as necessary throughout the duration of the experiment.

2.2. Resource capture

The proportion of photosynthetically active radiation (PAR) intercepted by the plant canopies was measured approximately every two weeks throughout the growing season to determine temporal patterns of canopy light interception. Light measurements were initiated in April once plant growth was observed and continued until 4 October in 2009 and 18 October in 2010. A 1 m quantum sensor bar (LI-COR Biosciences, Lincoln, NE) was placed on the ground to measure below-canopy PAR transmission, and a LI-COR point quantum sensor was held above the plant canopy to measure above-canopy PAR. Measurements were taken as 5 sec averages on sunny days between 10:00 and 14:00 hours, with two measurements per plot at each sampling date. Canopy light interception was calculated as the difference between the above-canopy PAR and below-canopy PAR divided by the above-canopy PAR.

We used a segmented regression, curve fitting technique to compare light interception among treatments. Two functions were fit to the light interception data over the course of the growing season using R (R: A Language and Environment for Statistical Computing, R Development Core Team, Vienna, Austria). A logistic curve was fit to the data from April through July. Choice of this function was motivated by its previous usefulness in describing patterns of light interception [39], the ease of interpreting its three parameters, and its close fit to our data. The equation of the logistic curve used was:

$$y = \frac{max}{1 + exp^{(\frac{x_{mid} - x}{scale})}}$$

where max is the maximum light interception, x_{mid} is the inflection point, and scale is the spread of the curve [40]. Max estimates the maximum plant canopy light interception over the range of x values (i.e. days) for the logistic function, which in our case was the maximum light interception through the end of July; x_{mid} describes the amount of time in the spring until rapid plant growth, and scale describes the rate with which canopy closure occurs. The logistic equation is undefined if light interception values are negative or zero. Consequently, in instances where measured light interception values were negative or zero, the experimental values were replaced by the dummy value 0.0001.

Linear functions were fit to the data from August through October in order to determine whether plant canopy light interception decreased after July, which is when peak aboveground biomass production occurs in prairies near our experimental site. We forced lines to begin fitting the data at the values predicted from the logistic curve for the end of

July. Both logistic and linear functions were fit to the data from each plot in order to generate predicted values for light interception over the growing season. The parameters determined from the logistic curve and the slope of the line were analyzed separately with analysis of variance (ANOVA) in SAS (SAS Institute, Cary, NC). A first-order autoregressive (AR(1)) covariance model was used to assess the effects of the treatments over the two years of the study. We used contrasts to make comparisons among treatments within the main effects and interactions tested by the ANOVA. Unless otherwise indicated, values were considered significant at $P \leq 0.05$.

2.3. Plant growth

Plant cover was measured using the point intercept method [41] in mid-May, late-July, and mid-September 2009 and 2010. The point intercept frame was 1 m^2, and two of the four quadrants of each plot were selected randomly at each sampling date. Within each sampling frame, a pin was dropped 24 times for a total of 48 pin drops per plot. The exterior 0.2 m of each plot was not sampled to reduce edge effects. With each pin drop, we recorded the number of hits and functional group identity of all living plant tissue. Therefore, our plant cover data provide estimates of living plant cover, whereas the light interception measurements provide estimates of standing aboveground plant material.

Using the plant cover data, we determined plant canopy duration by integrating the plant cover data over the growing season. We calculated the area under the curve beginning 1 April and ending 31 October, which was before and after plant growth was observed. Plant cover values of zero were used for 1 April and 31 October, and measured plant cover data were used for the May, July, and September sampling dates. Because the point intercept sampling took approximately 5 days to complete per sampling period, we used the midpoint of each sampling date when calculating canopy duration.

We assessed complementarity in the mixtures by calculating over- and underyielding values for canopy cover duration with the relative yields and relative yield totals. Over- and underyielding were calculated as the deviation in canopy duration of the mixtures compared to the expected canopy duration based on the performance of each functional group grown separately [42]. Positive values for relative yields of component functional groups indicated overyielding because of higher than expected canopy durations in mixture than as single functional groups, whereas negative values indicated underyielding. Positive values for relative yield totals for the entire mixture, which was the sum of the component functional-group values, indicated overyielding and that complementarity may have occurred.

In order to relate canopy duration to biomass production, we harvested all of the aboveground biomass in each plot after hard frost had occurred. Plants were harvested to approximately 2 cm above the soil surface, and the exterior 0.2 m of each plot was not included with the harvested biomass. The biomass was dried at 60°C for at least 48 hrs and then weighed.

Canopy duration and relative yields were analyzed with an AR(1) ANOVA covariance model, and contrasts were used to make comparisons among treatments as necessary. Unless otherwise indicated, values were considered significant at $P \leq 0.05$. The relationships between canopy duration and aboveground biomass production were examined using correlations for C_3 grasses, C_4 grasses, legumes, and mixtures with fertilized and unfertilized treatments combined.

3. Results

3.1. Resource capture

Both functional-group identity of the prairie plants and spring nitrogen fertilization affected all parameters of the fitted curves, and interactions between functional group and nitrogen fertilization were generally significant for all parameters ($P_{scale\_interaction} = 0.065$, others $P < 0.05$). The curves differed between years for all parameters except for the amount of time until rapid spring growth (i.e. x_{mid}; Table 1). Spring growth was stimulated by nitrogen fertilization for C_3 grasses ($P_{2009} = 0.02$, $P_{2010} < 0.0001$) and mixtures ($P_{2009} = 0.02$, $P_{2010} = 0.0001$) because fertilized C_3 grasses and fertilized mixtures had lower x_{mid} values than their unfertilized counterparts (Fig. 1a,b). Nitrogen fertilization had no effect, on the other hand, on the spring growth of the C_4 grasses ($P_{2009} = 0.6$, $P_{2010} = 0.9$) or legumes ($P_{2009} = 0.7$, $P_{2010} = 0.5$). In May, C_3 grasses were dominant in the fertilized mixtures, comprising 89% and 83% of plant cover in 2009 and 2010, and were less important in the unfertilized mixtures, comprising 73% and 31% of plant cover in 2009 and 2010 (Fig. 2a,d).

Rates of canopy closure (i.e. scale) were highly variable among functional groups and nitrogen fertilization levels (Table 1, Fig. 1). The only functional group that was affected by spring nitrogen fertilization was C_3 grasses for which the rate of canopy closure tended to increase with fertilization ($P_{2009} = 0.075$, $P_{2010} = 0.008$). Rates of canopy closure of C_4 grasses ($P_{2009} = 0.8$, $P_{2010} = 0.4$), legumes ($P_{2009} = 0.8$, $P_{2010} = 0.9$), and mixtures ($P_{2009} = 0.2$, $P_{2010} = 0.4$) were not affected by spring nitrogen fertilization.

Maximum canopy light interception by the end of July was estimated from the max parameter of the logistic curve, although the unfertilized C_3 and C_4 grasses had not reached their full-season maximum light interception by that date. The max values were increased with spring nitrogen fertilization for the C_3 grasses ($P_{2009} < 0.0001$, $P_{2010} < 0.0001$) and C_4 grasses ($P_{2009} = 0.02$, $P_{2010} = 0.02$), whereas fertilization did not affect max values of the legumes ($P_{2009} = 0.9$, $P_{2010} = 0.8$) or mixtures ($P_{2009} = 0.15$, $P_{2010} = 0.9$; Fig. 1).

Analyses of linear functions describing light interception after July indicated that the slopes for most treatments were either not different from zero ($P_{C4\_fertilized\_2009} = 0.3$, $P_{C4\_fertilized\_2010} = 0.4$, $P_{mixture\_unfertilized\_2009} = 0.9$, $P_{mixture\_unfertilized\_2010} = 0.8$, $P_{mixture\_fertilized\_2010} = 0.2$) indicating no change in light interception at the end of the growing season, or negative ($P_{mixture\_fertilized\_2009} = 0.009$, $P_{C3\_fertilized\_2009} < 0.0001$, $P_{C3\_fertilized\_2010} = 0.003$, $P_{all\_legumes} < 0.0001$), indicating reduced canopy light interception due to canopy senescence. The unfertilized C_3 and C_4 grasses had not reached full canopy development by the end of July, and the slopes of the lines tended to be positive from August to October ($P_{C3\_2009} = 0.08$, $P_{C3\_2010} < 0.0001$, $P_{C4\_2009} = 0.02$, $P_{C4\_2010} = 0.01$). Because canopy light interception of unfertilized C_3 and C_4 grasses tended to increase after July, and the fertilized C_3 grass canopy decreased and the fertilized C_4 canopy was not different from zero, there was a difference in end-of-season light interception between the fertilized and unfertilized C_3 grasses ($P_{2009} < 0.0001$, $P_{2010} < 0.0001$) and C_4 grasses ($P_{2009} = 0.02$, $P_{2010} = 0.02$). Nitrogen fertilization decreased the amount of light intercepted in the mixtures in 2009 after July ($P = 0.04$), but did not affect the amount of light intercepted after July in the legumes ($P_{2009} = 0.06$, $P_{2010} = 0.7$) or mixtures in 2010 ($P = 0.3$).

3.2. Plant growth

Functional group identity, spring nitrogen fertilization, and their interaction affected canopy duration (all $P < 0.0001$). Spring nitrogen fertilization increased canopy duration of the C_3 and C_4 grasses in both years (all $P < 0.0001$) and the mixtures in 2009 ($P = 0.0002$), but did not affect canopy duration of the legumes ($P_{2009} = 0.9$, $P_{2010} = 0.4$) or mixtures in 2010 ($P = 0.5$; Fig. 3). Overyielding was observed in the unfertilized mixtures but was not observed in the fertilized mixtures because of offsetting C_3 grass overyielding with legume underyielding in the fertilized mixtures (Table 2).

There was a positive correlation between canopy duration and aboveground biomass for all functional groups grown separately, and the relationship tended to be positive for the mixtures ($P_{slope} = 0.085$; Fig. 4). In the C_3 and C_4 grasses, the fertilized treatments had both higher canopy durations and aboveground biomass than their unfertilized counterparts. In the legumes and mixtures, on the other hand, the fertilized treatments did not always have higher canopy durations or aboveground biomass than the unfertilized treatments (Fig. 4).

4. Discussion

Previous studies have found that nitrogen fertilization generally has strong positive effects on the aboveground biomass production of prairie communities [43] and overall growth rates of prairie plants [44]. We found, however, differential responses to spring nitrogen fertilization among the functional groups represented in our experiment when growth dynamics and resource capture were measured throughout the growing season. Differential responses were particularly evident between C_3 and C_4 grasses. The growth of C_3 grasses, either alone or with other functional groups, was consistently stimulated by spring nitrogen fertilization, whereas C_4 grasses were stimulated by spring nitrogen fertilization only in the middle and late parts of the growing season. The later response of C_4 grasses to fertilization caused their abundance to be reduced in fertilized multi-functional group mixtures rather than increased, as was the case in unfertilized mixtures.

C_3 grasses were responsive to spring nitrogen fertilization for all measured characteristics: they had more rapid initiation of spring growth, more rapid canopy closure, higher canopy light interception through July, reduced canopy light interception at the end of the growing season, and greater canopy duration when plants were fertilized than not fertilized (Figs. 1a,b and 3). Fertilized C_3 grasses also produced more aboveground biomass than unfertilized C_3 grasses (Fig. 4a). These finding were consistent with those of other researchers regarding the high responsiveness of C_3 grasses to nitrogen [44,45].

C_4 grasses were responsive to spring nitrogen fertilization for some of the measured characteristics, but only those that occurred in the middle to late portions of the growing season: they had higher maximum canopy light interception in June and July, reduced canopy light interception at the end of the growing season, and greater canopy duration in fertilized versus unfertilized treatments (Figs. 1c,d and 3). Like the C_3 grasses, fertilized C_4 grasses produced more aboveground biomass than unfertilized C_4 grasses (Fig. 4b). The later phenologies of C_4 grasses were evident from their low plant cover values in May (Fig. 2a,d) and this is likely the reason for the lack of a stimulatory effect of spring nitrogen fertilization on resource capture early in the growing season.

The resource capture and growth of the legumes, in contrast to the grasses, was not affected by spring nitrogen fertilization, which is not surprising given the association that legumes have with nitrogen fixing bacteria in their roots [44].

The resource capture and growth of the mixtures generally were not different between the fertilized and unfertilized treatments, except for initiation of spring growth and canopy duration, due to offsetting impacts of priority effects in the fertilized mixtures and complementarity occurring in the unfertilized mixtures. Within the mixtures, there was earlier initiation of spring growth in the fertilized mixtures compared to the unfertilized mixtures and greater canopy duration in the fertilized mixtures in 2009 (Figs. 1g,h and 3a).

The fertilized mixtures were dominated by C_3 grasses in May, so the rapid initiation of spring growth the fertilized mixtures was likely due to the fact that the dominant functional group at that time had the strongest effect on the observed response [46]. The dominance of C_3 grasses in the fertilized mixtures in the spring of 2009 was also the major contributing factor to their increased canopy duration because that was the only time in the growing season when the fertilized mixtures had greater plant cover than the unfertilized mixtures (Fig. 2a,d).

Priority effects of C_3 grasses in the fertilized mixtures also contributed to the lack of an overall response of the mixtures to spring nitrogen fertilization, as evidenced by the lack of an effect of fertilization on the rate of canopy closure, maximum light interception, end-of-season light interception, and canopy duration in 2010. In the fertilized mixtures, the C_3 grasses became dominant in the spring and remained dominant throughout the rest of the growing season (Fig. 2), which is further supported by the overyielding of only C_3 grasses in the fertilized mixtures (Table 2). Both early spring growth [47] and nitrogen fertilization [34,45] have been found to increase C_3 grass dominance in other grasslands. C_4 grasses, on the other hand, became dominant in the unfertilized mixtures in July and September, as demonstrated by the tendency of the C_4 grasses to overyield in the unfertilized mixtures ($P_{2009} = 0.089$, $P_{2010} < 0.0001$; Table 2).

Both C_3 and C_4 grasses were stimulated by spring nitrogen fertilization in single functional group treatments, but the values for resource capture and growth were generally greater for C_4 grasses than C_3 grasses (Table 1, Fig. 3). When the mixtures were dominated by C_3

grasses, as they were in the fertilized mixtures, the responses of the mixtures were most similar to those of the C_3 grasses, which were lower than the responses of the C_4 grasses, demonstrating negative selection effects. Hooper [48] found negative selection effects in serpentine grasslands where annual plants established more rapidly than the more productive perennial bunchgrasses, which reduced the total productivity of the more diverse grasslands. The reverse was true with the unfertilized mixtures in the present study, especially in the middle and late parts of the growing season, when the C_4 grasses were able to become dominant. The lack of a stimulatory effect of spring nitrogen fertilization on mixture productivity was also evident in the lack of a strong relationship between canopy duration and aboveground biomass production in the mixtures (Fig. 4d).

Although priority effects of C_3 grasses were important in explaining many characteristics of the resource capture and growth of the mixtures, complementarity was observed in the unfertilized mixtures (Table 2). C_3 grasses were a major component of the plant cover of the unfertilized mixtures in May, whereas C_4 grasses and legumes became dominant in the unfertilized mixtures in July and September (Fig. 2). The presence of both C_3 and C_4 grasses in the unfertilized mixtures allowed temporal complementarity to occur because the unfertilized mixtures had rapid early spring growth characteristic of the C_3 grasses but also had high plant cover, resource duration, and aboveground biomass production characteristic of the C_4 grasses in the middle and late parts of the growing season.

Overyielding was not observed in the legumes in the unfertilized mixtures, but their relative yield values were positive (Table 2). This suggests that legumes also tended to contribute to higher yields in unfertilized mixtures through complementarity, whereas in the fertilized mixtures the legumes underyielded, presumably because they were suppressed by the faster growing C_3 grasses. Maintaining legumes is especially important in prairies managed for bioenergy production because the aboveground biomass, and therefore nutrients in that biomass, is removed annually. We expect the importance of the nitrogen-fixing capacity associated with legumes to increase over time with repeated harvests as nitrogen becomes more limiting to prairie productivity [49].

Having high functional group diversity would provide benefits for prairies managed for multiple ecosystem services including bioenergy production. We found that prairies containing C_3 grasses that received spring nitrogen fertilization had more rapid spring growth, but began senescing earlier in the fall, had lower plant cover throughout the growing season, and produced less aboveground biomass than C_4 grasses alone or in unfertilized multi-functional group mixtures. The presence of C_4 grasses in prairies was advantageous in extending the duration of plant cover, which would help reduce nutrient and sediment losses if the prairies were grown down-slope from annual crops, and in providing larger amounts of aboveground biomass, which would increase the amount harvestable bioenergy feedstocks. Yet unintentional spring nitrogen fertilization is likely to occur if prairies are grown down-slope from fertilized annual row crops, which may encourage the C_3 grasses to become dominant.

The fertilization levels used in this study were relatively high. Prairies receiving more modest nutrient inputs or nutrient inputs later in the growing season may maximize the production of multiple ecosystem services by producing large amount of biomass while still capturing resources throughout the whole growing season. Future experimental work testing that hypothesis would be useful in evaluating the agronomic and environmental impact characteristics of prairie-based bioenergy production systems.

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Table 1. Mean (SE) values for the x_{mid}, scale, and max parameters of the logistic curve and slope parameter of the line fitted to the intercepted photosynthetically active radiation (IPAR) data over the 2009 and 2010 growing seasons.

	x_{mid}		scale		max		slope	
Treatment	2009	2010	2009	2010	2009	2010	2009	2010
C_3 grass								
Fertilized	125(1)	126(1)	6.9(0.5)	11.2(1.5)	0.95(0.04)	0.87(0.01)	-0.0021(0.0004)	-0.0014(0.0003)
Unfertilized	136(3)	152(14)	12.2(3.3)	20.8(8.9)	0.66(0.02)	0.60(0.12)	0.0008(0.0004)	0.0021(0.0004)
C_4 grass								
Fertilized	138(1)	141(3)	5.0(0.7)	9.9(1.0)	0.99(0.003)	1.01(0.004)	-0.0005(0.0001)	-0.0004(0.0002)
Unfertilized	140(1)	142(3)	4.4(0.3)	12.2(1.5)	0.89(0.01)	0.87(0.02)	0.0010(0.0001)	0.0012(0.0001)
Legume								
Fertilized	156(2)	151(2)	5.9(1.0)	4.6(0.7)	0.95(0.02)	0.91(0.03)	-0.0050(0.0011)	-0.0045(0.0006)
Unfertilized	155(0.4)	154(3)	5.2(1.0)	4.3(0.8)	0.96(0.02)	0.92(0.01)	-0.0062(0.0004)	-0.0048(0.0003)
Mixture								
Fertilized	132(1)	132(3)	7.3(0.5)	10.5(2.0)	0.97(0.02)	0.92(0.01)	-0.0012(0.0006)	-0.0005(0.0001)
Unfertilized	144(2)	151(1)	11.3(1.4)	12.8(1.7)	0.91(0.02)	0.93(0.02)	0.0001(0.0006)	0.0001(0.0004)

Table 2. Overyielding and underyielding responses of C_3 grasses, C_4 grasses, and legumes in the fertilized and unfertilized mixtures in 2009 and 2010 as indicated by differences between observed relative yields and zero. Positive values indicate overyielding and negative values indicate underyielding.

	Fertilized mixture				Unfertilized mixture			
Year	C_3 grass	C_4 grass	Legumes	Total	C_3 grass	C_4 grass	Legumes	Total
2009	0.35*	-0.03	-0.26*	0.06	0.19	0.17	0.06	0.42*
2010	0.21*	0.05	-0.26*	-0.01	-0.16	0.49*	0.19	0.53*

*Indicates values significantly different from zero (P ≤ 0.05).

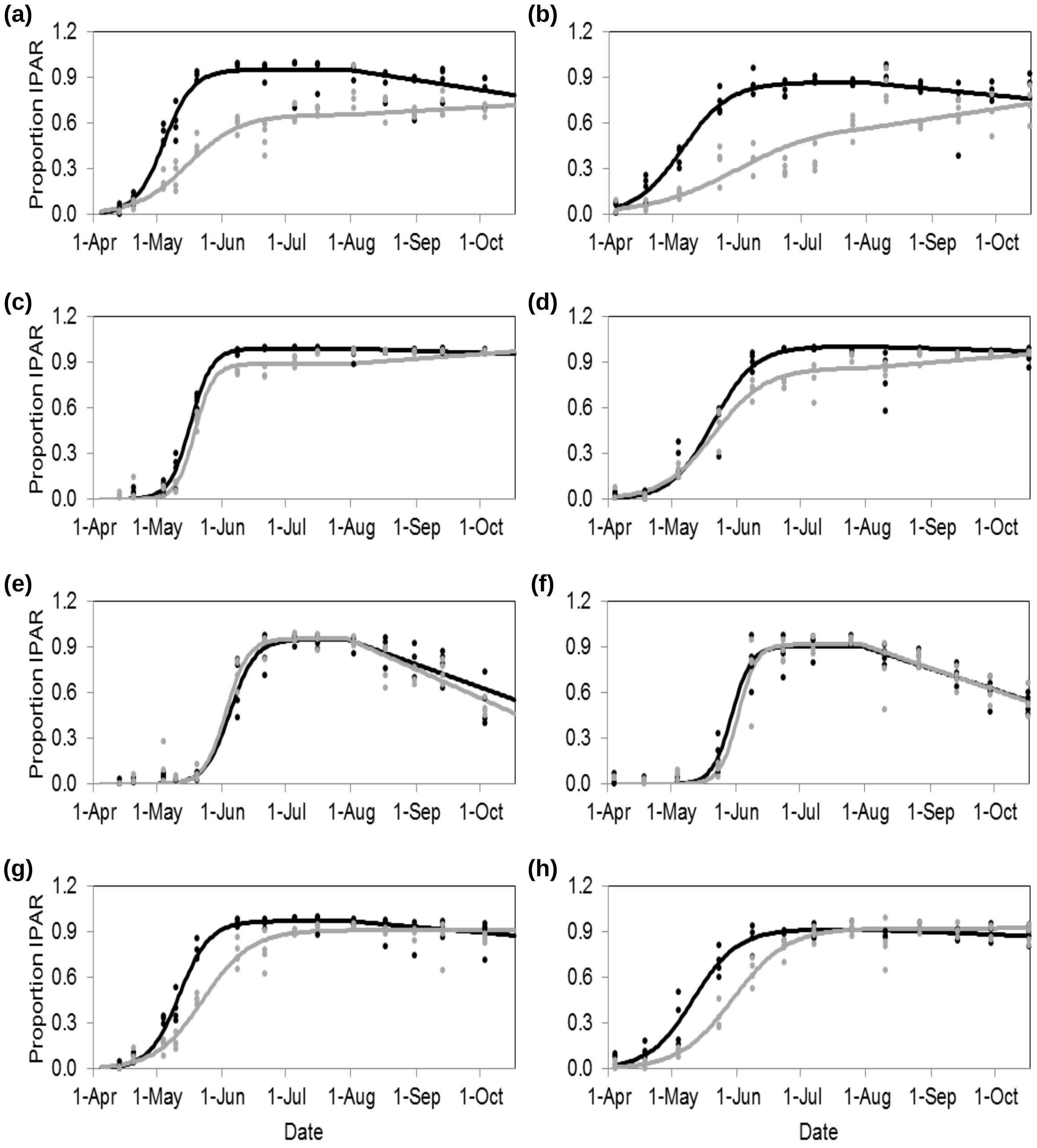

Figure 1. Curves fitted to the proportion of intercepted photosynthetically active radiation (IPAR) in functional group and N fertilization treatments using a logistic curve from April through July and a linear function beyond July. Predicted IPAR values are shown for unfertilized (gray line) and fertilized (black line) C_3 grasses (a,b), C_4 grasses (c,d), legumes (e,f), and mixtures (g,h) in 2009 (left column) and 2010 (right column). IPAR data for individual unfertilized (gray dots) and fertilized (black dots) plots are also shown.

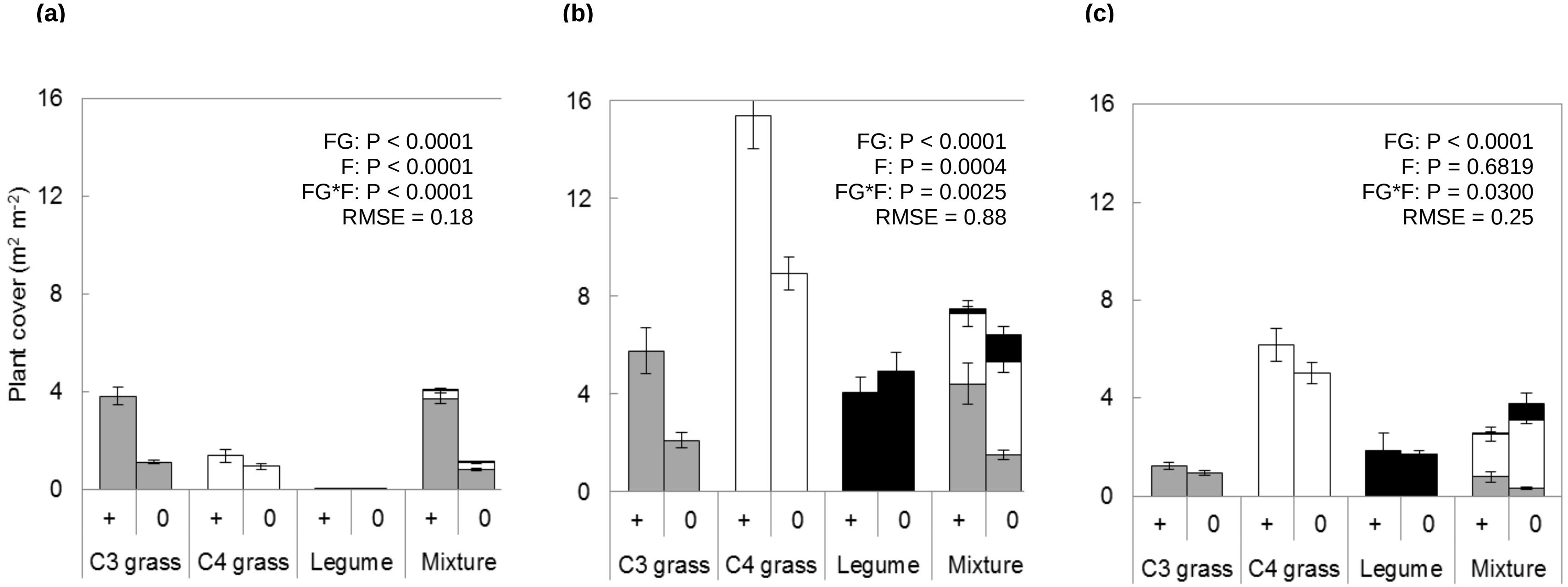

Figure 2. Mean (± SE) plant cover of C_3 grasses (gray bars), C_4 grasses (white bars), and legumes (black bars) in single functional group and mixture plots. "+" denotes fertilized treatments and "0" denotes unfertilized treatments in May (a,d), July (b,e), and September (c,f) of 2009 (a, b, c) and 2010 (d, e, f). Main effects of functional group (FG) and nitrogen fertilization (F), their interaction (FG*F), and root mean square errors (RMSE) from analyses of variance are also presented.

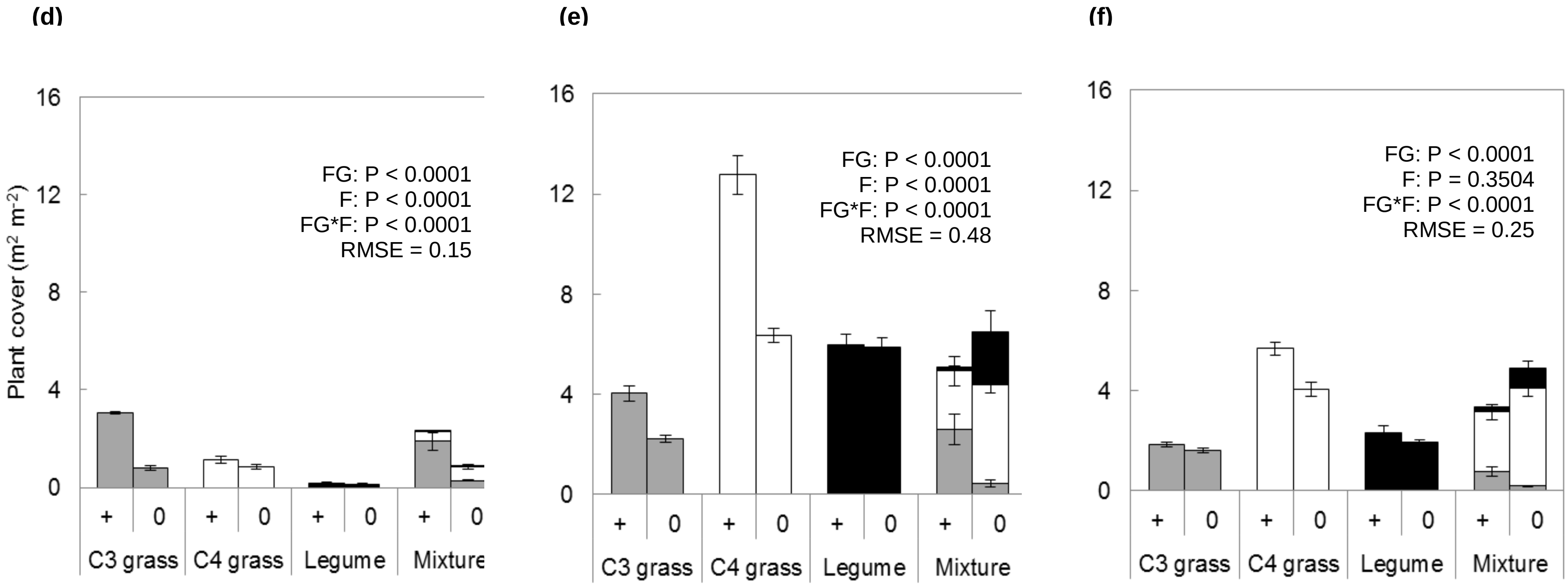

Figure 2. (continued)

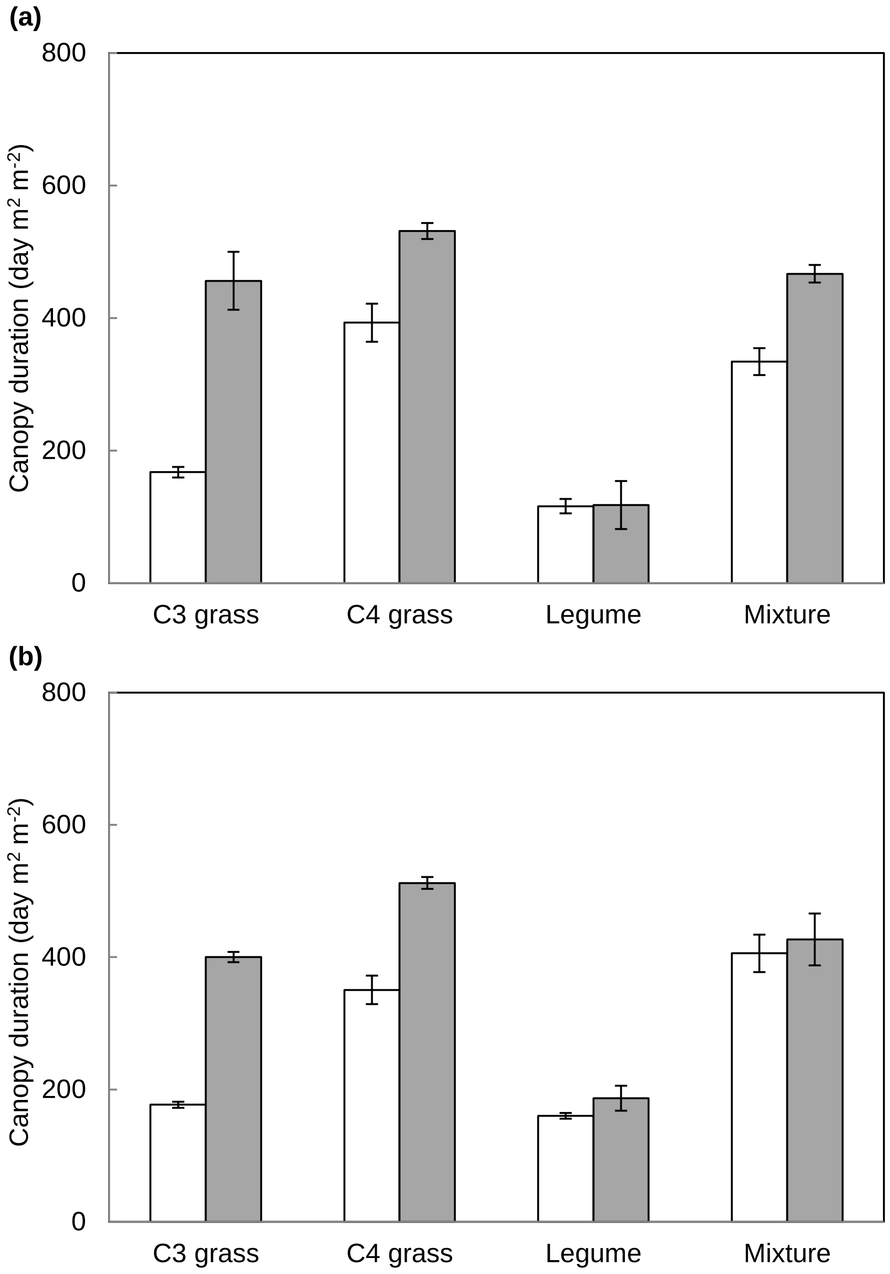

Figure 3. Mean (± SE) canopy duration for unfertilized (white bars) and fertilized (gray bars) C_3 grasses, C_4 grasses, legumes, and mixtures in 2009 (a) and 2010 (b). Main effects of functional group (FG) and nitrogen fertilization (F), their interaction (FG*F), and root mean square errors (RMSE) from analyses of variance are also presented.

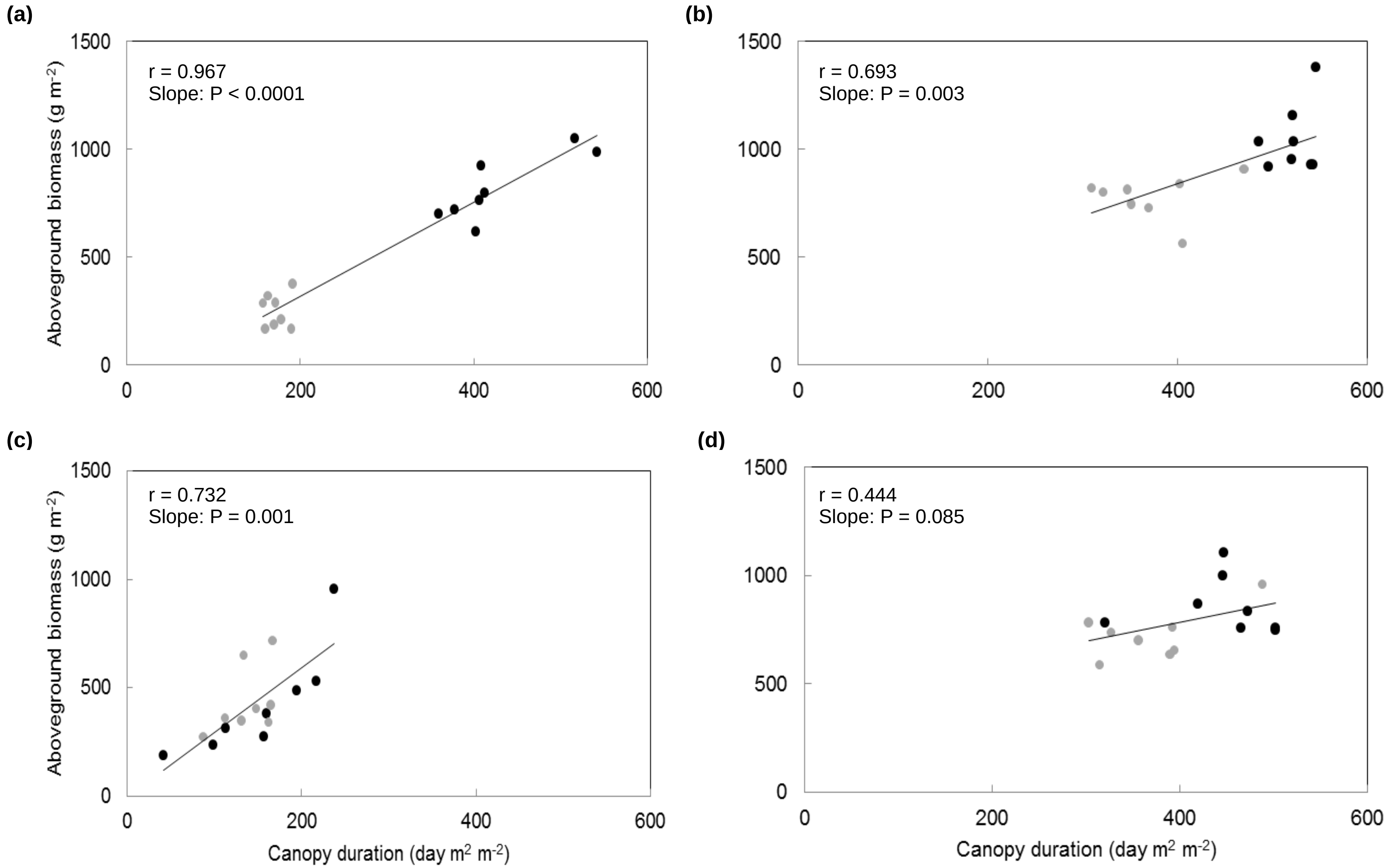

Figure 4. Relationship between aboveground biomass production and canopy duration for unfertilized (gray dots) and fertilized (black dots) C_3 grasses (a), C_4 grasses (b), legumes (c), and mixtures (d) averaged over 2009 and 2010. Correlation and significance values indicating whether slope of the relationship differs from zero are also presented.

CHAPTER 4. TRADEOFFS IN BIOMASS AND NUTRIENT ALLOCATION IN PRAIRIES AND CORN MANAGED FOR BIOENERGY PRODUCTION

A paper published in *Crop Science*

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Abstract

Prairie vegetation has the potential to serve as a bioenergy feedstock with favorable environmental impacts, but generally yields less than corn (*Zea mays* L.). To more fully assess the potential of prairie vegetation to serve as a biofuel feedstock, more needs to be understood about prairie responses to different management strategies, including fertilization. We hypothesized that (1) nitrogen fertilization would increase prairie productivity and nutrient content, and (2) fertilized prairie would produce similar amounts of total biomass as corn but would allocate more biomass and nutrients to roots. Our hypotheses were tested in a field experiment using prairie species representing different functional groups – C_3 grasses, C_4 grasses, legumes, and multi-functional group mixtures – grown with and without nitrogen fertilizer, as well as fertilized corn. Fertilized and unfertilized C_4 prairie grasses produced as much total biomass as corn, but allocated up to 65% of their biomass belowground compared to 3% for corn. Corn yields decreased over the three-year period of the study, whereas yields of fertilized C_4 grasses and multi-functional group mixtures were stable, and yields of unfertilized C_4 grasses and mixtures

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[1] Primary researcher and author

[2] Provided input at all stages and supervised the work

increased and became equal to their fertilized counterparts. Calculated annual costs for replacing nutrients removed in the harvested portions of corn and fertilized and unfertilized C_4 grasses were \$403, \$137, and \$40 ha^{-1}, respectively. Results of this experiment show tradeoffs among corn and prairie systems with respect to harvested biomass, root production, and nutrient exports, emphasizing the need for a multifaceted approach to fully evaluate bioenergy feedstock production systems.

Introduction

Agricultural systems in the United States are among the most productive in the world (NASS, 2009), but also contribute to environmental degradation such as soil erosion, greenhouse gas emissions, and the loss of biodiversity (Reganold et al., 2011). Consequently, many analysts have called for greater multifunctionality in agricultural systems, including the provision of a range of ecosystems services (e.g., soil, water, and wildlife conservation) in addition to food, feed, fiber, and fuel production (MEA, 2005; Jordan et al., 2007; Jordan and Warner, 2010). Bioenergy production has the opportunity to increase agricultural sustainability if bioenergy cropping systems are selected and managed to provide both biomass feedstocks and additional ecosystem services (Robertson et al., 2008; Tilman et al., 2009).

Two feedstocks that are candidates for bioenergy production in the Midwestern U.S. are corn (*Zea mays* L.) and prairie vegetation. Corn is grown throughout the region, and ethanol produced from corn grain is the dominant form of bioenergy produced in the U.S.

(RFA, 2011). Prairie vegetation has been discussed as an alternative bioenergy source that may enhance environmental sustainability by providing many ecosystem services (Tilman et al., 2006; Hill, 2007). Although both corn and prairie have been evaluated as potential bioenergy feedstocks (James et al., 2010), they differ greatly in their bioenergy profile.

Average corn grain yield for Iowa, Illinois, and Indiana – three states entirely in the U.S. Corn Belt – for 2011 was 8.5 Mg ha^{-1} (160 bu acre^{-1}; NASS, 2011), which corresponds to 16.6 Mg ha^{-1} total aboveground biomass assuming a harvest index of 0.51 (Lorenz et al., 2010). The aboveground biomass production of prairies can vary greatly depending on site conditions and prairie management. Reconstructed prairies in southern Minnesota produced approximately 5 Mg ha^{-1} of aboveground biomass without nitrogen fertilization and almost 8 Mg ha^{-1} with nitrogen fertilization (Camill et al., 2004). Reconstructed prairies in central Iowa, ranging in age from 4 to 12 years, produced between 3.4 and 8.6 Mg ha^{-1} of aboveground biomass with productivity generally increasing with prairie age (Maher et al., 2010). An annually burned portion of a native prairie in northeastern Kansas produced approximately 9.8 Mg ha^{-1} of aboveground biomass at lowland sites and approximately 4.5 Mg ha^{-1} of aboveground biomass at upland sites (Nippert et al., 2011).

Prairie composition can also affect prairie productivity. Prairies contain four broadly defined functional groups of species: cool-season (C_3) grasses, warm-season (C_4) grasses, leguminous forbs, and non-leguminous forbs (Kindscher and Wells, 1995; Craine et al., 2002). The functional groups differ in their phenology, nutrient use strategies and sources, and growth forms (Craine et al., 2002). Increasing dominance by C_4 grasses has been correlated with increasing aboveground biomass production (Adler et al., 2009), whereas high densities of non-leguminous forbs can be negatively associated with prairie biomass production (Kucharik et al., 2001).

Plants that have low nutrient concentrations in their aboveground biomass and perennial plants that retain nutrients in their roots for use in subsequent growing seasons are ideal for bioenergy feedstocks (Heaton et al., 2004; Fargione et al., 2010). Conversely, high nutrient concentrations in bioenergy feedstocks are undesirable because many nutrients become ash or residue in the conversion process, which can be damaging to processing equipment (McKendry, 2002). Most prairie plants are perennials and translocate nutrients to their roots at the end of the growing season (Sheedy et al., 1973; Risser and Parton, 1982). Corn, on the other hand, is an annual plant and large amounts of nutrients are removed when both the grain and stover are removed from the field (Hoskinson et al., 2007).

In addition to potentially having lower nutrient concentrations in the harvested biomass, prairie vegetation may provide numerous ecosystem services more effectively than annual row crops, including reduced soil erosion (Helmers et al. *In review*), reduced nutrient pollution (Schilling and Spooner, 2006), reduced greenhouse gas emissions (Davis et al., 2010), increased beneficial insect abundance (Gardiner et al., 2010), and increased habitat for wildlife (Fargione et al., 2009). The extensive root systems of prairie communities can reduce nitrogen and phosphorus emissions into water bodies (Lee et al., 1999; Schmitt et al., 1999), whereas corn systems can be “leaky” with regard to nutrients because no plant roots are growing in the spring when nutrient pollution is most likely to occur (Arbuckle et al., 2008). The composition of roots affects their recalcitrance to decomposition, and therefore their potential to sequester carbon. Prairie grass roots, particularly C_4 grasses, have higher carbon to nitrogen (C:N) ratios and are relatively more recalcitrant than those of legumes, forbs, and corn (Johnson et al., 2007; Fornara et al., 2009).

Management practices for prairies used for bioenergy production, including an annual harvest at the end of the growing season and fertilization, can be expected to alter prairie growth and nutrient composition. Harvesting prairie biomass late in the growing season, rather than burning, grazing, or haying, results in the removal of senesced biomass that is relatively nutrient-poor and dry (Parrish and Fike, 2005; Adler et al., 2006). Although nitrogen fertilization has been found to have a stimulatory effect on aboveground prairie biomass production (Reich et al., 2003; Camill et al., 2004), it has been found to reduce root production (Camill et al., 2004; Doll et al., 2009) and to affect prairie functional-group composition (Foster et al., 2009; Pan et al. 2010). In the research reported here, we compared contrasting prairie functional groups, grown with and without nitrogen fertilization, with corn with respect to harvestable biofuel feedstock production, nutrient allocation, and root production. We hypothesized (1) that nitrogen fertilization would increase prairie productivity and nutrient content, and (2) that fertilized prairie species would produce similar amounts of total biomass as corn but would allocate more biomass and nutrients to roots.

Materials and methods

Experimental design

Our hypotheses were tested in a field experiment that included both prairie and corn treatments organized as a randomized block design with four replicates. The prairie treatments comprised a factorial design with four groups of prairie plants with different functional-group compositions crossed with two levels of nitrogen fertilization. The four groups of prairie plants were: (1) a three-species mix of C_3 grasses (hereafter C_3 grasses); (2) a three-species mix of C_4 grasses (hereafter C_4 grasses); (3) a three-species mix of legumes (hereafter legumes); and (4) a nine-species mix of C_3 grasses, C_4 grasses, and legumes (hereafter mixture). The C_3 grasses used were prairie brome (*Bromus kalmii* A. Gray), Canada wildrye (*Elymus canadensis* L.), and slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners). The C_4 grasses used were big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.), and Indiangrass (*Sorghastrum nutans* (L.) Nash). The legumes used were Illinois bundleflower (*Desmanthus illinoensis* (Michx.) MacMill. Ex B.L. Rob. & Fernald), showy ticktrefoil (*Desmodium canadense* (L.) DC.), and round-headed bush clover (*Lespedeza capitata* Michx.). The mixtures contained all nine species listed above. All species are perennial and native to the county where the experiment was conducted. The levels of nitrogen fertilization were 0 kg N ha^{-1} yr^{-1} (hereafter unfertilized) and 150 kg N ha^{-1} yr^{-1} (hereafter fertilized). In addition to the prairie treatments, a continuous-corn treatment was also included, which was fertilized with 150 kg N ha^{-1} yr^{-1}. Nitrogen fertilizer was applied to the prairie and corn treatments as urea in 2008 and ammonium nitrate in 2009 and 2010 immediately before corn planting. In 2008, the fertilized prairie and corn treatments received an additional 45 kg N ha^{-1} on 24 June because soil test results indicated that additional nitrogen was recommended for optimal corn growth (Blackmer et al., 1997).

Study site

The experiment was conducted from May 2008 to November 2010 at the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone County, IA. Soils at the site are Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). Mean extractable phosphorus (P) and potassium (K) concentrations in the soil, as determined by the Mehlich-3 test, were 25 ppm and 165 ppm, respectively, at the beginning of the experiment, which are classified as “high” for agricultural production and not likely to be limiting to plant growth (Sawyer et al., 2011). Prior to initiation of the experiment the site had been used for corn, soybean, and oat production and was planted with soybean in 2007.

To hasten establishment of the prairie treatments and control initial plant diversity and density, prairie seedlings were transplanted into the field rather than sowing prairie seeds directly. Seedlings were grown from seed in a greenhouse beginning in late February 2008 and were transplanted into the field on 14 – 15 May 2008. Seedlings were planted into 2 x 2 m plots at a density of 54 plants m^{-2}. In the single functional group plots, each species was grown at a density of 18 plants m^{-2}, and in the mixtures, each of the nine species was grown at a density of 6 plants m^{-2}. All species locations within the plots were randomized. Tall fescue (*Festuca arundinacea* Schreb.) was seeded around the prairie plots and was not mowed to help minimize edge effects within the prairie plots. Prairie plots were watered for 2 weeks after transplantation and dead transplants were replaced through June 2008. Plots were hand weeded as necessary to remove inappropriate species. Corn plots (18 m x 10 m) were adjacent to prairie plots within each replicate block and were managed with farm machinery. A corn hybrid (DKC60-18) with 110-day relative maturity and corn rootworm, corn borer, and glyphosate resistance was planted at 79,000 seeds ha^{-1} in 76 cm rows on 13 May 2008, 22 April 2009, and 14 April 2010. Glyphosate was used as necessary for weed control in the corn plots.

Biomass production

Aboveground biomass

For the prairie systems, aboveground biomass was harvested after a hard frost, with harvests occurring on 31 October 2008, 19 October 2009, and 8 November 2010. Corn grain and stover were harvested after physiological maturity on 2 October 2008, 7 October 2009, and 5 October 2010. Prairie plants and corn were harvested by hand to approximately 2 cm above the soil surface. Although this harvest height is lower than what would likely be used for prairies and corn grown for bioenergy production (Parrish and Fike, 2005; Hoskinson et al., 2007), plants were cut close to the ground to measure maximum harvestable biomass. Harvest areas were the center 2.56 m^2 of the prairie plots and a total of 23 m^2 from the central eight rows of the corn plots. In the mixture treatments, one quarter of the harvested area was randomly selected and separated by functional group to determine the functional-group composition of the mixtures. Corn plants were separated into grain and stover components, with cobs being included in the stover component.

After the harvestable biomass was removed from the plots, the remaining plant residue was sampled to allow for a complete accounting of all aboveground biomass and nutrients. Within the prairie plots, residue was collected from two 0.1 m^2 areas within the center 2.56 m^2 of the plots. Because the corn plots were larger than the prairie plots, residue was collected from two 0.25 m^2 areas within the central eight rows of the plots. The harvested biomass and residue were dried at 60°C for at least 48 hr before being weighed. All weights are presented as dry biomass, including corn grain.

Roots

Soil cores were taken to 1 m depth in all plots using a hydraulic soil probe (Giddings Machine Co., Fort Collins, CO) following prairie residue harvest on 10-11 November 2010, which is after peak root production and at the start of post-harvest root decomposition in corn systems (Dwyer et al., 1996; Johnson et al., 2011). Four 5.08 cm internal-diameter cores were taken per plot and divided into four segments: 0-25 cm, 25-50 cm, 50-75 cm, and 75-100 cm. For the prairie systems, one soil core was taken from each of the four quadrants of the plots. For the corn systems, cores were taken within the central eight rows of the plots approximately 20 cm from corn rows. Roots were removed from the soil by elutriating the soil in strainers with 297 µm stainless steel screens for 3 hr (Wiles et al., 1996). Roots were removed from the remaining soil by sieving the portion of the sample that floated in water with 404 µm sieves followed by manually removing any remaining soil, stems, or plant residue present in the samples. Roots were then dried at 70°C for at least 4 hr before being weighed.

Carbon and nutrient allocation

Carbon, N, P, and K concentrations and C:N ratios were determined (1) in the harvested plant materials, (2) in biomass remaining on the field as residue, and (3) in the roots. A representative sample of plant tissue was ground to 2 mm with a centrifugal mill before nutrient content determinations were performed. Concentrations of C and N were determined by combustion analysis at the Soil and Plant Analysis Laboratory at Iowa State University in Ames, IA. Concentrations of P and K were determined by inductively coupled argon plasma spectroscopy at Harris Laboratory in Lincoln, NE. For the harvested biomass in the corn treatment, the grain and stover were analyzed separately, and harvested biomass nutrient concentrations were determined from the actual proportion of grain and stover in the harvested aboveground biomass. Due to the small quantity of corn roots, samples from two plots were combined in order to determine C, N, P, and K concentrations, resulting in two, rather than four, replicate samples.

Data analysis

Harvested biomass, residue, and prairie functional-group composition data were analyzed with repeated-measures ANOVAs using a compound symmetry covariance model with SAS software (SAS Institute, 2010). The residue data were natural log transformed to remove unequal variances. We used regression analyses to test for changes in harvested biomass from 2008 to 2010 for each treatment separately. Root biomass at all four depths was analyzed with an ANOVA model with depth nested within plot. Contrasts were used to test for differences among prairie functional groups at all depths. Total biomass – including harvested biomass, residue, and roots – from 2010 was analyzed with an ANOVA with Tukey's pairwise adjustment. Carbon, N, P, and K concentrations and contents were analyzed with repeated-measures ANOVAs using a compound symmetry covariance model. All treatment*year interactions were significant so the data from each year were analyzed separately, and pairwise comparisons among treatments within years were tested using Tukey's adjustment. All values were considered significant at $P \leq 0.05$.

Results

Biomass production

Aboveground biomass

Corn consistently produced more harvested biomass than any of the prairie systems, but corn yields decreased over the three years of the study ($P_{slope} < 0.0001$, $R^2 = 0.86$), with reductions in both corn grain and stover production (Figure 1). Corn grain and stover yields in 2010 were 75% and 77% those of the 2008 yields. Corn grain comprised 57% of the harvested corn biomass, and corn grain yields for 2008, 2009, and 2010 were 12.7 Mg ha^{-1}, 11.0 Mg ha^{-1}, and 9.5 Mg ha^{-1}, respectively (Figure 1). Corn produced 2.1 times the yield of the most productive prairie systems, the fertilized C_4 grasses, in 2008, but produced only 1.6 times the yield of the most productive prairie system in 2010 (Figure 1).

The prairies, on the other hand, produced either the same or more yield over the three years of the study, with the exception of the unfertilized C_3 grasses for which yields decreased ($P_{slope} = 0.0002$, $R^2 = 0.75$). Yields of the fertilized treatments with grasses – the C_3 grasses, C_4 grasses, and mixtures – did not change from 2008 to 2010 (C_3 grass: $P_{slope} = 0.79$; C_4 grass: $P_{slope} = 0.95$; mixture: $P_{slope} = 0.67$)(Figure 1). The yields of the unfertilized C_4 grasses, legumes, and mixtures, however, increased (C_4 grass: $P_{slope} = 0.013$, $R^2 = 0.48$; legume: $P_{slope} = 0.034$, $R^2 = 0.38$; mixture: $P_{slope} = 0.003$, $R^2 = 0.60$)(Figure 1). The yields of the fertilized legumes also tended to increase ($P_{slope} = 0.092$, $R^2 = 0.26$).

Among the prairie treatments, there was an overall stimulatory effect of nitrogen fertilization on yield ($P < 0.0001$), but fertilization effects varied among functional groups ($P_{functional\_group*fertilization} < 0.0001$)(Figure 1). For comparisons within functional groups, N fertilizer increased yield in the C_4 grasses in 2008 and 2009, the C_3 grasses in 2009 and 2010, and mixtures in 2008 (Figure 1), whereas legumes were never stimulated by N fertilizer. The yields of the unfertilized treatments of the C_4 grasses and mixtures increased and became more similar to those of the fertilized treatments over time and were not different from their unfertilized counterparts in 2010 (Figure 1). The composition of the unfertilized mixtures became more dominated by C_4 grasses from 2008 to 2010 and the legume component also increased (Figure 2). C_3 grasses, however, remained dominant in the fertilized mixtures during the study period (Figure 2).

The amount of residue remaining after the harvested biomass was removed differed among treatments ($P = 0.0010$) and years ($P = 0.0002$)(Figure 3). Among the prairie treatments, functional-group identity ($P = 0.0004$) and nitrogen fertilization ($P = 0.012$) affected the amount of residue remaining. Comparisons among all treatments, however, indicated that there were few differences in the amount of residue remaining over the three years of the study (Figure 3).

Roots

Corn produced approximately four times less root biomass to 1 m depth than the prairie functional group with the least root biomass, the unfertilized C_3 grasses, and approximately 22 times less biomass than the prairie functional group with the most root biomass, the unfertilized C_4 grasses (Figure 4). Among the prairie plants, functional-group identity ($P < 0.0001$), but not nitrogen fertilization ($P = 0.21$), affected root production, and there was no interaction between functional group and nitrogen fertilization ($P = 0.15$). C_4 grasses produced the most root biomass to 1 m depth, the C_3 grasses produced the least, and the mixtures and legumes produced an intermediate amount of roots (Figure 4).

Total root biomass was most strongly influenced by the mass of roots in the top 25 cm of soil in the prairie plants, especially in the treatments containing grasses (Figure 4). Seventy-six percent of the C_3 grass and mixture root mass and 72% of the C_4 grass root mass were found in the 0 to 25 cm stratum. In the legumes, 55% of the root mass was in the top 25 cm of soil. The root distribution of the corn differed from that of the prairie plants in that corn roots were more equally distributed in the soil to 1 m, although 47% of the root mass occurred in the top 25 cm of soil. At all of the soil strata below 25 cm, the C_4 grasses tended to produce more root biomass than the other prairie functional groups although differences were not significant (Figure 4).

Total biomass

Total biomass for 2010, including harvested biomass, residue, and roots, measured in the prairie and corn systems is presented in Figure 5. Corn and C_4 grasses had the highest total biomass, although the distribution of biomass to aboveground parts and roots differed greatly (Figure 5). More than 97% of the corn biomass was found aboveground, whereas 60% and 45% of the fertilized and unfertilized C_4 grass biomass, respectively, was found aboveground. Although the total biomass production of the mixtures was not statistically different from that of the corn and C_4 grasses, the mixtures produced only 72% of the corn and C_4 grass total biomass (Figure 5). The legumes and fertilized C_3 grasses had intermediate total biomass, and the unfertilized C_3 grasses had the least total biomass.

Carbon and nutrient allocation

Carbon concentrations in the harvested biomass and residue did not differ among treatments and were 424 and 371 g kg^{-1}, respectively (Table 1). The amounts of C harvested and in the residue, therefore, differed due to the amount of biomass produced (Table 2). Root C concentrations differed among treatments ($P < 0.0001$), including among prairie functional groups ($P < 0.0001$), but did not differ between nitrogen fertilization levels ($P = 0.19$). Legumes had the highest root C concentration at 428 g kg^{-1} and corn had the lowest root C concentration at 373 g kg^{-1} (Table 1). Root C concentrations were 393, 403, and 406 g kg^{-1} in the C_3 grasses, C_4 grasses, and mixtures, respectively (Table 1).

Nitrogen, P, and K in the harvested biomass, residue, and roots differed among treatments in both concentration and content, and differed among years for the harvested biomass and residue. The harvested corn biomass had higher concentrations of N, P, and K than the prairie treatments ($P_N < 0.0001$, $P_P < 0.0001$, $P_K = 0.009$)(Tables 3-5). Differences in nutrient concentrations were generally greatest between the corn and C_4 grasses, with corn having among the highest nutrient concentrations and C_4 grasses having among the lowest. Harvested corn biomass had average N, P, and K concentrations of 7.6, 1.8, and 5.7 g kg^{-1}, respectively, whereas harvested biomass of the fertilized C_4 grasses had average N, P, and K concentrations of 3.2, 0.7, and 4.3 g kg^{-1}, respectively (Tables 3-5). Concentrations of K were higher in the corn residue than the prairie residue ($P < 0.0001$), but N and P concentrations were higher in the prairie residue than the corn residue ($P_N < 0.0001$, $P_P = 0.005$). Legumes and corn had among the highest root N concentrations, whereas the unfertilized C_4 grasses had the lowest root N concentrations (Table 3). Root P concentrations were generally lowest in corn and the grasses with an average concentration of 0.7 g kg^{-1} and highest in the legumes at 2.1 g kg^{-1} (Table 3). Root K concentrations were lowest in the corn and highest in the legumes (Table 5). Among the prairie treatments, N concentrations in the harvested biomass and residue were higher in the fertilized than unfertilized treatments ($P_{harvested}$ = 0.043, $P_{residue} < 0.0001$), but were not higher in the roots (P = 0.24)(Table 3). Concentrations of P and K in the harvested biomass, residue, and roots were not affected by nitrogen fertilization (Tables 4-5).

Because corn produced large amounts of harvested biomass with high concentrations of nutrients, the amount of N, P, and K removed as harvested corn biomass was much greater than for the prairie treatments (Tables 6-8). Corn produced, on average, 1.9 times more harvested biomass than fertilized C_4 grasses (Figure 1), yet 2.7 times more N and K were harvested in the corn systems than fertilized C_4 grasses and 3.6 times more P was harvested in the corn than fertilized C_4 grasses (Tables 6-8). The differences in nutrient removal were even larger between corn and unfertilized C_4 grasses, with the corn producing 2.7 times more harvested biomass but removing 7.4 times more N, 7.7 times more P, and 6.7 times more K than the unfertilized C_4 grasses. The prairie roots contained more N, P, and K than the corn roots ($P_N < 0.004$, $P_P < 0.009$, $P_K = 0.008$)(Tables 6-8). Due to the low P and K concentrations in the corn roots, there were more than 25 and 75 times more P and K, respectively, in the unfertilized C_4 grass roots than there were in the corn roots. The relatively high N concentrations in the corn roots, however, resulted in the unfertilized C_4

grasses having only 9 times more root N. Both functional group and nitrogen fertilization had overall effects on N, P, and K contents in the harvested biomass and residue of the prairie treatments, whereas only functional group had overall effects on root N, P, and K contents (Tables 6-8).

The C:N ratios were higher in the harvested prairie biomass ($P < 0.0001$) and prairie roots ($P = 0.016$) than corn, but the C:N ratio of corn residue was higher than that of the prairie treatments ($P < 0.0001$). Among the prairie treatments, nitrogen fertilization decreased the C:N ratios of the mixture harvested biomass ($P = 0.0004$), residue ($P = 0.042$), and roots ($P = 0.041$) and C_4 grass residue ($P = 0.0004$) and roots ($P < 0.0001$), but not the harvested biomass, residue, or roots of the C_3 grasses and legumes or the harvested C_4 grass biomass. The harvested C_4 grass biomass had a C:N ratio of 141 which was the highest, whereas C_3 grass and legume harvested biomass C:N ratios were lowest among the prairie treatments at 70 and 66, respectively. The C:N ratios of the harvested biomass of the fertilized and unfertilized mixtures were 82 and 106, respectively. The unfertilized C_4 grass residue had the highest C:N ratio of 71, and the legume residue had the lowest C:N ratio of 30. The C:N ratios of the prairie roots ranged from 67 in the unfertilized C_4 grasses to 22 in the unfertilized legumes.

Discussion

The total above- and belowground biomass produced by one of the prairie functional groups – the C_4 grasses – was the same as that of corn in 2010 (Figure 5), which supported our first hypothesis. The C_4 grasses and corn all produced more than 20 Mg ha^{-1} of total biomass in 2010. Total biomass of the mixtures was not statistically different from that of the corn and C_4 grasses and was almost 15 Mg ha^{-1}.

The corn treatment produced more than twice the harvested biomass as most of the prairie systems, but corn yields decreased over time. Average corn grain yields for commercial farms in Boone County, IA, in 2008, 2009, and 2010 were 8.8 Mg ha^{-1}, 9.8 Mg ha^{-1}, and 8.6 Mg ha^{-1}, respectively (NASS, 2011), indicating that declining environmental conditions from 2008 to 2010 were not the cause of the declining corn yields in the present study. Continuous corn grown under no-till conditions with stover removal has been found to have declining yields over time on multiple soil types (Wilhelm et al., 2004; Blanco-Canqui et al., 2006; Varvel et al., 2008), though some studies have found no reductions in grain and stover yields on deep, glaciated soils (Blanco-Canqui et al., 2006) such as the ones in the present study.

The greater root production in the prairie treatments, particularly the C_4 grasses, than in the corn treatment would not contribute to the saleable products of the systems, but would contribute to the ecosystem services provided by the prairie systems and could help maintain their productivity by increasing soil organic carbon concentrations (Blanco-Canqui, 2010). The prairie grasses contained 74% of the total root biomass to 1 m in the top 25 cm of soil, and the dense sod formed by prairie grasses has been found to prevent soil erosion. Converting 10 – 20% of a row-cropped watershed to prairie vegetation has been found to reduce sediment losses due to water erosion by 95% compared to similar watersheds with

100% row crops (Helmers et al. *In press*). Removing corn stover, when combined with the small amount of roots produced by corn, has been found to exacerbate the potential for soil erosion even in no-till corn systems (Blanco-Canqui, 2010).

The fertilized prairie treatments did not consistently produce more biomass than unfertilized prairie treatments either in total biomass or harvested biomass, which did not support our hypothesis. Nitrogen fertilization never had a stimulatory effect on the legumes, probably due to the nitrogen fixing capacity of symbiotic microbes (Reich et al., 2003). In 2008 and 2009, the fertilized C_4 grasses and mixtures generally produced more harvested biomass than their unfertilized counterparts, but by 2010 the amount of harvested biomass produced in the fertilized and unfertilized C_4 grasses and mixtures did not differ (Figure 1). Heggenstaller at al. (2009) reported that fertilization with 140 kg N ha^{-1} yr^{-1} increased both total and aboveground biomass in forage varieties of the three C_4 grasses used in our study. However, wild genotypes of C_4 grass species, as were used in our study, may be less responsive to nitrogen fertilization than genotypes bred for forage production. The equivalent biomass production between the fertilized and unfertilized mixtures that we observed in 2010 was likely due to the increase in dominance by more productive C_4 grasses in the unfertilized mixtures and continued dominance by C_3 grasses in the fertilized mixtures (Figure 2).

We found that in the absence of nitrogen fertilization, harvestable biomass production in the mixtures and C_4 grasses did not differ statistically. This is at odds with the results of

Griffith et al. (2011) who found that unfertilized C_4 grass monocultures produced more harvestable biomass than unfertilized mixtures containing multiple functional groups.

As hypothesized, more nutrients were removed in the corn than the prairie systems, and the amounts of nutrients removed were disproportionately large compared to the amount of harvested biomass. The replacement costs for inorganic N, P, and K for the biomass removed from the corn, fertilized C_4 grass, and unfertilized C_4 grass harvested in 2010 would be $403, $137, and $40 per hectare, respectively (Duffy, 2011). The N and P concentrations in the residue, however, were higher in the prairie plants than the corn. Therefore, for the prairie plants, the more nutrient-dense portions of the aboveground biomass remained in the field as residue.

Among the prairie plants, N fertilization increased N concentrations in the harvested biomass and residue, but did not affect P or K concentrations in any plant parts or N concentrations in the roots, which partially supported our hypothesis. As with the harvested corn biomass, the increased N in the fertilized prairie harvested biomass would lead to lower quality feedstocks and increased need for N replacement through fertilization. The majority of the N, P, and K in the prairie plants, however, was in the roots, which would be available for plant utilization in subsequent growing seasons.

Corn roots contained less N than the prairie roots even though the N concentrations in the corn roots were higher than most of the prairie roots. Concentrations of P and K were much greater in the prairie roots than corn roots, however. The C:N ratio of the harvested corn biomass was 62, which was approximately half that of the fertilized C_4 grasses. The high C:N ratio of the C_4 grasses makes them more desirable bioenergy feedstocks (Monti et al., 2008). The C:N ratio of the corn roots was 2.6 times less than that of the unfertilized C_4 grasses, which was also the approximate difference in C:N ratio between corn roots and prairie roots from restored prairies in Illinois (Matamala et al., 2008).

In comparing corn and prairies for bioenergy production, there were tradeoffs among the amounts of harvested biomass, roots produced, and nutrients contained in the plants. Corn produced approximately twice the harvested biomass as even the most productive prairie plants. Yet corn produced up to 22 times less root mass than the prairie plants and contained higher nutrient concentrations in the harvested biomass. Because only harvested biomass is presently valued in bioenergy cropping systems, economic assessments of the profitability of corn versus grassland and prairie bioenergy cropping systems have found that corn systems are more profitable despite much larger input costs (James et al., 2010). In the present study, consistently high yields occurred with nitrogen fertilization in the prairie grasses, especially the C_4 grasses, but the unfertilized C_4 grasses produced large amounts of nutrient-poor harvested biomass by the third year and produced large amounts of high C:N root mass. The unfertilized mixtures produced a considerable amount of harvestable biomass and contained a significant proportion of legumes, which might replace some of the N removed in the harvested biomass. Based on these results, we suggest that choices of bioenergy feedstocks should include assessments of the tradeoffs among multiple production and environmental factors including, but not limited to, the ones assessed here.

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Table 1. Mean carbon (SE) concentrations of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. There were no differences among treatments for the harvested biomass and residue. Superscript letters by root values indicate differences among treatments.

	Biomass component						
	Harvested			Residue			Roots
Treatment	2008	2009	2010	2008	2009	2010	2010
	$g\ kg^{-1}$						
Corn	417(7)	415(0.5)	431(1)	352(17)	411(5)	412(4)	373(2)^{d}
C_3 grass							
Fertilized	410(3)	418(3)	428(2)	335(12)	399(2)	410(4)	395(6)^{cd}
Unfertilized	407(3)	409(1)	424(2)	321(20)	385(9)	379(16)	390(3)^{cd}
C_4 grass							
Fertilized	428(2)	423(2)	432(2)	351(16)	392(5)	362(23)	400(8)^{bcd}
Unfertilized	419(4)	413(2)	420(2)	296(10)	389(10)	364(19)	405(8)^{bcd}
Legume							
Fertilized	438(2)	435(1)	449(2)	333(27)	390(4)	401(11)	422(4)^{ab}
Unfertilized	441(1)	434(1)	446(3)	296(23)	380(10)	360(30)	434(1)^{a}
Mixture							
Fertilized	413(3)	418(2)	430(1)	331(24)	408(5)	407(06)	401(5)^{bcd}
Unfertilized	411(2)	415(4)	428(2)	381(7)	396(1)	385(19)	410(5)^{abc}

Table 2. Mean carbon (SE) contents of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component							
	Harvested			Residue			Roots	Total
Treatment	2008	2009	2010	2008	2009	2010	2010	2010
	kg ha^{-1}							
Corn	9118(100)^{a}	8136(183)^{a}	7158(219)^{a}	1223(194)^{ab}	1109(166)^{bc}	1473(223)^{a}	175(37)^{d}	8398(636)^{ab}
C_3 grass								
Fertilized	3027(241)^{cd}	3651(341)^{bc}	3270(257)^{bc}	1398(217)^{ab}	1844(324)^{ab}	1087(190)^{abc}	896(268)^{cd}	5253(622)^{cd}
Unfertilized	2013(118)^{efg}	1179(179)^{e}	908(109)^{d}	1203(214)^{ab}	1128(214)^{bc}	549(139)^{bc}	915(190)^{cd}	2371(153)^{e}
C_4 grass								
Fertilized	4443(341)^{b}	4396(496)^{b}	4518(172)^{b}	737(91)^{ab}	1235(89)^{abc}	681(130)^{bc}	3234(384)^{b}	8433(440)^{a}
Unfertilized	2402(201)^{de}	3135(294)^{c}	3357(94)^{bc}	590(34)^{b}	918(66)^{c}	512(62)^{c}	4642(737)^{a}	8512(686)^{a}
Legume								
Fertilized	1178(173)^{fg}	1377(335)^{e}	2355(677)^{c}	692(147)^{ab}	919(145)^{c}	1269(95)^{ab}	1863(701)^{c}	5488(1041)^{cd}
Unfertilized	1025(130)^{g}	1775(360)^{de}	2103(382)^{cd}	763(69)^{ab}	1110(162)^{bc}	875(206)^{abc}	1428(163)^{cd}	4406(389)^{d}
Mixture								
Fertilized	3462(460)^{bc}	3862(326)^{bc}	3395(112)^{bc}	1135(124)^{ab}	1970(274)^{a}	1152(278)^{abc}	1647(61)^{c}	6194(305)^{bc}
Unfertilized	2053(51)^{def}	2867(155)^{cd}	3272(287)^{bc}	1456(301)^{a}	1274(201)^{abc}	807(155)^{abc}	2097(245)^{bc}	6176(388)^{bc}

Table 3. Mean nitrogen (SE) concentrations of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component						
	Harvested			Residue			Roots
Treatment	2008	2009	2010	2008	2009	2010	2010
	--	--	--	g kg^{-1}	--	--	--
Corn	7.7(0.5)^{ab}	7.3(0.5)^{a}	7.9(0.4)^{a}	5.3(0.7)^{c}	4.4(0.3)^{c}	4.0(0.4)^{d}	14.6(0.4)^{abc}
C_3 grass							
Fertilized	9.7(0.5)^{a}	4.5(0.7)^{b}	6.9(0.7)^{ab}	9.8(0.9)^{a}	7.4(0.7)^{b}	11.1(1.3)^{bc}	11.9(1.5)^{abc}
Unfertilized	6.4(1.0)^{bc}	5.1(0.4)^{b}	6.9(0.6)^{ab}	7.2(0.7)^{abc}	6.8(0.6)^{bc}	9.5(1.2)^{cd}	11.0(0.2)^{bcd}
C_4 grass							
Fertilized	3.1(0.3)^{c}	3.4(0.1)^{bc}	3.1(0.2)^{d}	7.2(0.7)^{abc}	8.1(0.7)^{b}	9.4(0.8)^{cd}	9.8(0.2)^{cd}
Unfertilized	3.1(0.1)^{c}	2.4(0.1)^{c}	3.1(0.2)^{d}	5.9(1.5)^{bc}	3.9(0.4)^{c}	7.1(0.5)^{cd}	6.0(0.1)^{d}
Legume							
Fertilized	7.1(0.6)^{ab}	7.3(0.5)^{a}	7.1(0.6)^{ab}	9.5(0.7)^{ab}	12.0(1.0)^{a}	16.9(1.9)^{a}	16.8(0.9)^{ab}
Unfertilized	6.1(0.4)^{bc}	7.8(0.9)^{a}	6.2(0.9)^{abc}	9.2(0.4)^{ab}	12.6(0.8)^{a}	15.4(0.7)^{ab}	19.9(1.9)^{a}
Mixture							
Fertilized	7.6(1.3)^{ab}	4.5(0.4)^{b}	4.8(0.2)^{bcd}	9.3(0.5)^{ab}	6.4(0.2)^{bc}	10.8(0.8)^{bc}	11.8(0.6)^{bc}
Unfertilized	5.8(0.1)^{bc}	3.7(0.03)^{bc}	4.0(0.9)^{bcd}	5.8(0.1)^{bc}	5.3(0.2)^{bc}	9.9(1.6)^{bc}	9.8(1.1)^{cd}

Table 4. Mean phosphorus (SE) concentrations of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component						
	Harvested			Residue			Roots
Treatment	2008	2009	2010	2008	2009	2010	2010
	$g\ kg^{-1}$						
Corn	1.4(0.1)^{a}	1.5(0.2)^{a}	2.4(0.1)^{a}	0.7(0.1)^{d}	0.6(0.2)^{cd}	1.3(0.3)^{a}	0.7(0.1)^{b}
C_3 grass							
Fertilized	1.4(0.1)^{a}	0.9(0.04)^{ab}	1.2(0.03)^{b}	1.5(0.1)^{a}	1.0(0.04)^{abc}	1.4(0.2)^{a}	1.0(0.1)^{b}
Unfertilized	1.2(0.04)^{ab}	1.2(0.04)^{ab}	1.6(0.03)^{ab}	1.1(0.1)^{abc}	0.9(0.02)^{bcd}	1.3(0.1)^{a}	1.2(0.01)^{b}
C_4 grass							
Fertilized	0.8(0.1)^{b}	0.6(0.2)^{b}	0.8(0.1)^{b}	1.1(0.1)^{abc}	0.9(0.1)^{cd}	1.2(0.1)^{a}	1.0(0.04)^{b}
Unfertilized	1.1(0.2)^{ab}	0.9(0.1)^{ab}	0.9(0.1)^{b}	0.9(0.04)^{cd}	0.6(0.04)^{d}	0.9(0.05)^{a}	0.8(0.05)^{b}
Legume							
Fertilized	0.9(0.1)^{ab}	1.5(0.3)^{a}	1.7(0.4)^{ab}	1.1(0.03)^{bc}	1.3(0.1)^{ab}	1.5(0.3)^{a}	2.1(0.1)^{a}
Unfertilized	1.1(0.1)^{ab}	1.4(0.1)^{a}	1.5(0.3)^{ab}	1.1(0.1)^{bc}	1.4(0.1)^{a}	1.6(0.1)^{a}	2.1(0.3)^{a}
Mixture							
Fertilized	1.4(0.1)^{a}	0.9(0.05)^{ab}	1.3(0.1)^{b}	1.4(0.1)^{ab}	0.9(0.1)^{cd}	1.4(0.1)^{a}	1.3(0.1)^{b}
Unfertilized	1.5(0.3)^{a}	1.2(0.1)^{ab}	1.3(0.1)^{b}	1.2(0.04)^{abc}	0.8(0.1)^{cd}	1.2(0.1)^{a}	1.2(0.05)^{b}

Table 5. Mean potassium (SE) concentrations of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component						
	Harvested			Residue			Roots
Treatment	2008	2009	2010	2008	2009	2010	2010
	------g kg^{-1}------						
Corn	$5.9(0.5)^{a}$	$5.1(0.2)^{a}$	$6.0(0.6)^{a}$	$4.5(0.6)^{ab}$	$5.3(1.3)^{a}$	$7.4(1.2)^{a}$	$0.9(0.1)^{d}$
C_3 grass							
Fertilized	$6.5(0.3)^{a}$	$4.7(0.4)^{ab}$	$5.1(0.7)^{ab}$	$4.7(0.3)^{a}$	$3.0(0.1)^{b}$	$3.9(0.1)^{b}$	$2.2(0.5)^{bcd}$
Unfertilized	$5.4(0.3)^{a}$	$4.3(0.4)^{ab}$	$4.9(0.3)^{ab}$	$4.0(0.3)^{abc}$	$3.0(0.2)^{b}$	$4.9(0.8)^{b}$	$2.4(0.3)^{bcd}$
C_4 grass							
Fertilized	$4.9(0.8)^{a}$	$3.0(0.8)^{b}$	$5.0(0.3)^{ab}$	$4.2(0.3)^{ab}$	$2.2(0.1)^{b}$	$3.5(0.3)^{b}$	$2.9(0.2)^{bc}$
Unfertilized	$5.1(0.4)^{a}$	$3.9(0.1)^{ab}$	$3.7(0.1)^{b}$	$3.0(0.1)^{bc}$	$2.0(0.1)^{b}$	$2.7(0.2)^{b}$	$3.1(0.1)^{abc}$
Legume							
Fertilized	$5.6(0.2)^{a}$	$3.5(0.2)^{ab}$	$4.5(0.5)^{ab}$	$2.7(0.1)^{c}$	$2.4(0.2)^{b}$	$2.2(0.1)^{b}$	$4.5(0.5)^{a}$
Unfertilized	$5.1(0.4)^{a}$	$4.1(0.5)^{ab}$	$3.7(0.6)^{b}$	$2.7(0.2)^{c}$	$2.3(0.1)^{b}$	$2.6(0.2)^{b}$	$4.5(0.1)^{a}$
Mixture							
Fertilized	$6.1(0.5)^{a}$	$5.0(0.4)^{a}$	$4.9(0.5)^{ab}$	$3.9(0.3)^{abc}$	$2.9(0.2)^{b}$	$3.5(0.3)^{b}$	$2.9(0.1)^{bc}$
Unfertilized	$5.7(0.7)^{a}$	$4.4(0.3)^{ab}$	$4.7(0.4)^{ab}$	$4.1(0.3)^{abc}$	$2.3(0.1)^{b}$	$3.2(0.5)^{b}$	$2.4(0.03)^{bc}$

Table 6. Mean nitrogen (SE) contents of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component							
	Harvested			Residue			Roots	Total
Treatment	2008	2009	2010	2008	2009	2010	2010	2010
	kg ha^{-1}							
Corn	170(11)^{a}	143(9)^{a}	132(10)^{a}	17(0.5)^{bc}	12(1)^{cd}	14(3)^{bc}	7(1)^{c}	140(20)^{ab}
C_3 grass								
Fertilized	71(2)^{b}	40(8)^{bc}	54(9)^{b}	42(10)^{a}	35(9)^{ab}	30(8)^{abc}	26(8)^{bc}	110(21)^{b}
Unfertilized	31(4)^{c}	14(2)^{c}	15(3)^{c}	26(4)^{abc}	20(4)^{abcd}	13(2)^{bc}	26(5)^{bc}	53(2)^{c}
C_4 grass								
Fertilized	31(3)^{c}	36(4)^{bc}	33(1)^{bc}	15(2)^{c}	26(4)^{abcd}	18(4)^{bc}	79(8)^{a}	130(11)^{ab}
Unfertilized	18(1)^{c}	19(3)^{bc}	25(2)^{c}	12(3)^{c}	10(2)^{d}	10(1)^{c}	69(9)^{a}	104(8)^{b}
Legume								
Fertilized	19(3)^{c}	23(7)^{bc}	34(6)^{bc}	19(3)^{bc}	27(3)^{abcd}	55(9)^{a}	75(27)^{a}	164(28)^{a}
Unfertilized	14(2)^{c}	33(10)^{bc}	27(2)^{bc}	24(3)^{abc}	37(7)^{a}	38(9)^{ab}	64(4)^{a}	129(12)^{ab}
Mixture								
Fertilized	65(15)^{b}	43(7)^{b}	38(2)^{bc}	37(2)^{ab}	31(6)^{abc}	30(7)^{abc}	48(1)^{ab}	117(8)^{b}
Unfertilized	26(5)^{c}	25(2)^{bc}	31(7)^{bc}	22(4)^{bc}	17(3)^{bcd}	21(5)^{bc}	50(9)^{ab}	103(18)^{b}

Table 7. Mean phosphorus (SE) contents of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component							
	Harvested			Residue			Roots	Total
Treatment	2008	2009	2010	2008	2009	2010	2010	2010
	kg ha^{-1}							
Corn	30(2)^{a}	30(4)^{a}	39(0.4)^{a}	2(0.3)^{c}	2(0.5)^{c}	4(1)^{ab}	4(0.1)^{c}	49(1)^{bc}
C_3 grass								
Fertilized	10(0.7)^{bc}	8(0.6)^{b}	9(1)^{bc}	6(1)^{a}	5(0.7)^{a}	4(1)^{ab}	23(7)^{bc}	37(9)^{c}
Unfertilized	6(0.4)^{cd}	4(0.6)^{b}	3(0.4)^{d}	4(0.7)^{abc}	3(0.5)^{bc}	2(0.3)^{ab}	27(6)^{bc}	32(5)^{c}
C_4 grass								
Fertilized	7(0.9)^{bcd}	6(2)^{b}	8(0.6)^{bc}	2(0.3)^{c}	3(0.4)^{bc}	2(0.4)^{ab}	78(7)^{a}	89(7)^{ab}
Unfertilized	6(0.9)^{cd}	7(1)^{b}	7(0.7)^{bc}	2(0.1)^{c}	1(0.2)^{c}	1(0.1)^{b}	93(13)^{a}	102(13)^{a}
Legume								
Fertilized	2(0.3)^{d}	4(0.1)^{b}	7(0.5)^{bc}	2(0.4)^{c}	3(0.3)^{abc}	5(1)^{a}	96(42)^{a}	108(42)^{a}
Unfertilized	3(0.5)^{d}	6(1)^{b}	6(0.6)^{cd}	3(0.4)^{bc}	4(0.5)^{ab}	4(0.7)^{ab}	67(5)^{ab}	76(6)^{ab}
Mixture								
Fertilized	11(2)^{b}	8(0.9)^{b}	10(0.8)^{b}	6(0.3)^{ab}	4(0.8)^{ab}	4(1)^{ab}	52(2)^{ab}	65(3)^{ab}
Unfertilized	7(1)^{bcd}	9(1)^{b}	10(1)^{b}	5(0.9)^{abc}	2(0.3)^{bc}	3(0.5)^{ab}	60(7)^{ab}	72(8)^{ab}

Table 8. Mean potassium (SE) contents of the harvested biomass and residue from 2008 – 2010 and roots from 2010. Total biomass values include harvested biomass, residue, and roots from 2010 only. Statistical analyses were performed on each year separately for the harvested biomass and residue. Superscript letters indicate differences among treatments for each biomass component*year combination.

	Biomass component							
	Harvested			Residue			Roots	Total
Treatment	2008	2009	2010	2008	2009	2010	2010	2010
	kg ha^{-1}							
Corn	129(12)^{a}	100(6)^{a}	112(9)^{a}	16(4)^{ab}	14(4)^{a}	26(6)^{a}	5(0.3)^{d}	148(20)^{cd}
C_3 grass								
Fertilized	48(4)^{b}	41(6)^{b}	39(6)^{bc}	20(3)^{a}	14(3)^{a}	11(4)^{b}	54(28)^{cd}	105(29)^{d}
Unfertilized	26(2)^{bc}	13(2)^{de}	11(1)^{d}	15(2)^{ab}	9(1)^{ab}	5(1)^{b}	60(18)^{cd}	75(17)^{d}
C_4 grass								
Fertilized	53(12)^{b}	31(9)^{bc}	52(5)^{b}	9(1)^{ab}	7(0.8)^{ab}	6(0.9)^{b}	228(24)^{b}	286(20)^{ab}
Unfertilized	28(1)^{bc}	30(4)^{bcd}	29(1)^{bcd}	6(0.3)^{b}	5(0.4)^{b}	4(0.5)^{b}	347(52)^{a}	381(52)^{a}
Legume								
Fertilized	15(2)^{c}	11(2)^{e}	22(4)^{cd}	6(1)^{b}	6(0.9)^{b}	7(0.9)^{b}	222(104)^{b}	250(105)^{bc}
Unfertilized	12(2)^{c}	17(5)^{cde}	16(2)^{cd}	7(1)^{b}	7(1)^{ab}	6(1)^{b}	149(18)^{bc}	172(19)^{bcd}
Mixture								
Fertilized	52(10)^{b}	45(2)^{b}	39(5)^{bc}	16(1)^{ab}	14(3)^{a}	11(4)^{b}	119(4)^{bcd}	168(7)^{bcd}
Unfertilized	29(4)^{bc}	30(3)^{bc}	37(6)^{bc}	15(4)^{ab}	8(1)^{ab}	7(1)^{b}	175(20)^{bc}	218(24)^{bcd}

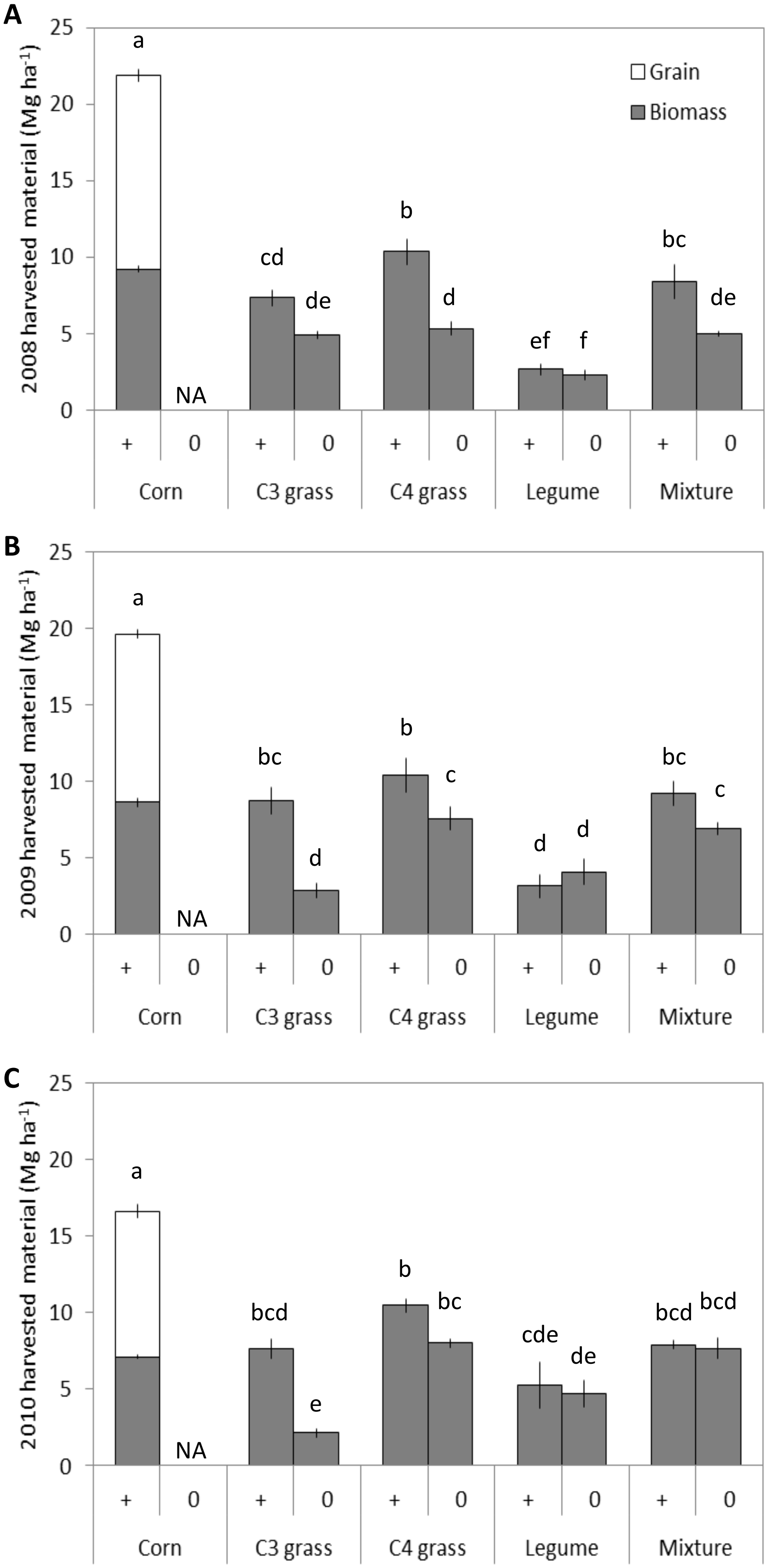

Figure 1. Mean harvested material (± SE) of corn grain and other harvested biomass of prairie and corn treatments (“+” denotes fertilized treatments and “0” denotes unfertilized treatments) in (a) 2008, (b) 2009, and (c) 2010. Lower-case letters above bars represent differences among treatments within years at P ≤ 0.05. “NA” indicates treatment not included in the present study.

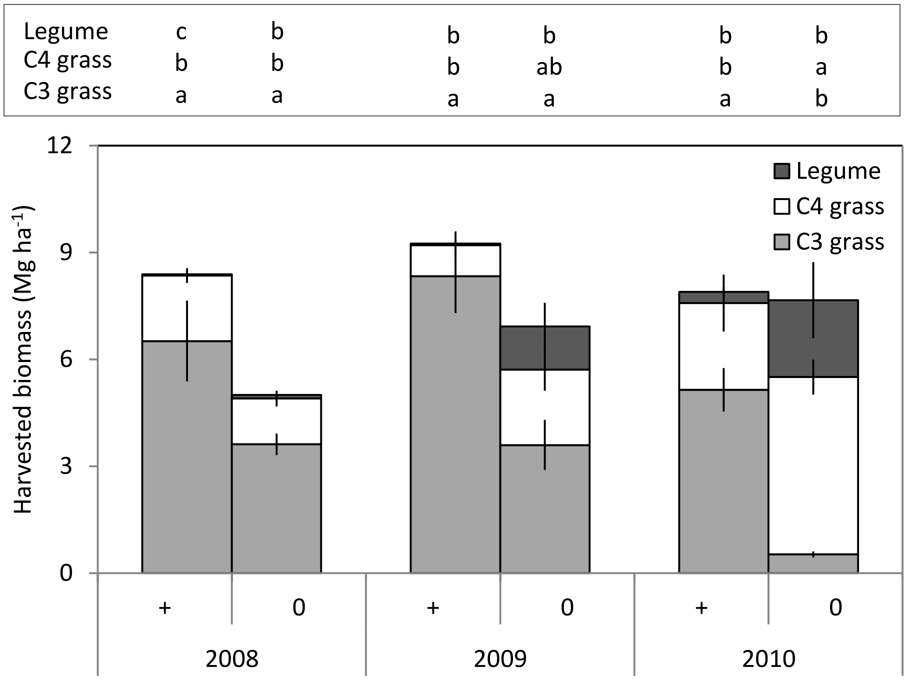

Figure 2. Mean harvested biomass (± SE) of C_3 grasses, C_4 grasses, and legumes in the mixtures (“+” denotes fertilized mixtures and “0” denotes unfertilized mixtures) in 2008, 2009, and 2010. Letters above the figure indicate differences among C_3 grasses, C_4 grasses, and legumes within each treatment at $P \leq 0.05$.

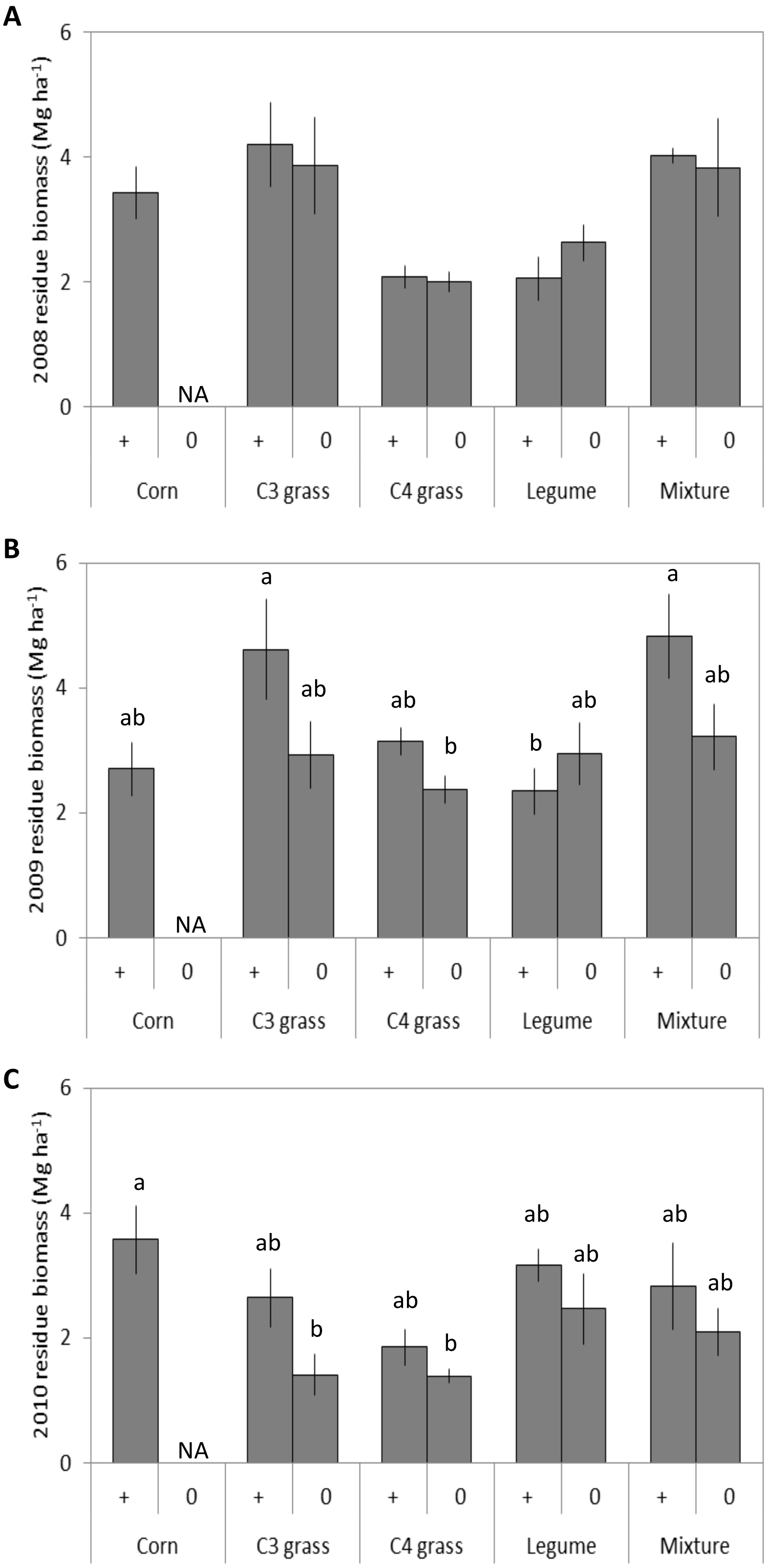

Figure 3. Mean residue (± SE) of prairie and corn treatments ("+" denotes fertilized treatments and "0" denotes unfertilized treatments) in (a) 2008, (b) 2009, and (c) 2010. Lower-case letters above bars represent differences among treatments within years at $P \leq 0.05$. There were no significant differences among treatments in 2008. "NA" indicates treatment not included in the present study.

0-5 cm	c	a	bc	b
25-50 cm	a	a	a	a
50-75 cm	b	a	b	b
75-100 cm	c	ab	a	bc
Total	c	a	bc	b

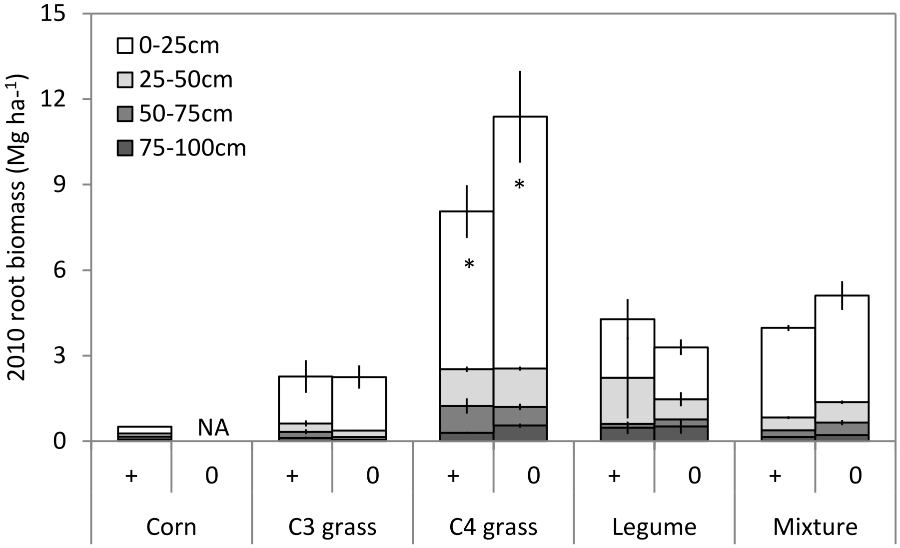

Figure 4. Mean root biomass (± SE) of prairie and corn treatments (“+” denotes fertilized treatments and “0” denotes unfertilized treatments) for 0-25 cm, 25-50 cm, 50-75 cm, and 75-100 cm depth increments. Letters above the figure indicate differences among prairie functional groups within each depth increment. Corn produced fewer roots than any of the prairie treatments. Asterisks indicate pairwise differences between fertilized and unfertilized treatments within a depth increment. “NA” indicates treatment not included in the present study.

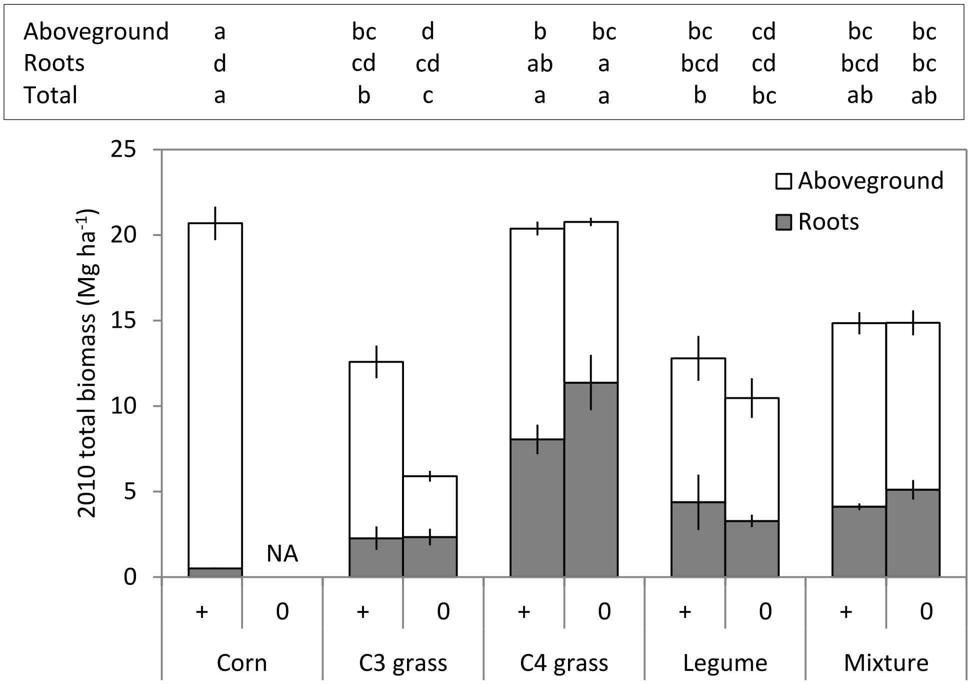

Figure 5. Mean total biomass (± SE) of aboveground biomass and roots in prairie and corn treatments ("+" denotes fertilized treatments and "0" denotes unfertilized treatments) in 2010. Letters above the figure represent differences in aboveground biomass, roots, and total biomass among treatments at $P \leq 0.05$. "NA" indicates treatment not included in the present study.

CHAPTER 5. FUNCTIONAL GROUP AND FERTILIZATION AFFECT THE COMPOSITION AND BIOENERGY YIELDS OF PRAIRIE PLANTS

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Abstract

Prairies used for bioenergy production have potential to generate marketable products while enhancing environmental quality, but little is known about how prairie species composition and nutrient management affect the suitability of prairie biomass for bioenergy production. We determined how functional-group identity and nitrogen fertilization affected feedstock characteristics and estimated bioenergy yields of prairie plants, and compared those prairie characteristics to that of corn stover. We tested our objectives with a field experiment that was set up as a 5 x 2 incomplete factorial design with C_3 grasses, C_4 grasses, legumes, and multi-functional group mixtures grown with and without nitrogen fertilizer; a fertilized corn treatment was also included. We determined cell wall, hemicellulose, cellulose, and ash concentrations; ethanol conversion ratios; gross caloric ratios; aboveground biomass production; ethanol yields; and energy yields for all treatments. Prairie functional-group identity affected the biomass feedstock characteristics, whereas nitrogen fertilization did not. Functional group and fertilization had

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[1] Primary researcher and author
[2] Provided input at all stages and supervised the work
[3] Conducted laboratory analyses
[4] Provided laboratory resources and assisted writing methods section

a strong effect on aboveground biomass production, which was the major predictor of ethanol and energy yields. C_4 grasses, especially when fertilized, had among the most favorable bioenergy characteristics with high estimated ethanol conversion ratios and non-grain biomass production and relatively high gross caloric ratios and low ash concentrations. The bioenergy characteristics of corn stover, from an annual C_4 grass, were similar to those of the biomass of perennial C_4 grasses. Both functional-group composition and nitrogen fertility management were found to be important in optimizing bioenergy production from prairies.

Introduction

The production of energy from plant biomass may either exacerbate current environmental degradation caused by agricultural production or may present an opportunity to enhance environmental quality (Robertson *et al.* 2008). Growing plant biomass for bioenergy production is likely to lead to increased environmental degradation if it results in more land being put into intensive agricultural production either through removal of land from conservation programs (Secchi *et al.* 2009) or conversion of existing grasslands and forests to produce bioenergy or food and feed crops displaced by bioenergy production (Fargione *et al.* 2008, Searchinger *et al.* 2008). Intensifying production activities on existing land, such as increasing nutrient inputs or removing all crop residues, may also exacerbate environmental damage (Tilman *et al.* 2009). Alternatively, environmental quality might be enhanced by bioenergy production if perennial plants, rather than annual plants, are used because perennial plants greatly reduce soil erosion rates and the associated phosphorus pollution (Kort *et al.* 1998, Tomer *et al.* 2010, Helmers *et al.* 2012); reduce nitrogen pollution (Helmers *et al.* 2012); provide habitat to animals (Liebman *et al.* 2011); and require fewer nutrient inputs and less management (Lynd 1996; Schmer *et al.* 2008). Furthermore, new bioenergy cropping systems could increase landscape diversity by introducing additional crops to the landscape or the crops themselves could be multi-species mixtures. Diverse mixtures of prairie plant species have been considered for bioenergy production and would be a bioenergy feedstock that could both enhance environmental quality and generate marketable products (Tilman *et al.* 2006; Hill 2007).

Most of the research on feedstock conversion of herbaceous materials into biofuels has been conducted on single-species materials, such as corn (*Zea mays* L.) stover and switchgrass (*Panicum virgatum* L.) (U.S. DOE 2011, but see Adler *et al.* 2009; DeMartini & Wyman 2011). Prairie vegetation, however, would be a heterogeneous mix comprised of multiple species. Constructed prairies, which are established on sites not currently in prairie and which may be the prairies used for bioenergy production, can be a mix of species from a pool of hundreds of potential species (Packard & Mutel 1997). Characterizing the biomass of all of these species would be difficult. However, in prairies there are four broadly-defined functional groups: cool-season (C_3) grasses, warm-season (C_4) grasses, leguminous forbs, and non-leguminous forbs (Kindscher & Wells 1995), which could be used as a basis for characterizing prairie biomass.

Studies comparing differences in forage quality among these functional groups have found differences in biomass characteristics, particularly among the C_3 grasses, C_4 grasses, and legumes (Vázquez-de-Aldana *et al.* 2000; Hatfield *et al.* 2007). Forages, however, are harvested early in the growing season when there are lower concentrations of cell wall components, which are not easily digested by livestock (Akin 1993), and when the biomass has higher nutrient concentrations (Adler *et al.* 2006). Desirable characteristics of biomass used for bioenergy production, however, include biomass with high concentrations of the energy-dense cell wall components (McKendry 2002); biomass with low nutrient concentrations because plant nutrients become ash in the conversion to bioenergy (McKendry 2002, Adler *et al.* 2006); and low moisture content (Mitchell *et al.* 2008). Harvesting prairies after plant senescence maximizes the above characteristics in addition to maintaining prairie stand health (U.S. DOE 2011). Therefore, there is a need to characterize post-senescence prairie biomass characteristics.

Nitrogen fertilization has been recommended for prairie plants grown for bioenergy, particularly C_4 prairie grasses, which have been heavily studied, in order to enhance aboveground biomass production (Parrish & Fike 2005; Heggenstaller *et al.* 2009). The stimulatory effect of nitrogen fertilization on aboveground biomass production has been found to be smaller for mixtures of prairie plants containing switchgrass, however, than for monocultures of switchgrass (Wang *et al.* 2010). Nitrogen fertilization may affect the bioenergy feedstock characteristics of prairie plants (Sanderson *et al.* 2007). The primary objective of our research was to determine how functional-group identity and nitrogen fertilization affected the feedstock characteristics and bioenergy yields of prairie plants. Our secondary objective was to compare the prairie feedstock characteristics and bioenergy yields to those of corn stover, which has been widely proposed as a potential bioenergy feedstock (U.S. DOE 2011).

Materials and Methods

Study Site and Experimental Design

We conducted a field experiment from May 2008 through November 2009 at the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone County, IA (42°00’N, 93°43’W). Soils at the site were Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) and Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls), which are both common soil types for central Iowa. Mean precipitation during the growing season (April through November) at the site is 84 cm. The growing season precipitation in 2008, which had spring flooding, was 115 cm, and the precipitation in 2009 was 94 cm. Prior to initiation of the field experiment the site had been used for corn and soybean production and was planted with soybean in 2007.

We studied the interaction between prairie functional-group identity and nitrogen fertilization in a factorial design, with four classes of prairie functional groups and two levels of nitrogen fertilization. The four classes of prairie functional groups were: (1) a 3-species mix of cool-season grasses (hereafter C_3 grasses), (2) a 3-species mix of warm-season grasses (hereafter C_4 grasses), (3) a 3-species mix of leguminous forbs (hereafter legumes), and (4) a 9-species mix of the above listed species (hereafter mixture). The C_3 grasses used were prairie brome (*Bromus kalmii* A. Gray), Canada wildrye (*Elymus canadensis* L.), and slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners). The C_4 grasses used were big bluestem (*Andropogon gerardii* Vitman), switchgrass, and Indian grass (*Sorghastrum nutans* (L.) Nash). The legumes used were Illinois bundleflower (*Desmanthus illinoensis* (Michx.) MacMill. Ex B.L. Rob. & Fernald), showy ticktrefoil (*Desmodium canadense* (L.) DC.), and round-headed bush clover (*Lespedeza capitata* Michx.). The levels of nitrogen fertilization were (1) 0 kg N ha^{-1} yr^{-1} and (2) 195 and 150 kg N ha^{-1} yr^{-1} in 2008 and 2009, respectively. In addition to the prairie plots, we included a fertilized continuous corn treatment, which resulted in the experiment being arranged as a 5 x 2 incomplete factorial design (because of the lack of an unfertilized corn treatment) arranged as randomized complete blocks with four replicates. The fertilizer rates used in this study, although above the fertilizer rates recommended for some C_4 grasses (Vogel *et al.* 2002, Heggenstaller *et al.* 2009), were chosen because they were recommended for corn production, and we sought to maintain parity among all of the fertilized treatments. The fertilized plots received 150 kg N ha^{-1} on 13 May 2008 and 18 April 2009. An additional 45 kg N ha^{-1} was applied to the fertilized plots on 24 June 2008 because the late-spring soil nitrate test indicated that additional nitrogen fertilization was recommended for optimal corn growth, whereas no additional nitrogen fertilization was recommended in 2009 (Blackmer *et al.* 1997).

Prairie seedlings were transplanted into 2 m x 2 m plots to hasten plant establishment. Seedlings were grown from seed in a greenhouse beginning in late February 2008 and were transplanted into the field on 14 – 15 May 2008. Seedlings were planted at a density of 54 plants m^{-2}. In the single functional group plots, each species was grown at a density of 18 plants m^{-2}, and in the mixtures, each of the nine species was grown at a density of 6 plants m^{-2}. All species locations within the plots were determined randomly. Prairie plots were watered for 2 weeks after transplantation as necessary and dead transplants were replaced through June 2008. Plots were hand weeded in 2008 and 2009 as necessary.

Corn was grown in larger plots (18 m x 10 m) adjacent to the group of prairie plots within each block. Location of the corn plot relative to the group of prairie plots within each block was determined randomly. Larger corn plots were used so that the corn could be managed with farm machinery. A corn hybrid (DKC60-18) with 110-day relative maturity and corn rootworm, corn borer, and glyphosate resistance was planted at 79,000 seeds ha^{-1} in 76 cm rows on 13 May 2008 and 22 April 2009. Glyphosate was used as necessary for weed control in the corn plots.

Biomass and Diversity Measurements

Aboveground prairie biomass was harvested on 31 October 2008 and 19 October 2009, after hard frost. The inner 2.56 m^2 of the each prairie plot was harvested approximately 2 cm above ground level by hand to determine aboveground biomass. This harvest height is lower than the 5-10 cm cut height that has been recommended for switchgrass managed for bioenergy (Parrish and Fike 2005), but we chose this height in order to estimate the maximum potentially harvestable biomass (see Jarchow and Liebman [2012] for data regarding the amount of prairie and corn residue remaining after harvest). Within the hand harvested areas of the mixture plots, a 0.64 m^2 area was separated into functional groups while harvesting to determine functional-group diversity. All samples were dried at 60°C for at least 48 hrs, and then weighed and ground to 2 mm using a centrifugal mill.

Corn grain and biomass were harvested on 2 October 2008 and 7 October 2009, after the grain had reached physiological maturity. Both the corn grain and corn stover were removed from the plots. Corn yields were determined by hand harvesting a 23 m^2 area within the center of each plot. Corn plants were cut to approximately 2 cm above ground level, which is lower than the 40 cm cut height that has been recommended for corn stover harvest for biomass energy production (Hoskinson *et al.* 2007). The plants were then separated into grain and stover components, which were dried at 60°C for at least 48 hrs. Samples were then weighed and ground to 2 mm using a centrifugal mill.

Feedstock Characteristic Measurements

Bioenergy may be produced either by direct combustion of plant biomass to produce heat and electricity or by converting the biomass into biofuels such as ethanol or hydrocarbons (EUBIA 2007). The efficiency with which plant biomass is converted into bioenergy significantly relies on the interaction between the conversion technologies used and the feedstock characteristics. The focus of this paper is on prairie and corn stover feedstock characteristics rather than on the bioenergy conversion technologies.

Feedstock characteristics relevant to the production of bioenergy from direct combustion include heating value, moisture content, elemental composition, and ash properties

(Jenkins *et al.* 1998). However, the heating value (also called the gross caloric ratio) defines the energy content of the biomass and is one of the most important biomass parameters for predicting direct combustion performance (Sheng & Azevedo 2005). The gross calorific ratio of a fuel is defined as the amount of heat released by a specified quantity of biomass once it is combusted.

Biochemical conversion of plant biomass into biofuels is a multi-step process that involves physical size reduction, pretreatment, hydrolysis of the cell wall polysaccharides to monomeric sugars and fermentation. The last two steps are generally combined into a single process known as simultaneous saccharification and fermentation (SSF) (Dowe & McMillan 2001). Evaluation of the biochemical conversion potential of biomass using the complete conversion process is not practical when processing a large number of samples due to the time-consuming nature of the procedure and the need for specialized equipment (Isci *et al.* 2008; Hansey *et al.* 2010).

An alternative, and less time consuming, method for estimating the biochemical conversion potential of biomass is using detergent fiber analysis (Van Soest 1967), which is used routinely by forage quality laboratories. Detergent fiber analyses have been found to be highly predictive of fermentation-based biofuel yields from cellulosic biomass and have been used successfully to predict biofuel yield potential (Lorenz *et al.* 2009; Hansey *et al.* 2010). Detergent fiber analyses estimate the cell wall components of the plant, which are comprised of hemicellulose, cellulose, and lignin, with small amounts of ash and protein (Moore *et al.* 2007).

We used an ANKOM-200 Fiber Analyzer (ANKOM Technology, Macedon, NY) to measure the hemicellulose and cellulose concentrations in the plant biomass. Lignin concentrations were not estimated because detergent fiber analyses underestimate lignin concentrations and do so differentially among functional groups (Hatfield *et al.* 2007). Duplicate samples were run for all plots from 2008 and 2009, and only corn stover was analyzed from the corn plots. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were extracted sequentially with neutral detergent solution, acid detergent solution, and 72% sulfuric acid, respectively. We measured ash concentrations gravimetrically after heating the samples to 525°C for 3 hrs in a muffle furnace. Hemicellulose was estimated as the difference between ADF and NDF values. The difference between ADL and ADF values was estimated as cellulose.

The efficiency with which the plant biomass could be converted into bioenergy was estimated based on ethanol conversion ratios and gross caloric ratios. The ethanol conversion ratio is an estimate of the amount ethanol that can be produced per gram of biomass, and is a useful indicator of potential ethanol yields. Ethanol conversion ratios were calculated from the hemicellulose and cellulose concentrations with a conversion efficiency of 51% (i.e. 0.51 g ethanol produced per 1 g hemicellulose or cellulose) (Adler *et al.* 2009). Our ethanol conversion ratios, therefore, assume that 100% of both the hemicellulose and cellulose were converted to ethanol. Different conversion processes have different efficienies with which hemicellulose and cellulose are converted to ethanol.

The gross caloric ratio is an appropriate indicator of bioenergy yields if direct combustion or thermochemical conversion of biomass are used to produce bioenergy (Sanderson *et al.* 2007). Gross caloric ratios were determined by bomb calorimetry with a 6200 Isoperibol Calorimeter (Parr Instrument Company, Moline, IL) at the Central Analytical Lab at the University of Arkansas.

Estimated Bioenergy Yields

Aboveground prairie and corn stover biomass yields were used to estimate the ethanol and energy yields. Ethanol yields of the prairie assemblages and corn stover were determined from the ethanol conversion ratios and biomass yields. Ethanol yields from the corn grain were estimated with an ethanol conversion ratio of 0.487 mL ethanol per gram dry corn grain, which was the ethanol conversion ratio achieved by commercial corn grain ethanol processing plants in 2008 (Shapouri *et al.* 2010). Therefore, the ethanol yields from corn grain are realized ethanol yields, whereas those from the prairie biomass and corn stover are theoretical maxima. We used the theoretical maxima because cellulosic ethanol conversion processes have not yet become commercialized, and therefore, it is unknown what the realizable cellulosic ethanol conversion ratios will be. Energy yields were determined from the gross caloric ratios and biomass yields.

Statistical Analyses

We analyzed feedstock characteristics and bioenergy yields of the prairie plants and corn using repeated measures analysis of variance (ANOVA) with a first-order autoregressive covariance model in PROC MIXED in SAS (SAS Institute, Cary, NC). Repeated measures analysis was used because samples were analyzed over multiple growing seasons. The treatment effect in the model had nine levels because all treatments were considered separately due to the incomplete factorial design of the experiment. Main effects of functional group and fertilizer and the functional group*fertilizer interaction were assessed only on the prairie treatments using contrasts. Contrasts were also used to test differences among pairwise comparisons of the treatments. When there were no interactions between treatment effects and year, data are presented as averages over the 2008 and 2009 growing seasons. We used regression procedures in PROC REG in SAS to examine the effect of biomass yields on ethanol and energy yields. Principal components analysis (PCA) was used to analyze relationships among feedstock characteristics of the treatments (PC-ORD, MjM Software Design, Gleneden Beach, OR). We standardized all feedstock characteristics to zero before the analysis because the units differed among the characteristics.

Results

Feedstock Characteristics

The cell wall component of the plants, which is primarily hemicellulose, cellulose, and lignin, differed between years and among treatments (Table 1), comprising between 70% and 77% of the total plant biomass (Fig. 1, Table S1). Hemicellulose and cellulose concentrations also differed between years and among treatments. Among the prairie treatments, the effects of functional group were significant, whereas those of nitrogen fertilization were not. There were also no significant functional group*fertilizer interactions. Because nitrogen fertilization did not affect the structural composition of the prairie plants, the data are averaged over the fertilizer treatments (Fig. 1, but see Table S1 for data from each treatment in each year).

Hemicellulose and cellulose concentrations were most similar among those treatments containing grasses; the legumes contained more cellulose and less hemicellulose than the grasses. Among the treatments containing grasses, the structural composition of the C_3 grasses and mixtures were not different. Hemicellulose concentrations were 273 and 276 mg g^{-1} in the C_3 grasses and mixtures, respectively, and both had a cellulose concentration of 384 mg g^{-1}. Although the mixtures were planted with maximal functional group evenness, the functional group diversity at the end of the growing seasons in 2008 and 2009 did not have maximal evenness (Table 2). The C_3 grasses were dominant in the mixtures, producing more than 72% of the biomass in the mixtures on average, with concomitant decreases in C_4 grass and legume biomass production. The structural composition of the C_4 grasses and corn were similar, which was not surprising because corn is a grass with C_4 photosynthesis. Both had 308 mg g^{-1} of hemicellulose, and 418 and 408 mg g^{-1} of cellulose in the C_4 grasses and corn, respectively.

Ash concentrations comprised less than 2% of the cell wall components. The C_3 grasses had the highest ash concentrations at 17.6 mg g^{-1}. The mixtures, corn, and C_4 grasses had intermediate ash concentrations that ranged from 12.3 to 8.6 mg g^{-1}. Ash concentrations were lowest in the legumes at 5.1 mg g^{-1}.

Ethanol conversion ratios and gross caloric ratios of the prairie plants and corn differed between years and among treatments (Table 1). Prairie functional group affected the values of both ratios, whereas nitrogen fertilization did not. Because there were no functional group*fertilizer interactions, we present means by functional groups (Table 3). Ethanol conversion ratios were greatest in the C_4 grasses and corn; least in the legumes; and intermediate in the C_3 grasses and mixtures, with a 19% difference in ethanol conversion ratios between the C_4 grasses and legumes. The legumes had the highest gross caloric ratios; the C_3 grasses had the lowest gross caloric ratios; and the C_4 grasses, mixtures, and corn had intermediate gross caloric ratios. The range of the gross caloric ratios, however, was small; there was only a 7% difference in the ratios between the legumes and the C_3 grasses.

Relationships among the feedstock characteristics are displayed in the PCA bi-plot (Fig. 2). Axes 1 and 2 explained 80.7% and 19.1% of the variability in the data, respectively. Axis 1 separated the data primarily based on the cell wall concentrations and the ethanol conversion ratios, which were positively associated with one another. The C_4 grasses were positively associated with high ethanol conversion ratios in particular but also cell wall concentrations, whereas the C_3 grasses tended to be negatively associated with those feedstock characteristics. Axis 2 separated the data based on high gross caloric ratios and cellulose concentrations versus high ash and hemicellulose concentrations. Legumes were strongly positively associated with high gross caloric ratios and cellulose concentrations. The C_3 grasses and mixtures were not distinct from one another and tended to be positively associated with high ash concentrations. The corn stover was highly variable between 2008 and 2009 with respect to Axis 1, but was more similar along Axis 2, generally having high hemicellulose concentrations.

Estimated Bioenergy Yields

The amount of biomass harvested differed between years and among treatments, and within the prairie treatments both functional group and fertilization had a strong effect on the amount of biomass produced. Although there was an interaction between treatment effects and year in biomass production ($F_{treatment*year} = 2.9$, $P = 0.012$), the effects of fertilization and functional group had a much larger effect on biomass production (see Table 1). The treatment*year interaction occurred because the biomass of the unfertilized C_3 grasses was lower in 2009 than 2008, whereas the biomass production increased or stayed the same in all other treatments. In order to focus on major trends in the data, we present biomass yields averaged over 2008 and 2009 (Fig. 3a). Nitrogen fertilization increased biomass production in the C_3 grasses, C_4 grasses, and mixtures, but did not increase the legume yield. The most biomass was produced in the fertilized C_4 grasses, fertilized mixtures, and corn stover, whereas the legumes and unfertilized C_3 grasses produced the least biomass. Average corn grain yield from 2008 and 2009 was 11.8 Mg ha^{-1} (dry weight), which was greater than the biomass produced by any of the prairie treatments. The total aboveground yield (grain plus stover) from the corn treatment was 20.8 Mg ha^{-1}.

The differences in biomass produced among the treatments, rather than ethanol conversion or gross caloric ratios, were the dominant factor affecting the ethanol and energy yields. Biomass yield predicted 98% of the variability in ethanol yields (ethanol yield in L ha^{-1} = 541*biomass in Mg ha^{-1} - 279; R^2 = 0.96; RMSE = 303) and more than 99% of the variability in energy yields (energy yield in GJ ha^{-1}= 17.0*biomass in Mg ha^{-1} + 0.615; R^2 = 0.99; RMSE = 4.18). Therefore, ethanol and energy yields were greatest in the fertilized C_4 grasses at 5500 L ethanol ha^{-1} and 179 GJ ha^{-1}, respectively. The ethanol yield from the corn grain was 5750 L ethanol ha^{-1}, and total ethanol yield from the corn grain and stover was 10400 L ethanol ha^{-1}.

Discussion

Both functional group and nitrogen fertilization affected prairie plants grown for bioenergy production. The feedstock characteristics of the prairie plants, however, only differed based on the functional-group identity. Between C_3 and C_4 grasses, C_4 grasses have been found to have higher hemicellulose concentrations, whereas cellulose concentrations have been found to be more variable (Jefferson *et al.* 2004). Plants with C_3 photosynthesis generally have higher ash concentrations than plants with C_4 photosynthesis (Bakker & Elbersen 2005). Although the C_3 grasses in our study had higher ash concentrations than the C_4 grasses, the legumes, which have C_3 photosynthesis, had very low ash concentrations.

In this study, the structural composition of the mixtures was most similar to the C_3 grasses. C_3 grasses were dominant in the mixtures, which demonstrates that knowing even the dominant functional group in a prairie can provide a means of estimating the characteristics of composite prairie biomass. Although this study did not include non-leguminous forbs, forbs are often a relatively minor component of constructed prairies (Baer *et al.* 2002, Polley *et al.* 2005, Foster *et al.* 2009, Pan *et al.* 2010) and therefore should have a lesser effect on overall prairie biomass characteristics.

We calculated an average ethanol conversion ratio for the prairie biomass at 0.483 mL g^{-1} based on the conversion of all hemicellulose and cellulose into ethanol. The values reported here were similar to, but more variable than, ethanol conversion ratios determined for biomass from multiple conservation grasslands (Adler *et al.* 2009), but higher than the ethanol conversion ratio of 0.38 mL g^{-1} determined for switchgrass using the ERG Biofuel Analysis Meta-Model (EBAMM) which is not based on the theoretical maximum for conversion (Schmer *et al.* 2008). Actual ethanol conversion ratios that can be obtained from a feedstock will be lower than those presented here and will depend on the specific conversion process being used and the efficiency with which five- and six-carbon sugars can be fermented (Casler *et al.* 2009). The conversion of hemicelluloses to ethanol, in particular, can be problematic due to the heterogeneity of hemicellulose molecules (Chandel *et al.* 2011). The gross caloric ratios presented here are similar to the gross caloric ratios of herbaceous bioenergy feedstocks, including C_3 and C_4 grasses, legumes, and corn (Scurlock 2000; McKendry 2002; Cantrell *et al.* 2009; Hu *et al.* 2010).

The PCA bi-plot (Fig. 2) highlights the relationships among the feedstock characteristics and treatments. We found that gross caloric ratios and ash concentrations were inversely related, which is the relationship that has been found consistently with many single-species feedstocks and multi-species grassland vegetation (Sheng & Azevedo 2005; Florine *et al.* 2006). Legumes had high gross caloric ratios and low ash concentrations, whereas the C_3 grasses and mixtures had relatively high ash concentrations. Gross caloric ratios and ethanol conversion ratios were nearly orthogonal to one another, indicating that the two values were not correlated, which has been found in other studies even though both values are related to the carbon concentration in the plant biomass (Sheng & Azevedo 2005). The C_4 grasses were most strongly associated with high ethanol conversion ratios. The feedstock characteristics in the corn stover differed between 2008 and 2009, which resulted in only a loose association with high hemicellulose concentrations. Corn stover feedstock characteristics have been found to vary between years; the year in which corn stover is harvested has been found to contribute to variations in feedstock characteristics more than the location where the corn was grown or the variety of corn used (Templeton *et al.* 2009).

In order to estimate the amount of bioenergy that can be produced on a given amount of land, both the feedstock characteristics and the aboveground productivity must be considered. Our results demonstrated, however, that the amount of aboveground biomass produced was the primary determinant of the ethanol and energy yields. Understanding feedstock characteristics, however, is necessary for optimizing bioenergy conversion processes or determining the best possible use for a feedstock with a given composition (Hays 2008).

Nitrogen fertilization generally increased the amount of aboveground biomass produced in the prairie systems, but there were large differences in biomass production among functional groups. We found that the fertilized C_4 grasses were the most productive of the treatments in terms of non-grain biomass, potential cellulosic ethanol yields, and energy yields. C_4 grasses have been heavily researched as biofuel feedstocks because of their high productivity (Heaton *et al.* 2004), and estimated ethanol yields have been found to increase with increasing proportions of C_4 grasses in grassland mixtures (Adler *et al.* 2009). When the corn grain was considered in analyses, however, the corn systems are more productive than any of the prairie systems. The corn systems had nearly twice the productivity and estimated ethanol yields than even the fertilized C_4 grasses, which is consistent with other analyses comparing corn and prairie systems for bioenergy production (James *et al.* 2010).

Comparing between corn and prairie systems – and among prairie systems – for bioenergy production requires evaluating tradeoffs among total output, desirable feedstock characteristics, and environmental services provided. This analysis focused on tradeoffs among bioenergy yields and feedstock characteristics in prairie systems with differing functional-group identities and nitrogen fertilization. We found that ethanol and energy yield estimates were driven almost exclusively by the amount of biomass produced even though the characteristics of the feedstocks differed among functional groups. Therefore, the functional-group composition of prairies managed for bioenergy production is likely to be important to land managers primarily as it affects aboveground biomass production, whereas the differences in feedstock characteristics among the prairie functional groups and corn stover are likely to be important to bioenergy plant engineers. Consequently, growing prairies for bioenergy production may entail managing both prairie diversity and nutrient inputs to affect biomass quality and quantity.

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Table 1. Effects of treatment, year, functional group, and fertilizer on the response variables from repeated measures analysis of variance. Prairie and corn treatments were included in treatment and year analyses, whereas only prairie treatments were included in functional group and fertilizer analyses. Separate tests were run for all response variables.

	F values			
Response variable	Treatment (df = 8,24)	Year (df = 1,27)	Functional group (df = 3,24)	Fertilizer (df = 1,24)
Cell wall	3.0^{*}	27.4^{***}	6.8^{**}	0.3
Hemicellulose	92.7^{***}	5.6^{*}	48.8^{***}	0.02
Cellulose	8.4^{***}	26.2^{***}	10.6^{***}	0.8
Ash	4.5^{**}	5.0^{*}	6.9^{**}	0.4
Ethanol conversion ratio	8.5^{***}	19.4^{***}	8.8^{***}	0.4
Gross caloric ratio	15.6^{***}	36.5^{***}	13.6^{***}	1.6
Biomass	49.4^{***}	5.7^{*}	20.6^{***}	90.6^{***}
Energy yield	46.4^{***}	10.1^{**}	20.6^{***}	89.5^{***}
Ethanol yield	50.2^{***}	17.4^{***}	25.2^{***}	80.1^{***}

$^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$

Table 2. Percentage of mean (± SE) aboveground biomass of legumes, C_3 grasses, and C_4 grasses from the unfertilized and fertilized multi-functional group mixtures in 2008 and 2009.

	Percent of total mixture biomass			
Functional	2008		2009	
group	Unfertilized	Fertilized	Unfertilized	Fertilized
Legume	2.1 ± 0.9	0.5 ± 0.2	19.5 ± 11.3	0.4 ± 0.3
C_3 grass	72.1 ± 4.9	76.3 ± 3.9	50.8 ± 7.1	89.4 ± 4.5
C_4 grass	25.8 ± 4.5	23.2 ± 4.0	29.7 ± 7.8	10.2 ± 4.5

Table 3. Mean (± SE) ethanol conversion ratios (ethanol) and gross caloric ratios (energy) of legumes, C_3 grasses, C_4 grasses, mixtures and corn stover. Treatments are averaged over years and nitrogen fertilization levels. Superscripted letters after SE indicate differences among treatments at $P \leq 0.05$ for ethanol conversion ratios and gross caloric ratios separately.

Treatment	Feedstock characteristics	
	Ethanol (mL g^{-1})	Energy (kJ g^{-1})
Legume	0.443 ± 0.007^{c}	19.93 ± 0.07^{a}
C_3 grass	0.479 ± 0.012^{b}	16.77 ± 0.14^{c}
C_4 grass	0.529 ± 0.046^{a}	17.17 ± 0.10^{b}
Mixture	0.481 ± 0.007^{b}	17.02 ± 0.14^{b}
Corn stover	0.522 ± 0.026^{a}	17.03 ± 0.17^{bc}

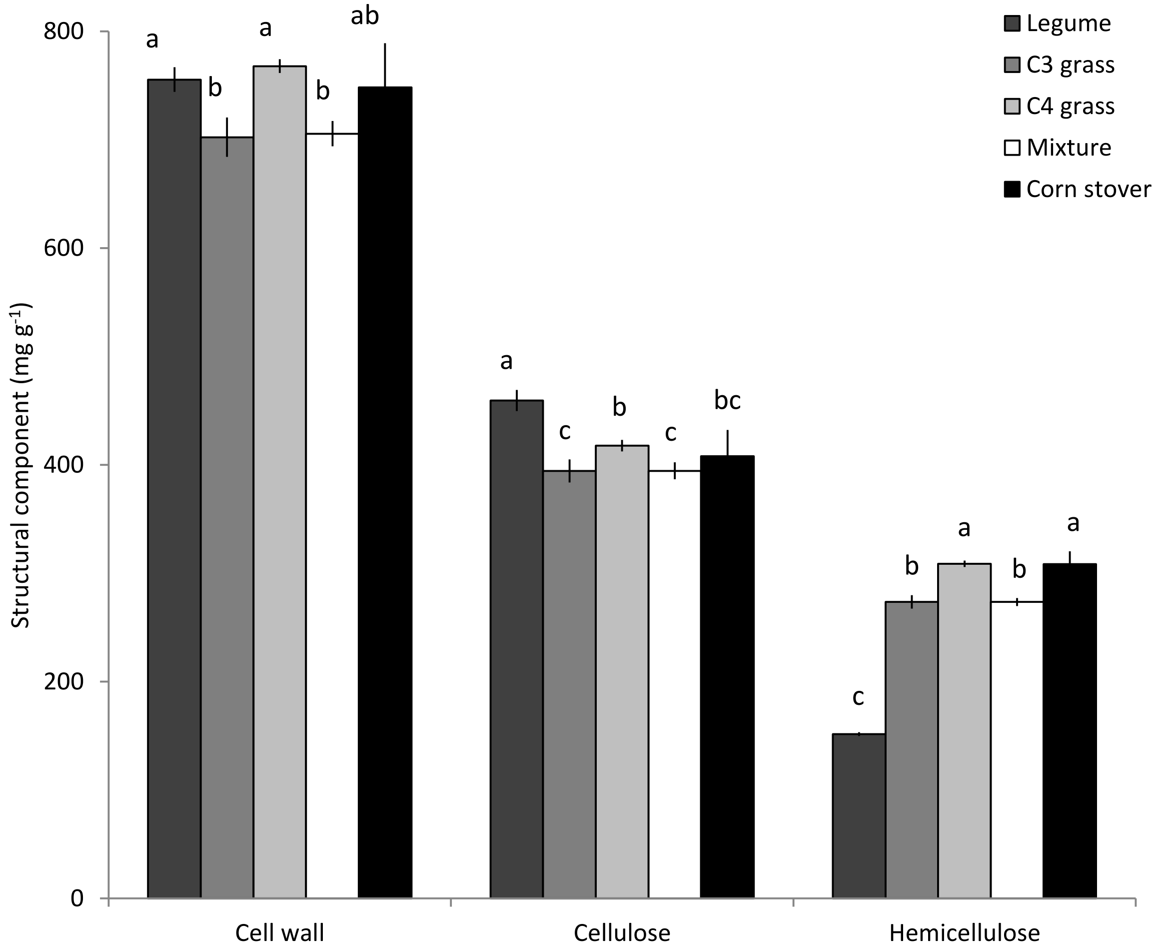

Fig. 1. Mean (± SE) cell wall, cellulose, and hemicellulose concentrations of legumes, C_3 grasses, C_4 grasses, mixtures, and corn stover. Treatments are averaged over years and nitrogen fertilization levels. Within each structural component, letters above bars indicate differences among treatments at $P \leq 0.05$.

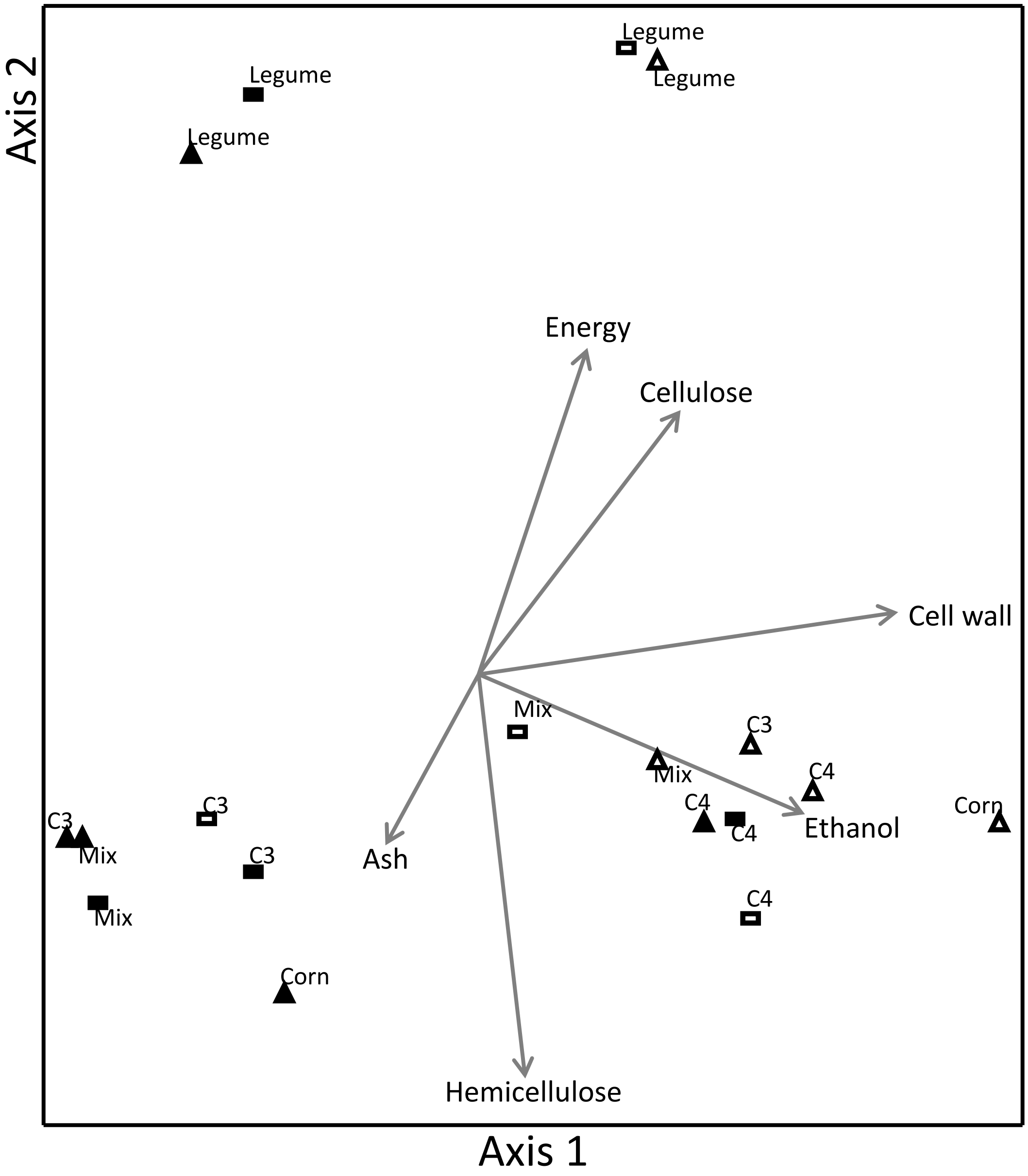

Fig. 2. Principal component analysis bi-plot of the feedstock characteristics of the prairie and corn treatments. Treatment means are displayed for unfertilized (*squares*) and fertilized (*triangles*) treatments in 2008 (*closed symbols*) and 2009 (*open symbols*). Arrows indicate the magnitude of the contribution of each of the measured feedstock characteristics. Treatments located close to a feedstock-characteristic arrow are more positively related to that characteristic.

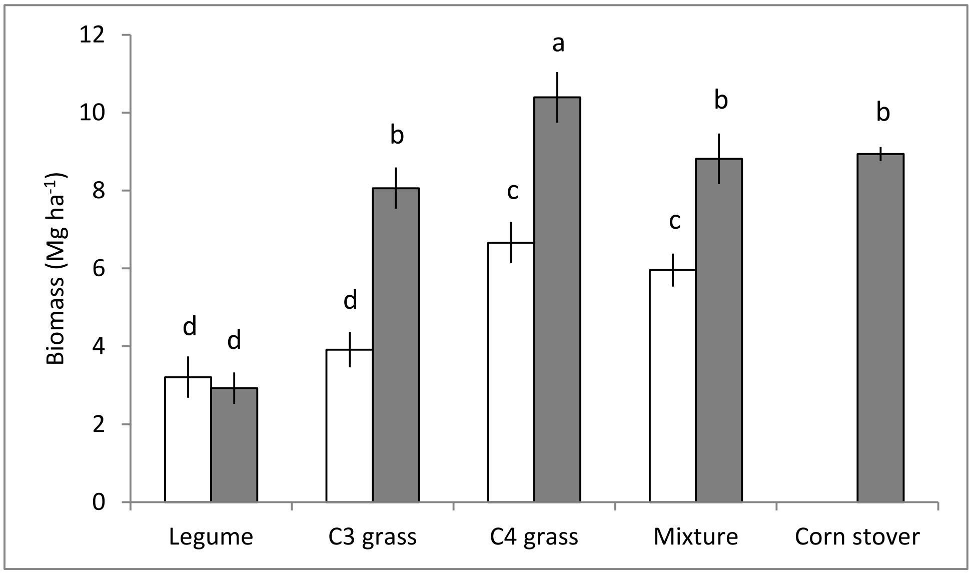

Fig. 3. Mean (± SE) aboveground biomass production of unfertilized and fertilized legumes, C_3 grasses, C_4 grasses, mixtures, and corn stover. Treatments were averaged over 2008 and 2009. Letters above bars indicate differences among treatments at $P \leq 0.05$. Aboveground biomass production was highly predictive of ethanol yields ($R^2 = 0.96$) and energy yields ($R^2 = 0.99$); see text for prediction equations.

Supporting information

Table S1. Mean (± SE) cell wall, cellulose, hemicellulose, and ash concentration of corn stover and fertilized and unfertilized C_3 grasses, C_4 grasses, legumes, and mixtures in 2008 and 2009.

	Biomass characteristic							
	Cell wall		Cellulose		Hemicellulose		Ash	
Treatment	2008	2009	2008	2009	2008	2009	2008	2009
	-----mg g^{-1}-----							
Corn stover	688 ± 72	808 ± 13	374 ± 44	442 ± 5	295 ± 23	322 ± 3	12.9 ± 4.4	9.6 ± 2.6
C_3 grass								
Fertilized	663 ± 17	773 ± 4	356 ± 10	428 ± 4	265 ± 3	290 ± 4	23.3 ± 6.6	14.7 ± 2.2
Unfertilized	689 ± 6	684 ± 63	377 ± 1	377 ± 35	276 ± 4	263 ± 24	21.2 ± 3.3	11.0 ± 3.3
C_4 grass								
Fertilized	763 ± 14	780 ± 10	419 ± 12	423 ± 12	301 ± 3	307 ± 3	9.8 ± 3.7	6.1 ± 1.7
Unfertilized	766 ± 12	763 ± 17	415 ± 11	414 ± 12	307 ± 6	319 ± 9	6.1 ± 1.7	9.5 ± 2.5
Legume								
Fertilized	716 ± 7	791 ± 14	428 ± 10	491 ± 16	151 ± 3	153 ± 4	7.4 ± 3.3	5.4 ± 3.2
Unfertilized	727 ± 26	787 ± 13	435 ± 18	483 ± 13	148 ± 3	154 ± 4	3.2 ± 2.3	4.4 ± 2.1
Mixture								
Fertilized	664 ± 10	759 ± 5	356 ± 7	418 ± 6	266 ± 7	288 ± 2	15.2 ± 3.3	12.5 ± 4.1
Unfertilized	663 ± 10	737 ± 7	361 ± 11	403 ± 10	271 ± 5	278 ± 11	13.1 ± 4.6	8.5 ± 4.0

CHAPTER 6. CONCLUSIONS

The goal of my dissertation research was to compare prairie and corn systems managed for bioenergy production with a focus on the prairie systems. One of the important results of this research was to highlight the tradeoffs in the production of ecosystem services, including provisioning, regulating, and supporting services (MEA 2005), between the prairie and corn systems and among the prairie systems.

We found that the corn systems were incredibly productive in terms of harvestable biomass; they produced approximately twice the harvestable biomass as even the most productive prairie systems. The biomass harvested from the corn systems, however, was high in nutrients, which would need to be replaced in order to maintain the productivity of the system. Nutrient replacement costs were approximately three and ten times greater in the corn systems than the fertilized and unfertilized C_4 grass systems, respectively, which were among the most productive prairie systems.

Corn produced ten times less root mass than the average prairie root mass. Increased root production is associated with increased soil organic matter concentrations (Blanco-Canqui 2010) which help maintain soil productivity (Anderson 2008). The prairie roots were also more likely to contribute to long-term carbon storage than corn due to greater production of roots lower in the soil profile and with higher C:N ratios in the roots (Johnson et al. 2007, Matmala et al. 2008). The dense sod of living prairie roots near the soil surface which was not found in the corn systems has been found to prevent soil erosion (Helmers et al. 2012).

Among the prairie systems there were also tradeoffs in the production of ecosystem services. Nitrogen fertilization increased the aboveground biomass production in all of the prairie systems except for the single-functional-group legume treatments. Nitrogen fertilization did not, however, increase root production in the prairie plants, which has been found in the COBS prairie systems also (R. Dietzel *unpublished data*). The fertilization decreased the C:N ratios of the roots, which likely lead to increased root decomposition as has been found in corn systems (Russell et al. 2009).

We had mixed results regarding the effect of nitrogen fertilization on prairie diversity. In the COBS experiment, where a high-diversity seed mix was used, species diversity was lower in the fertilized prairie in June over the study period, but species diversity was higher in the fertilized prairie in August by the end of the study period due to increased species evenness, particularly due to increased forb abundance. In the DIVN experiment, which did not contain non-leguminous forbs, nitrogen fertilization consistently reduced diversity due to continued dominance by C_3 grasses. The presence of forbs in prairies, as was exemplified in the fertilized prairie in the COBS experiment, is necessary to provide food resources for insects that provide the ecosystem services of pollination and pest control (Isaacs et al. 2009). The large reduction in legume abundance with nitrogen fertilization, which was consistent between both experiments, would likely reduce the input of fixed atmospheric nitrogen into the systems, although the amount of nitrogen fixed by legumes in prairie systems has been estimated to be small (Woodmansee 1978).

In the DIVN experiment, where we tested the effects of functional groups separately, the C_4 grasses were among the most productive. Grassland systems with only one functional group, however, would lack the structural heterogeneity of diverse prairies, and therefore would provide inferior animal habitat than prairies (Robertson et al. 2011). Furthermore, these grasslands would not exhibit phenological complementarity of resource use. In the DIVN experiment, phenological complementarity occurred in the unfertilized multi-functional group mixtures where C_3 grasses were more dominant in the spring and C_4 grasses were dominant in the fall. Phenological complementarity also occurred in the COBS experiment. In both prairie treatments there was a shift from Canada wildrye (*Elymus canadensis* L.) in June to the C_4 grasses, particularly big bluestem (*Andropogon gerardii* Vitman), in August. In the fertilized prairie, the prairie also had greater forb abundance in August than June.

Having the data to quantify differences in the production of many ecosystem services among alternative agricultural land uses, including the tradeoffs among them, is necessary if we want to move towards more multifunctional agricultural systems. Multifunctional agricultural systems are managed to produce agricultural commodities in addition to a wide range of ecosystem services (Jordan et al. 2007, Jordan and Warner 2010). Current agricultural systems are often managed to maximize the production of agricultural commodities at the expense of other ecosystem services (Robertson and Swinton 2005), but there is a need to manage agricultural systems to optimize the production of many ecosystem services (Foley et al. 2005, Jarchow and Liebman 2011). The market does not currently value most ecosystem services, however, even though they are known to be tremendously valuable (Costanza et al. 1997). Valuation of more ecosystem services, through governmental policies for example, would aid in the expansion of multifunctional agricultural systems on the landscape (Jordan and Warner 2010, Jarchow et al. 2012).

The research that I conducted for my dissertation is one step in the process of quantifying how prairies could be used for bioenergy production within multifunctional agricultural systems. Additional research quantifying the production of many ecosystem services, such as regulation of greenhouse gas emissions, water purification, provision of wildlife habitat, and soil protection and enhancement, are needed. My research was conducted on prime agricultural land, and research is also needed to compare the production of ecosystem services of bioenergy cropping systems at multiple locations on the landscape, including land that is considered marginal for agricultural production. Because prairies are perennial systems that change over time, long-term research is needed to study the effects of managing prairies for bioenergy production on the functioning of the prairies over decadal time scales.

In addition to studying how managing prairies for bioenergy production affects the ecology of the systems, the management of prairies for bioenergy production should be compared to other prairie management regimes. In particular, research is needed to assess how an annual post-senescence harvest for bioenergy feedstock production differs from more commonly studied prairie disturbances including mowing, grazing, and burning. Mowing prairies for hay is distinct from post-senescence harvesting even though similar harvesting equipment can be used. Hay is often harvested during the summer when there is near maximum aboveground biomass production but while the biomass still has high nutritional quality (Kansas Biological Survey 2009). Summer mowing for hay production has been found to maintain species diversity in native prairies by decreasing the abundance of C_4 grasses (Collins *et al.* 1998).

Animal grazing differs from post-senescence harvesting because animals often selectively graze rather than completely removing all aboveground biomass above a harvest height as with post-senescence harvesting. Bison (*Bos bison* L.) grazing in particular has been found to increase prairie diversity (Hartnett *et al*. 1996, Collins *et al.* 1998) because bison preferentially graze C_4 grasses and avoid forbs thereby increasing forb abundance and richness (Steuter & Hidinger 1999). Although domestic livestock are less selective towards C_4 grasses than bison, livestock still predominantly eat C_4 grasses and have been found to increase prairie diversity (Steuter & Hidinger 1999, Collins & Smith 2006).

Burning prairies differs from post-senescence harvesting because burning results in the differential return of nutrients to the prairie based on what forms of the nutrients are produced during combustion. Historically most prairie fires are believed to have occurred during the summer due to lightning strikes (Howe 1994). Currently most prairie burns are conducted in the spring (Howe 1994, Howe 1995). Spring burning decreases C_3 grass and forb abundance while increasing C_4 grass abundance (Howe 1995, Collins *et al.* 1998). The less commonly studied winter burning, which is more similar to the timing of post-senescence harvests, has been found to increase forb and C_3 grass abundance compared to late-spring burning (Towne & Owensby 1984).

Reconstructing and managing prairies for bioenergy production has the potential to allow prairies to be reincorporated back into the landscape. The specific management regime of growing prairies for bioenergy production, including a post-senescence annual harvest and possible nitrogen fertilization, will affect the ecology of the systems. Therefore, research, including the type of research included in this dissertation, is needed to assess how this novel use of prairies affects their composition and functioning.

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