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The Effect of Nitrogen, Phosphorus, and Potassium Fertilizers on Prairie Biomass Yield, Ethanol Yield, and Nutrient Harvest

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Abstract Native prairie plants can be managed to provide biomass for cellulosic ethanol production; however, there is inadequate information in northern latitudes regarding the effects of fertilizers on biomass and ethanol yields. We evaluated biomass yield, land ethanol yield (theoretical ethanol production per unit area), and nutrient harvest in grasslands managed across a gradient of nitrogen (N), phosphorus (P), and potassium (K) fertilizers at three locations in MN, USA, from 2008 to 2009. The Austin and Lamberton locations were planted with a mixture of prairie plants, while the Rosemount location was solely switchgrass (*Panicum virgatum* L.). Model-based estimations of agronomically optimum nitrogen rates (AONRs) for land ethanol yield were determined for five of six site-year environments. Five response functions were modeled for land ethanol yield, each predicting a unique AONR with varying degrees of confidence. The linear plateau function was best-supported for four of six environments. Agronomically optimum nitrogen rates ranged from 61 to 87 kg N ha^{-1} and, on average, yielded 3,160, 2,090, and 3,180 L ethanol ha^{-1} at Austin, Lamberton, and Rosemount, respectively. On average, predicted ethanol yields increased 52 % when fertilized at AONRs compared to yields without fertilizer. Phosphorus and K fertilizers did not affect land ethanol yield. Nitrogen, P, and K removed during biomass harvest increased with N fertilization and averaged 31, 6, and 20 kg ha^{-1} at the AONRs. Nitrogen use efficiency declined with N fertilization during drier years. Modest rates of N fertilizer (between 60 and 90 kg N ha^{-1}) can maximize cellulosic ethanol production in established northern latitude grasslands. Soil P and K should be monitored as nutrients are removed during repeated biomass harvests.

Keywords Biomass yield · Cellulosic ethanol · Switchgrass · Prairie · Nitrogen fertilization · Agronomically optimum nitrogen rate

Introduction

The US Department of Agriculture estimates that more than 50 billion L of advanced biofuels will be produced from dedicated energy crops by 2022 to meet the larger national target of 80 billion L [1]. One advanced biofuel is cellulosic ethanol, which is an alternative transportation fuel that can be derived from perennial, non-food crops to limit greenhouse gas emissions and promote energy security [2]. Perennial grasses such as switchgrass (*Panicum virgatum* L.), Miscanthus (*Miscanthus*×*giganteus*), and big bluestem (*Andropogon gerardii* Vitman) have been identified as potential dedicated energy crops for cellulosic ethanol based on their relatively high yields and their adaptability to a broad range of growing conditions [3]. Much of the research on dedicated energy crops has focused on maximizing yields by growing them in monoculture [4, 5]. However, mixtures of native perennial plants that include species from multiple plant functional groups—such as warm-season (C4) grasses, cool-season (C3) grasses, legumes, and non-legume forbs—can increase biomass yields [6, 7] and provide additional ecosystem services compared to monocultures [8–10]. Grasslands with a mixture of grasses and legumes produced

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more biomass when harvested in autumn than most monocultures across eight study sites in MN, USA [11]. In other studies, C4 grass-legume bicultures had greater harvestable biomass and belowground carbon accumulation than monocultures [9].

Although cellulosic biofuel feedstocks may be harvested from fields sown with dedicated energy crops, mixed-species biomass from marginal land has direct greenhouse gas mitigation potential that rivals dedicated energy crops [12]. For example, there are more than 1.4 million ha of perennial grassland seeded in the Conservation Reserve Program (CRP) in Minnesota, North Dakota, and South Dakota. Perennial grassland biomass yield from marginal land enrolled in the CRP was as high as 7.9 Mg ha^{-1} without fertilization [13], but the bioenergy production potential of these lands managed with fertilization is uncertain.

Fertilization may be needed to replace nutrients removed during annual biomass harvest to avoid depleting the soil reserve and subsequently lowering biomass yields. For example, available soil phosphorus (P) decreased at some sites in North and South Dakota after 5 years of annual switchgrass harvest, suggesting that P fertilizer may be necessary for long-term harvest sites [14]. Nitrogen in harvested biomass can be substantial in high-yielding, nitrogen (N)-fertilized systems as demonstrated by Guretzky et al. [15], who reported harvest N rates of 85 kg N ha^{-1} in switchgrass biomass fertilized at 90 kg N ha^{-1}. Although potassium (K) harvest has been reported for switchgrass, big bluestem [16], and mixed-species grasslands [17], the implications of K harvest from grasslands are less understood. Nutrient removal through harvest of monoculture bioenergy crops vary by species [18] and fertilization rates [16]. Therefore, determining nutrient harvest from dedicated energy crops and mixed-species grasslands across locations and a fertilizer gradient is essential for planning economically viable, agronomically productive, long-term bioenergy operations.

Lignocellulose from energy crops and mixed-species grasslands can be used for ethanol production. Maximum theoretical ethanol potential can be estimated based on the concentration of fermentable sugars within biomass lignocellulose [19]. Previous studies reported an average theoretical ethanol potential of 405 L Mg^{-1} in switchgrass harvested in ND, USA [20], 450 L Mg^{-1} in mixed-species biomass from conservation grasslands in MN, USA [21], and 388 L Mg^{-1} in C4-dominated grasslands in MN, USA [22]. Furthermore, multiplying theoretical ethanol potential by biomass yield provides a measure of ethanol potential per unit area, hereafter referred to as land ethanol yield. Estimates of land ethanol yield range from 1,100 L ha^{-1} from conservation grassland biomass [21] to 5,500 L ha^{-1} for fertilized C4-dominated grasslands [7] in the Upper Midwest, USA. Fertilizer rates that maximize land ethanol yield are unknown for mixed-species grassland biomass in the Upper Midwest, USA.

Land ethanol yield is strongly influenced by biomass yield. The effect of fertilization on biomass yield has been studied for various bioenergy feedstocks to identify optimal fertilization rates [5, 23–25]. In most studies, linear regression was used to fit various response functions to identify the N fertilization rate at which biomass yields are maximized: the agronomically optimum N rate (AONR). Examples of AONRs for switchgrass managed for bioenergy in the Midwestern US ranged from 60 to 120 kg ha^{-1} [26, 27]. However, many studies reporting AONRs do not report statistical reliability with their estimates. Failing to include confidence intervals or other measures of statistical uncertainty in AONR estimates can lead to over or under-application of fertilizers and suboptimal crop production [28]. Methods to calculate uncertainty of AONRs have been reported for corn production [29].

Determining the AONR that maximizes land ethanol yield of mixed-species grasslands harvested after senescence will provide useful information to increase production efficiency. Our objectives were to measure mixed-species grassland and switchgrass biomass and ethanol yield across a range of N fertilizer rates, determine whether responses were affected by P or K fertilization, and identify an AONR based on land ethanol yield for three regions of MN, USA. Another objective was to measure the effect of fertilization on biomass nutrient harvest to determine nutrient removal and N use efficiency of harvested biomass across fertilizer treatments and environments.

Methods

Site Description

Research was conducted on established stands of native perennial plants at sites in Austin, Lamberton, and Rosemount, MN, in 2008 and 2009 (Table 1). The Austin and Lamberton sites were restored in 2005 to a diverse mixture of native grasses and forbs. The average canopy cover was 64 % perennial grasses, 35 % forbs, and 2 % weeds at Austin and 62 % perennial grasses, 16 % forbs, and 23 % weeds at Lamberton. The most prominent grass species at both polyculture sites were switchgrass, big bluestem, and indiangrass (*Sorghastrum nutans* (L.) Nash). Common forbs at Austin were Canada goldenrod (*Solidago canadensis* L.), yellow coneflower (*Ratibida pinnata* (Vent.) Barnh.), and black-eyed Susan (*Rudbeckia hirta* L.). Common forbs at Lamberton were Maximilian sunflower (*Helianthus maximilani* Schrad.), daisy fleabane (*Erigeron strigosus* Muhl. ex Willd.), and black-eyed Susan. Common weeds at Austin and Lamberton were green foxtail (*Setaria viridis* (L.) Beauv.), common ragweed (*Ambrosia artemisiifolia* L.), and

Table 1 Site description of three experimental locations in MN, USA

Location	GPS coordinates	Soil description	Grassland type	pH	Organic matter (%)	N (kg ha^{-1})	Bray P (ppm)	K (ppm)
Austin	43° 40″ N 92° 58″ W	Sargeant silt loam (fine-loamy, mixed, superactive, mesic Aquic Glossudalfs)	Mixed-species	5.9	3	2.5	12	126
Lamberton	44° 14″ N 95° 18″ W	Ves clay loam (fine-loamy, mixed superactive mesic Calcic Hapludolls)	Mixed-species	7.2	3.8	2.7	8	172
Rosemount	44° 44″ N 93° 7″ W	Waukegan silt loam (fine-silty over sandy, mixed mexic, Typic Argiudoll)	Switchgrass monoculture	6.8	4.3	2.8	49	160

Canada thistle (*Cirsium arvense* (L.) Scop.). The Rosemount site was seeded to a commercially marketed switchgrass variety, "Sunburst," in 2005. Initial stands at all locations had >95 % ground cover prior to fertilizer application in 2008. All locations were rain-fed (Table 2).

Experimental Design and Field Methods

The experimental design was a randomized complete block with four replications per location. Treatments were applied in a complete factorial arrangement of either N and P or N and K. Plots were 3 m×3 m and received variable rates of N fertilizer (0, 56, 112, 168, and 224 kg N ha^{-1}) as ammonium nitrate (34-0-0) that were combined in a factorial arrangement with variable rates of P or K fertilizer depending on initial soil fertility tests. For the low P soils at Austin and Lamberton, P was applied at rates of 0, 34, 67, 101, and 135 kg P ha^{-1} as triple super phosphate (0-46-0) and for the low K soil at Rosemount, K was applied at 0, 45, 90, 135, and 179 kg K ha^{-1} as potassium chloride (0-0-60). Fertilizers were broadcast in May of 2008 and 2009.

Biomass yield was determined by harvesting and weighing a representative 1 m×1 m area to a 1.5-cm stubble height within each plot in early November each year following a killing frost (−2 °C). A subsample of the harvested material from each plot was oven-dried at 57 °C to adjust biomass yields for moisture concentration (g kg^{-1}); thus, yields were expressed on a dry matter basis. Each subsample was then ground and analyzed for cell wall polysaccharides using a combination of wet chemistry [30] and near-infrared reflectance spectroscopy (NIRS) [31]. Equations for NIRS were developed using the software program Calibrate (NIRS 3 version 4.0, Infrasoft International, Port Matilda, PA) with the modified partial least squares regression option [32]. Ethanol potential was calculated using the energy ethanol yield calculator (http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html), which was based on biomass 5- and 6-carbon sugar concentrations with the following equation:

$$\text{Theoretical ethanol yield } \mathrm{L\,Mg^{-1}}$$
$$((\%\,\text{Arabinose} + \%\,\text{Xylose}) \times 737.55)$$
$$+ ((\%\,\text{Glucose} + \%\,\text{Galactose} + \%\,\text{Mannose}) \times 720.66)$$

Land ethanol yield was calculated by multiplying ethanol potential by biomass yield. Biomass N was determined by combustion and P and K by inductively coupled plasma spectrometry using standard procedures at a commercial laboratory (Agvise Laboratories, Benson, MN). Nutrient harvest was calculated by multiplying biomass nutrient concentrations by biomass yield.

Table 2 Precipitation and 30-year averages for each month of the growing season from 2008 to 2009 at three locations in MN, USA

Month	Austin			Lamberton			Rosemount		
	2008	2009	30-year average[a]	2008	2009	30-year average	2008	2009	30-year average
	Precipitation (mm)								
April	155	74	90	75	38	75	118	57	74
May	100	111	110	82	41	83	68	34	103
June	216	149	124	91	82	106	117	100	120
July	79	60	121	85	42	95	71	47	114
August	74	86	112	15	88	93	77	198	120
September	41	30	88	54	71	84	58	15	92
October	57	191	60	107	138	52	51	160	73
Total	722	701	705	509	500	588	560	611	622

[a] 30-year average=1978–2008

Statistical Analysis

Data were first analyzed as a complete block design with a factorial arrangement. Data from each location were analyzed separately due to variation in plant species composition and fertilizer type. The effect of N, P, and K fertilizer and year were determined using analysis of variance (ANOVA) with α=0.05. Fertilizers were analyzed as factored variables when used with ANOVA for all response variables. Fisher's least significant difference (LSD; P=0.05) was used to identify differences in means between levels of significant factor predictors (no more than five comparisons per test). When fertilizers were significant based on ANOVA, they were analyzed as continuous predictor variables using linear regression. We analyzed year as a fixed effect to quantify the changes in yield response to N fertilizer through time. Since these crops were perennial and possibly experiencing changes in root biomass or morphology during maturation, we expected that aboveground yields would vary in response to fertilizers through time.

Agronomically Optimum Nitrogen Rate

Agronomically optimum nitrogen rates were determined for land ethanol yield by fitting five response models to the data. The five response models were linear (LR), quadratic (QD), square root quadratic (SQD), linear plateau (LRP), and quadratic plateau (QDP; Table 3). The use of these models for estimating optimum fertilizer rates for crops is described by Cerrato and Blackmer [33] and Bullock and Bullock [34]. The model equations were reparameterized from their original form to include a parameter that identifies the optimum of each function (β_2; Table 3). The β_2 parameter is equivalent to the AONR. Reparameterization allowed estimation of standard errors and confidence intervals (CIs) of β_2, and thus AONR, directly from the regression analysis. This method is described in detail by Hernandez and Mulla [29] and Jaynes [28]. Reparameterized models required non-linear regression, which was analyzed for each site-year environment using the nls function in the R "stats" package [35].

After fitting all functional response models to observed land ethanol yields, CIs were generated for the parameter estimates by bootstrapping the data (n=9,999) using the nlsBoot function in the R package "nlstools" [36]. Confidence intervals for the AONR parameter and goodness of fit as determined by Akaike information criterion adjusted for small sample size (AICc) were used to select one model for reporting AONR (hereafter the predictor model). The AONR was used from the predictor model for each environment to estimate all other response variables (biomass yield, nutrient harvest, and nitrogen use efficiency) at this N rate. We used a two-step process for selecting the predictor model: (1) ranked the models by AICc score with the lowest score identifying the superior model [37] and (2) assessed the CI of the AONR for reasonableness. In many cases, the difference in AICc among competing models was less than two points, which does not provide strong evidence of differentiation among a pair of non-nested models [38]. If multiple top models were within two AICc points, we selected the model with the smallest CI/AONR ratio as the predictor model (Table 4). Figure 1 illustrates how multiple models that fit the data similarly can generate AONRs and CIs that are considerably different. Our two-step method for determining a predictor model is based on the variation explained by the model (accuracy of parameter estimation) and confidence of its predictive capabilities (precision of parameter estimation). Since the LR model does not estimate an AONR, the LR model was selected if its AICc score was more than two points less than any other model with a CI/AONR ratio less than one. This method does not rely on P values from a statistical test for model selection like methods used by Boyer et al. [27].

After selecting a predictor model to estimate an AONR and its associated CI for each environment, we sequentially fit the same five functions to all other response variables: biomass yield, theoretical ethanol potential, N, P, and K harvest, and N use efficiency (NUE). Here, NUE is the difference in biomass yield between fertilized plots and unfertilized plots, divided by the amount of fertilizer added;

$$\text{NUE} = (\text{Yield}_X - \text{Yield}_0)/X$$

where Yield_X is the average biomass yield in plots fertilized with N at a rate of Xkg N ha^{-1} and Yield_0 is the average biomass yield of unfertilized plots (rate=0 kg N ha^{-1}).

Table 3 Equations for original response functions and reparameterized response functions from five response models used to predict agronomically optimum nitrogen rates for land ethanol yield

Model	Abbreviation	Original response function	Reparameterized response function
Linear	LR	$Y=\beta_0+\beta_1X$	No reparameterization required
Quadratic	QD	$Y=\beta_0+\beta_1X+\beta_2X^2$	$Y=\beta_0+2\beta_1\beta_2X+\beta_1X^2$
Square root quadratic	SQD	$Y=\beta_0+\beta_1X+\beta_2X^{0.5}$	$Y=\beta_0+(0.5\beta_1/\beta_2^{0.5})X+\beta_1X^{0.5}$
Linear plateau	LRP	$Y=\beta_0+\beta_1X$ for $X<k$	$Y=\beta_0+\beta_1X$ for $X<\beta_2$
		$Y=\beta_0+\beta_1k$ for $X>k$	$Y=\beta_0+\beta_1\beta_2$ for $X>\beta_2$
Quadratic plateau	QDP	$Y=\beta_0+\beta_1X+\beta_2X^2$ for $X<k$	$Y=\beta_0+\beta_1X+(-\beta_1/2\beta_2)X^2$ for $X<\beta_2$
		$Y=\beta_0+\beta_1k+\beta_2k^2$ for $X>k$	$Y=\beta_0+(\beta_1\beta_2)/2$ for $X>\beta_2$

Table 4 Akaike information criterion (*AICc* adjusted for small sample size), agronomically optimum nitrogen rate (AONR), and 95 % confidence intervals (CI) from five models based on different response functions used to select predictor models to estimate AONR for six site-year environments

Location[a]	Function[b]	AICc	AONR kg N ha^{-1}	2.50 %	97.50 %	CI	CI/AONR %
Aus08	LR[c]	1,516.5	NA	NA	NA	NA	NA
	QD	1,516.9	285	182	2,277	2,095	735
	SQD	1,515.4	>224	–[d]	–		
	LRP	1,523.1	91	67	141	74	81
	QDP	1,516.9	299	170	1,617	1,448	484
Aus09	LR	1,539.9	NA	NA	NA	NA	NA
	QD	1,518.4	131	119	152	33	25
	SQD[c]	1,509.2	87	71	122	51	60
	LRP	–	–	–	–		
	QDP	–	–	–	–		
Lam08	LR	1,490.8	NA	NA	NA	NA	NA
	QD	1,490.1	178	131	661	530	298
	SQD	1,489.2	273	143	22,860	22,717	8,322
	LRP[c]	1,489.4	73	59	148	89	122
	QDP	1,489.4	108	67	439	372	344
Lam09	LR	1,445.8	NA	NA	NA	NA	NA
	QD	1,445.7	242	168	1,970	1,802	745
	SQD	1,443.1	1,800	–	–		
	LRP[c]	1,446.7	71	58	112	54	76
	QDP	1,446.7	104	64	232	168	162
Ros08	LR	1,569.5	NA	NA	NA	NA	NA
	QD	1,559.4	175	149	243	94	54
	SQD	1,554.7	245	137	2,698	2,561	1,045
	LRP[c]	1,555.3	70	58	102	44	63
	QDP	1,555.3	102	67	173	106	105
Ros09	LR	1,510.3	NA	NA	NA	NA	NA
	QD	1,492.7	149	133	181	48	32
	SQD	1,486.9	129	90	281	191	148
	LRP[c]	1,486.9	61	57	83	26	43
	QDP	1,486.9	78	62	137	75	96

NA not applicable

[a] Site-year environments include Austin in 2008 (Aus08), Austin in 2009 (Aus09), Lamberton in 2008 (Lam08), Lamberton in 2009 (Lam09), Rosemount in 2008 (Ros08), and Rosemount in 2009 (Ros09)

[b] See Table 3 for response function abbreviations

[c] Model selected as predictor model

[d] Models did not converge

We selected the top model for each response at each environment based solely on lowest AICc. We omitted the step of assessing CIs of the parameter estimates since we were less concerned with parameter estimate precision than determining the best model fit. Instead, we predicted the response at the level of the AONR based on land ethanol yield. This value is not a predicted parameter in the modeled response. Confidence and prediction intervals for estimates other than the coefficients are not currently available for non-linear models.

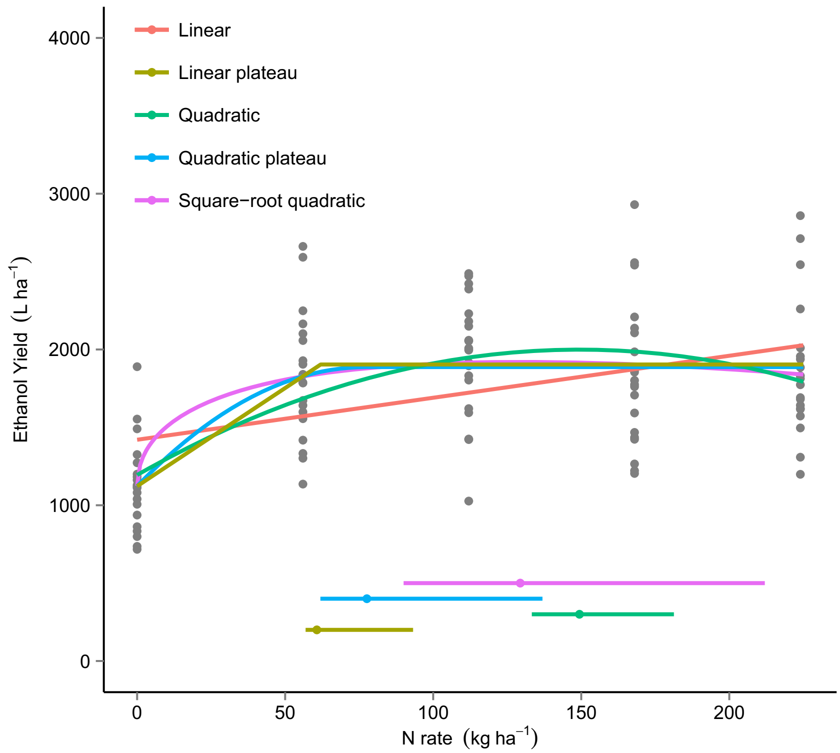
Results and Discussion

Biomass Yield

Average biomass yield in unfertilized plots was 4.9, 3.7, and 4.6 Mg ha^{-1} at Austin, Lamberton, and Rosemount, respectively. At Austin, biomass yield declined slightly from 2008 to 2009 (Table 5), which may be associated with a decrease in rainfall at that location (Table 2). Precipitation and biomass yield were similar in both years at Lamberton (Tables 2 and 5). Rosemount experienced a 57 % decline in biomass yield despite receiving more total precipitation in 2009 than 2008. However, 2009 precipitation at Rosemount was not evenly distributed and there were heavy events in August and October and unusually dry periods in May, July, and September. Except for Austin in 2008, all sites and years received less cumulative precipitation during each growing season than the 30-year average (Table 2).

Nitrogen fertilization increased biomass yield at all locations. At Austin and Rosemount, the effect of N differed by year (Table 6). Therefore, we analyzed the effect of N on biomass yields in 2008 and 2009 separately for all locations. In 2008, observed biomass yields peaked at the greatest

Fig. 1 Measured land ethanol yield at five nitrogen fertilization rates at Rosemount in 2009. Also shown are model fit curves from five response functions along with the agronomically optimum nitrogen rate and 95 % confidence intervals for each model



applied N fertilizer rate of 224 kg N ha^{-1} at all locations. There was a 46, 30, and 44 % increase in biomass yield at the largest N fertilization rate (224 kg N ha^{-1}) compared to unfertilized biomass at Austin, Lamberton, and Rosemount, respectively. Compared to 2008, yield responses were similar in 2009 at Lamberton, but peaked at lesser N rates at Austin and Rosemount in 2009 (Table 5). In 2009, maximum biomass yields were greater than unfertilized yields by 100, 49, and 79 % at Austin (56 kg N ha^{-1}), Lamberton (224 kg N ha^{-1}), and Rosemount (112 kg N ha^{-1}), respectively. Averaged across years, P fertilization did not affect biomass yield at Austin and Lamberton and K fertilization did not affect biomass yield at Rosemount (Table 6).

Table 5 Average (standard error; n=20) biomass yield by N fertilizer rates, best-fit model and parameter estimates explaining variation in biomass yield, agronomically optimum N fertilizer rate (AONR), and predicted yield at AONR for grassland biomass at three locations in 2008 and 2009

Location	Year	Biomass yield (Mg ha^{-1})						Regression analysis					
		N fertilizer rate (kg N ha^{-1})						Parameter estimates				AONR[b] (kg N ha^{-1})	Biomass yield at AONR
		0	56	112	168	224	Mean	Model[a]	β_0 (intercept)	β_1	β_2 (max)		
Austin	2008	6.1 (0.1)	7.3 (0.3)	7.8 (0.2)	8.2 (0.3)	8.9 (0.2)	7.7 (0.1)	LR	6.4	0.01	NA	NA	NA
	2009	3.7 (0.3)	7.4 (0.4)	6.8 (0.4)	7.0 (0.4)	6.2 (0.4)	6.2 (0.2)	SQD	3.8	0.73	92.9	87	7.3
	Mean	4.9 (0.2)	7.3 (0.2)	7.3 (0.2)	7.6 (0.3)	7.6 (0.3)	6.9 (0.1)						
Lamberton	2008	4.0 (0.3)	4.7 (0.2)	4.9 (0.2)	4.8 (0.2)	5.2 (0.3)	4.7 (0.1)	SQD	4.1	0.11	414.7	73	4.8
	2009	3.5 (0.2)	4.5 (0.1)	4.6 (0.2)	4.8 (0.1)	5.2 (0.2)	4.8 (0.1)	SQD	3.5	0.13	1243.6	71	4.4
	Mean	3.8 (0.2)	4.6 (0.1)	4.8 (0.1)	4.8 (0.1)	5.2 (0.2)	4.6 (0.1)						
Rosemount	2008	6.8 (0.3)	8.8 (0.3)	9.3 (0.3)	9.4 (0.3)	9.8 (0.2)	8.8 (0.2)	SQD	6.9	0.31	374.5	70	8.9
	2009	2.4 (0.1)	4.0 (0.2)	4.3 (0.2)	4.1 (0.2)	4.3 (0.2)	3.8 (0.1)	LRP	2.4	0.03	66.0	61	4.2
	Mean	4.6 (0.4)	6.4 (0.4)	6.8 (0.4)	6.8 (0.5)	7.1 (0.5)	6.3 (0.2)						

NA not applicable

[a] See Table 3 for response function abbreviations

[b] Agronomically optimum nitrogen rate (AONR) based on biomass yield

Table 6 *P* values from analysis of variance for fertilizer and year effects on biomass yield, theoretical ethanol potential, land ethanol yield, biomass nutrient concentrations and nutrient harvest. Nitrogen (N), phosphorus (P), and potassium (K) fertilizers were analyzed as factor variables for this analysis

	Treatment	Biomass yield	Eth potential[a]	LEY[b]	Nutrient concentrations			Nutrient harvest		
					N	P	K	N	P	K
Austin	N	*<0.001*[c]	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
	P	0.300	0.088	0.521	*0.032*	*0.002*	0.077	*0.018*	*0.001*	*0.017*
	Year	*<0.001*	*0.037*	*<0.001*	*<0.001*	*0.006*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
	N×P	0.108	0.060	0.066	0.275	0.061	0.062	*0.032*	0.118	0.100
	N×year	*<0.001*	0.603	*<0.001*	0.215	0.472	0.078	*0.001*	0.057	*<0.001*
	P×year	0.183	*0.032*	0.530	0.338	0.058	0.918	0.062	*0.025*	0.211
	N×P×year	0.945	0.290	0.973	0.879	0.816	0.660	0.879	0.847	0.275
Lamberton	N	*<0.001*	*0.011*	*<0.001*	*<0.001*	0.261	*0.011*	*<0.001*	*0.002*	*<0.001*
	P	0.217	*0.021*	0.345	0.421	*<0.001*	*0.036*	0.146	*<0.001*	*0.020*
	Year	0.054	*<0.001*	0.188	*<0.001*	0.339	0.504	*<0.001*	0.650	0.186
	N×P	0.864	0.144	0.846	0.225	0.109	0.217	0.482	0.242	*0.037*
	N×year	0.639	0.065	0.692	0.282	0.541	0.889	0.327	0.198	0.856
	P×year	0.855	0.129	0.654	0.516	0.906	0.921	0.730	0.796	0.984
	N×P×year	0.964	0.362	0.941	0.192	0.657	0.808	0.206	0.477	0.917
Rosemount	N	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
	K	0.141	0.584	0.129	0.307	0.527	*<0.001*	0.961	0.629	*<0.001*
	Year	*<0.001*	*<0.001*	*<0.001*	*0.012*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
	N×K	0.565	0.715	0.654	0.505	0.257	*<0.001*	0.507	0.155	*0.011*
	N×year	0.194	*<0.001*	0.166	*<0.001*	0.076	0.267	*0.002*	0.100	*<0.001*
	K×year	0.322	0.778	0.295	0.989	0.436	0.165	0.933	0.529	0.390
	N×K×year	0.410	0.852	0.529	0.806	0.904	0.721	0.174	0.465	0.393

Eth potential theoretical ethanol potential, *LEY* land ethanol yield (biomass yield×Eth potential) [c] Italicized P values significant at $\alpha = 0.05$

In mixed-species grasslands at Austin, the biomass yield response to N fertilization was predicted by the LR model in 2008 and the SQD model in 2009 (Table 4 and 5). The best-supported model at Lamberton was SQD during both years. The SQD and LRP models were best-supported for the switchgrass monocultures at Rosemount in 2008 and 2009, respectively.

The positive effect of N fertilizer on biomass yield in this study is consistent with other fertilization research on switchgrass and mixed-species grasslands [27, 39–41]. Biomass yield plateaued at moderate N rates consistently at Lamberton and Rosemount similar to other studies [39]. The mixed-species plantings at Austin responded differently to N compared to Lamberton and Rosemount and shifted from LR in 2008 to SQD in 2009. The delayed plateau response from 2008 to 2009 could have been due to a number of environmental changes beyond the scope of this project. For instance, a relatively larger yield decrease in unfertilized plots from 2008 to 2009—possibly due to resource limitation by N or another resource—would add curvature to the response function. However, data were not available to identify the mechanism responsible for the changing response function at Austin.

Theoretical Ethanol Potential

Average theoretical ethanol potential in unfertilized plots was 448, 435, and 479 L Mg^{-1} of biomass at Austin, Lamberton, and Rosemount, respectively. Theoretical ethanol potential was similar in both years at Austin, increased in 2009 at Lamberton, and decreased in 2009 at Rosemount (Table 7). Other studies reported greater ethanol potential in grasslands dominated by C4 grasses compared to C3 grasses [13, 22], likely because of a greater concentration of total carbohydrates in C4 grasses [19]. Despite the presence of C3 grasses and forbs in the mixed-species grasslands at Austin and Lamberton, we did not consistently observe lower ethanol potential at these sites compared to switchgrass monoculture at Rosemount.

Theoretical ethanol potential decreased where N fertilizer was applied at all locations except for Lamberton in 2008, where no relationship was observed [23] (Table 7). Phosphorus fertilization also affected theoretical ethanol potential at Austin and Lamberton (Table 6). When considered a categorical variable, a significant interaction between P fertilizer and year was apparent at Austin (F=2.72, P=0.03), but

Table 7 Treatment averages (standard error; n=20), best-supported predictor model and parameter estimates, and theoretical ethanol potential at AONR for three locations in 2008 and 2009

Location	Year	Theoretical ethanol potential (L Mg^{-1})						Regression analysis					
		N fertilizer rate (kg N ha^{-1})						Parameter estimates				AONR[b]	Ethanol potential at AONR
		0	56	112	168	224	Mean	Model[a]	β_0 (intercept)	β_1	β_2		
Austin	2008	448 (3)	447 (4)	435 (3)	435 (4)	428 (5)	439 (2)	LR	449	−0.09	NA	NA	NA
	2009	447 (6)	446 (5)	429 (5)	428 (4)	417 (5)	433 (3)	LR	449	−0.14	NA	87	436
	Mean	448 (3)	447 (3)	432 (3)	432 (3)	423 (4)	436 (2)						
Lamberton	2008	407 (6)	412 (5)	408 (6)	416 (6)	402 (5)	409 (2)	NS	NA	NA	NA	73	409[c]
	2009	463 (2)	453 (4)	441 (5)	449 (3)	440 (5)	449 (2)	LRP	463	−0.20	0.178	71	450
	Mean	435 (6)	432 (5)	425 (5)	433 (4)	421 (4)	429 (4)						
Rosemount	2008	485 (1)	481 (1)	477 (2)	473 (2)	466 (2)	476 (1)	LR	485	−0.080	NA	71	480
	2009	473 (1)	460 (1)	450 (1)	443 (2)	435 (1)	452 (1)	SQD	473	−0.095	−1.097	61	459
	Mean	479 (1)	471 (2)	463 (2)	458 (3)	451 (3)	464 (2)						

NA not applicable, *NS* not significant

[a] See Table 3 for response function abbreviations

[b] Agronomically optimum nitrogen rate (AONR) based on land ethanol yield

[c] Mean ethanol potential at Lamberton in 2008

when P fertilizer was modeled as a continuous variable using linear regression, a weak, non-significant relationship was observed (P=0.07, R^2=0.03). Therefore, an effect of P on theoretical ethanol potential was only determined at Lamberton. The response of theoretical ethanol potential to fertilization was relatively small compared to the response of biomass yield. In light of this finding and its economic implications, the following discussion is focused on land ethanol yield.

Land Ethanol Yield

Average land ethanol yield in unfertilized plots was 2,197, 1,619, and 2,218 L ha^{-1} at Austin, Lamberton, and Rosemount, respectively (Table 8). Land ethanol yield declined from 2008 to 2009 by 20 % at Austin and 59 % at Rosemount and was similar between years at Lamberton. Averaged across treatments, Rosemount had the greatest land ethanol yield in 2008 (4,197 L ha^{-1}) followed by Austin (3,348 L ha^{-1}) and Lamberton (1,938 L ha^{-1}; LSD= 200 L ha^{-1}). This changed in 2009 as land ethanol yields ranked largest to smallest at Austin (2,686 L ha^{-1}), Lamberton (2,011 L ha^{-1}), and Rosemount (1,722 L ha^{-1}; LSD=227 L ha^{-1}). The relatively drastic decline in biomass yield at Rosemount translated to a significant decline in land ethanol yield from 2008 to 2009 (Table 8).

The relationship between N rate and land ethanol yield was positive at all locations. At Austin, the predictor model used to estimate AONR was LR in 2008 and SQD in 2009. The predictor models were LRP at Lamberton and Rosemount during both years (Fig. 1, Table 8). Phosphorus and K fertilizers did not affect land ethanol yield at any location or year (Table 6).

Two or more models were similar in estimating variation in land ethanol yield at all environments except Austin in 2009. At environments where multiple models were similar in AICc, CIs were important for choosing the predictor model (Table 4, Fig. 1). For instance, at Rosemount in 2009, the SQD, LRP, and QDP models differed in AICc by less than one (Table 8), and all three fit the data well based on visual assessment (Fig. 1). The SQD model estimated an AONR with a relatively large CI (Fig. 1; Table 4). The LRP and QDP models estimated AONRs that were similar, but the LRP had a smaller CI relative to its estimate; therefore, it was selected as the predictor model (Table 4). At Lamberton in 2009, the AICc score for the LRP model was more than two points less than the next lowest model score, indicating that it explained the most variation in the data. However, this model estimated an AONR of 1,800 kg N ha^{-1}, which far exceeds a reasonable N fertilization rate. Small CIs are a desired trait for predicting AONR, but they should not be used to compare the accuracy among other models [28]. Nonetheless, small CIs are an appropriate qualitative measure for choosing a predictor model when multiple models do not generate similar distributions for AONR estimates [28].

If a bioenergy industry grows and a market for biomass stabilizes, it will be necessary to account for biomass prices to determine *economically* optimum N rates. Also, as cellulosic ethanol facilities expand to production capacity, realized conversion efficiency rates will be available and necessary for

Table 8 Treatment averages (standard error; n=20), best-supported predictor model and parameter estimates, and land ethanol yield at AONR for three locations in 2008 and 2009

Location	Year	Land ethanol yield (L ha^{-1})						Regression analysis				
		N fertilizer rate (kg N ha^{-1})						Parameter estimates				LEY at AONR[b]
		0	56	112	168	224	Mean	Model[a]	β_0 (intercept)	β_1	β_2 (AONR)	
Austin	2008	2,730 (50)	3,250 (130)	3,380 (80)	3,570 (130)	3,800 (100)	3,350 (60)	LR	2,860	4	NA	NA
	2009	1,600 (110)	3,250 (170)	2,940 (190)	2,990 (220)	2,620 (190)	2,690 (100)	SQD	1,620	331	87	3,160
	Mean	2,200 (110)	3,250 (110)	3,160 (110)	3,300 (130)	3,230 (140)	3,030 (60)					
Lamberton	2008	1,640 (100)	1,940 (70)	2,020 (80)	2,020 (70)	2,080 (120)	1,940 (40)	LRP	1,640	5	73	2,040
	2009	1,600 (70)	2,030 (70)	2,020 (90)	2,130 (60)	2,270 (90)	2,010 (40)	LRP	1,600	8	71	2,140
	Mean	1,620 (60)	1,980 (50)	2,020 (60)	2,080 (50)	2,180 (80)	1,970 (30)					
Rosemount	2008	3,310 (140)	4,240 (140)	4,420 (130)	4,430 (140)	4,590 (80)	4,200 (70)	LRP	3,310	17	71	4,480
	2009	1,120 (70)	1,830 (90)	1,950 (90)	1,840 (110)	1,870 (100)	1,720 (50)	LRP	1,120	13	61	1,890
	Mean	2,220 (190)	3,040 (210)	3,180 (210)	3,140 (220)	3,230 (230)	2,960 (100)					

NA not applicable

[a] See Table 3 for response function abbreviations

[b] Land ethanol yield (LEY) at the agronomically optimum nitrogen rate (AONR)

calculating economically optimum N rates. In our analysis and others [21], maximum theoretical ethanol potential was calculated because realized efficiencies are not yet available.

Nutrient Harvest

Various interactions between fertilizers and time influenced nutrient harvest at all locations (Table 6). Since N was the only fertilizer that affected yield, we focus on the effects of N on nutrient harvest.

Nutrients harvested in aboveground biomass varied by location and year (Table 6). In 2008, average N harvest in unfertilized plots was similar at all locations averaging 29 kg ha^{-1} (Table 9). Nitrogen harvest declined at all locations in 2009, averaging 15 kg ha^{-1} at Austin and Lamberton and 8 kg ha^{-1} at Rosemount (Table 9). As expected, the patterns in nutrient harvest closely followed the patterns observed in biomass yield. Nitrogen fertilization affected N harvest at all locations and in all years (Tables 6 and 9). The positive relationship was LR at Lamberton and Rosemount during both years, LR at Austin in 2008, and QD at Austin in 2009 (Table 9). At environments where AONRs were identified for land ethanol yield, it is clear that the AONRs were well above the amount of N removed in the biomass at those locations (Table 9).

In 2008, average P harvest in unfertilized plots was 5, 2, and 9 kg ha^{-1} at Austin, Lamberton, and Rosemount, respectively. Phosphorus harvest declined at Austin and Rosemount in 2009 (Table 9). The effect of N fertilization on P harvest varied by location and year (Table 6). Averaged over both years, P harvest was 105, 32, and 64 % greater in plots fertilized with 224 kg N ha^{-1} compared to unfertilized plots at Austin, Lamberton, and Rosemount, respectively. Nitrogen fertilization did not affect P harvest at Lamberton in 2008 but did generate a LR response in 2009 (Table 9). The relationship between N fertilization and P harvest was LRP during both years at Rosemount, LR at Austin in 2008, and LRP at Austin in 2009 (Table 9).

In 2008, average K harvest in unfertilized plots was 17, 11, and 28 kg ha^{-1} at Austin, Lamberton, and Rosemount, respectively. Potassium harvest declined at all sites in 2009 (Table 9). Averaged over both years, K harvest was 133, 80, and 75 % greater in plots fertilized with 224 kg N ha^{-1} compared to unfertilized plots at Austin, Lamberton, and Rosemount, respectively. At Austin, a LR relationship was observed between N fertilizer rate and K harvest in 2008, followed by a SRQ relationship in 2009. A LR relationship was observed for both years at Lamberton and a LRP relationship for both years at Rosemount (Table 9).

Nutrient harvest can be considered a consequence of increased biomass growth from N fertilization and assessed at the AONR for land ethanol yield. The N removed annually with biomass harvest is replaced at the AONRs we identified. Therefore, biomass yields should not be limited by N with continuous N fertilization. This is not the case for P and K. Since our results suggest that P and K fertilizers do not affect biomass yields on these soils in the short term, we do not recommend investing in their application annually. In unfertilized plots, P and K harvest was low compared to other reported values [15]; however, we observed significant increases in P and K harvest with N fertilization. Therefore,

Table 9 Treatment averages (standard error; n=20), agronomically optimum N fertilizer rate (AONR), and nutrient harvest at AONR for grassland biomass at three locations in 2008 and 2009

Location	Year	N fertilizer rate (kg N ha^{-1})						Model[a]	AONR[b] (kg N ha^{-1})	Removal at AONR
		0	56	112	168	224	Mean			
		Biomass N harvest (kg N ha^{-1})								
Austin	2008	33 (2)	40 (2)	52 (2)	64 (4)	80 (4)	54 (2)	LR	NA	NA
	2009	15 (1)	31 (2)	44 (4)	51 (3)	50 (3)	38 (2)	QD	87	39
	Mean	24 (2)	36 (2)	48 (2)	58 (3)	65 (4)	46 (2)			
Lamberton	2008	23 (3)	33 (4)	33 (2)	35 (2)	41 (3)	33 (1)	LR	73	30
	2009	15 (1)	23 (1)	26 (1)	32 (2)	38 (2)	27 (1)	LR	71	23
	Mean	19 (2)	28 (2)	29 (1)	33 (1)	39 (2)	30 (1)			
Rosemount	2008	30 (2)	43 (2)	55 (3)	62 (3)	80 (3)	54 (2)	LR	70	44
	2009	8 (1)	17 (1)	28 (1)	32 (2)	42 (3)	26 (1)	LR	61	18
	Mean	19 (2)	30 (2)	41 (3)	46 (3)	61 (4)	40 (2)			
		Biomass P harvest (kg P ha^{-1})								
Austin	2008	5 (0)	6 (0)	7 (0)	8 (1)	9 (1)	7 (0)	LR	NA	NA
	2009	3 (0)	5 (0)	6 (0)	6 (0)	6 (0)	5 (0)	LRP	87	5
	Mean	4 (0)	6 (0)	6 (0)	7 (0)	8 (1)	6 (0)			
Lamberton	2008	2 (0)	2 (0)	2 (0)	2 (0)	2 (0)	2 (0)	NS	73	NA
	2009	2 (0)	2 (0)	2 (0)	2 (0)	3 (0)	2 (0)	LR	71	2
	Mean	2 (0)	2 (0)	2 (0)	2 (0)	3 (0)	2 (0)			
Rosemount	2008	9 (0)	12 (1)	13 (1)	12 (0)	13 (1)	12 (0)	LRP	70	12
	2009	2 (0)	4 (0)	5 (0)	5 (0)	5 (0)	4 (0)	LRP	61	4
	Mean	5 (1)	8 (1)	9 (1)	8 (1)	9 (1)	8 (0)			
		Biomass K harvest (kg K ha^{-1})								
Austin	2008	17 (1)	24 (1)	28 (2)	33 (2)	44 (5)	29 (1)	LR	NA	NA
	2009	11 (1)	19 (1)	20 (2)	21 (2)	21 (2)	18 (1)	SRQ	87	20
	Mean	14 (1)	21 (1)	24 (1)	27 (2)	33 (3)	24 (1)			
Lamberton	2008	11 (1)	14 (1)	16 (1)	15 (1)	18 (2)	15 (1)	LR	73	14
	2009	9 (1)	12 (1)	15 (2)	15 (1)	18 (2)	14 (1)	LR	71	12
	Mean	10 (1)	13 (1)	16 (1)	15 (1)	18 (1)	14 (1)			
Rosemount	2008	28 (2)	39 (2)	44 (2)	41 (2)	46 (2)	40 (1)	LRP	70	42
	2009	6 (0)	13 (1)	14 (1)	12 (1)	13 (1)	12 (0)	LRP	61	13
	Mean	17 (2)	26 (2)	29 (3)	26 (3)	29 (3)	25 (1)			

NA not applicable, *NS* not significant

[a] See Table 3 for response function abbreviations

[b] Agronomically optimum nitrogen rate (AONR) based on land ethanol yield

we suggest that P and K be monitored with soil tests and added to soils when needed. Phosphorus harvest was 5, 2, and 4 kg ha^{-1} at AONRs identified for Austin, Lamberton, and Rosemount in 2009 (Table 9), which are low compared to other reported P harvest values between 8 and 13 kg ha^{-1} for four different grass species fertilized at 140 kg N ha^{-1} [16]. The effects of nutrient removal from biomass harvest on soil properties were reported by Schmer et al. [14] who found an average annual decrease in soil available P of 1.5 kg P ha^{-1} year^{-1} after 5 years of switchgrass harvest. At this rate of decline, the authors stated that it was unlikely that available P limited biomass yield during the study.

Potassium is an essential mineral for plant physiological and biochemical function in harvested grasslands [42]; however, little research has been done on the effect of biomass removal on soil K. Application of K fertilizer at 100 kg K ha^{-1} during 2 of 5 years prevented changes in soil K concentration in a continuous corn system where all remaining stover was harvested (which included about 34 kg K ha^{-1}) following grain harvest for 5 years [43]. Although the current study observed lower K removal rates compared to 100 % corn stover harvest, research has not quantified the short- or long-term effect of K removal on soil K reserves and biomass yield in perennial grassland bioenergy systems.

Nitrogen Use Efficiency

In 2008, nitrogen use efficiency (NUE) did not change with N fertilization at Austin (P=0.06) and Lamberton (P=0.12), where it averaged 15.2 and 7.6 kg biomass kg N^{-1}, respectively (Fig. 2). At Rosemount in 2008, the SRQ model best explained the decrease in NUE and predicted NUE of 30.8 kg biomass kg N^{-1} at the AONR. In 2009, the SQR model best explained the decrease in NUE in response to N fertilization at all locations. The predicted NUE at the AONR was 42.2, 15.7, and 27.3 kg biomass kg N^{-1} at Austin, Lamberton, and Rosemount, respectively.

Reduced NUE with increased N fertilization has been observed for orchardgrass [44] and other dryland forage grass species [45] when grown in monoculture. Diminishing NUE with associated increases in N fertilization rates suggests that other resources, possibly other nutrients such as P or K, become more limiting for productivity in N-fertilized systems [45]. Our results show that application of P or K along with N did not prevent decreases in NUE with increasing N fertilizer rates. It is possible that moisture limited NUE at higher N fertilizer rates, therefore explaining the observed relationship between NUE and N fertilization. Austin and Lamberton received more precipitation in 2008 compared to 2009, which may explain why NUE was constant across N fertilization rates in 2008, but not in 2009.

Nitrogen use efficiency is one metric by which biomass producers can measure return on fertilizer investment. Although perennial bioenergy crops have lower N losses in fertilized systems compared to annual crops like corn [46], perennials allocate a smaller proportion of assimilated N to increase harvestable biomass [16]. Nitrogen use efficiency in corn grain and stover averaged 35.4 kg biomass kg N^{-1} when fertilized at 168 kg N ha^{-1} at Lamberton in 2008 and 2009 [23], which is greater than the average Lamberton grassland

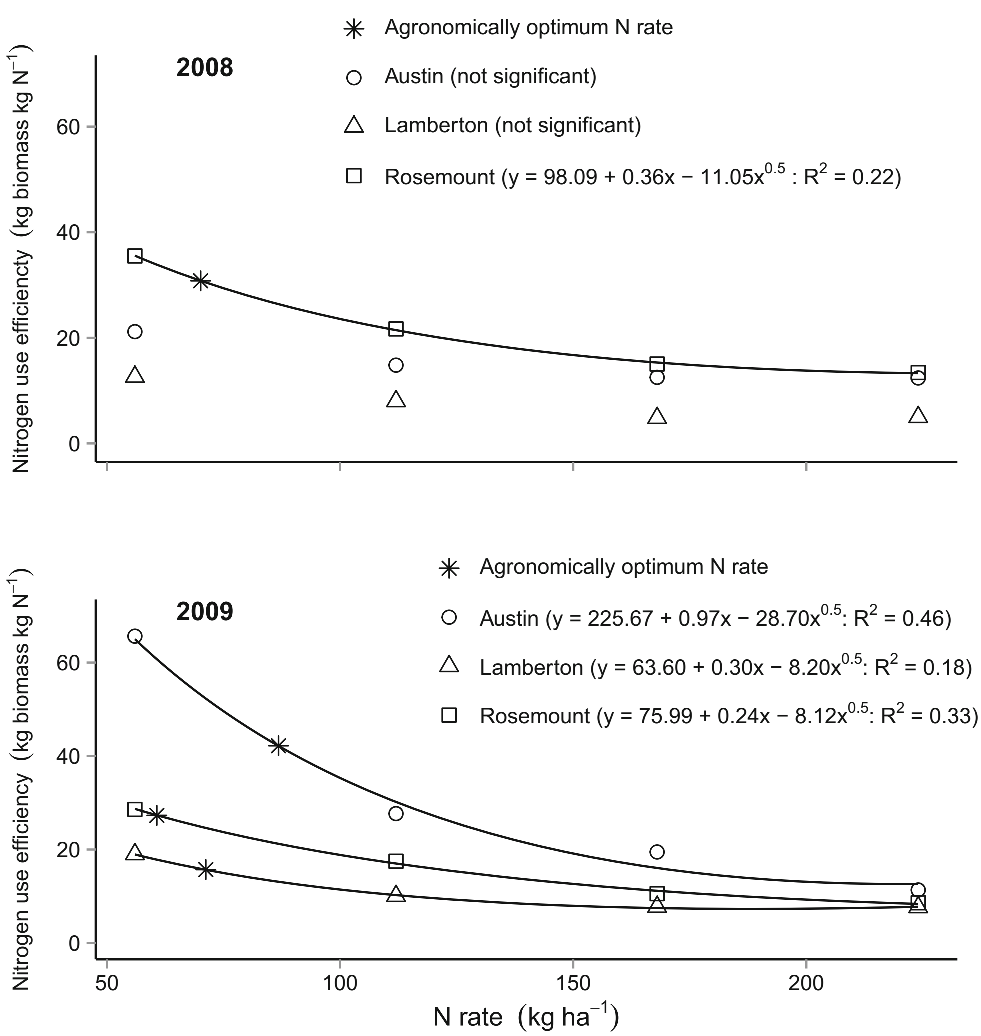


Fig. 2 Average nitrogen use efficiency (NUE) at four N fertilization rates (56, 112, 168, and 224 kg N ha^{-1}) for three locations in 2008 and 2009. Also shown is the best-supported model fit for NUE at each site-year environment, with the agronomically optimum nitrogen rate based on land ethanol yield for each environment

NUE of 11.7 kg biomass kg N^{-1} we estimated at AONR. Although we did not measure root biomass, it is likely that the plants at Austin, Lamberton, and Rosemount used N to increase root biomass, which would explain relatively low values of NUE at these sites compared to annual crops. A study of switchgrass and big bluestem grown in monoculture reported that root biomass—and the concentration of N in the root biomass—increased in response to N fertilizer [16], thus showing that N fertilizer was allocated to tissues not harvested for biomass. Allocation of N fertilizer to root biomass in perennial grasses managed for bioenergy provides other conservation benefits including long-term crop management and carbon sequestration, thus should not be considered a negative consequence of fertilization.

Fertilizer Recommendation

Based on AONR estimates from regression models and their 95 % CIs, we recommend applying N fertilizer at rates between 60 and 90 kg N ha^{-1} annually to maximize cellulosic ethanol yield from established switchgrass and mixed-species grassland biomass in MN, USA. Although largely driven by biomass yield, this recommendation incorporates effects of N fertilizer on plant carbohydrate concentrations and therefore is slightly lower than our recommendation for maximizing biomass yield. Our recommended N fertilizer range of 60–90 kg N ha^{-1} is similar to a reported range for maximizing switchgrass biomass yield [39] (56–112 kg N ha^{-1}) and lower than rates reported by Heggenstaller et al. [16] and Waramit et al. [24] (140 kg N ha^{-1}).

Summary and Conclusions

In established mixed-species grasslands and switchgrass monocultures, N fertilization consistently increased biomass and land ethanol yield, while P and K fertilizers had no effect. We identified agronomically optimum N rates (AONRs) and associated confidence intervals based on land ethanol yield (L ethanol ha^{-1}) for five of six environments, which ranged from 61 to 87 kg N ha^{-1}. Averaged across years, N fertilizer applied at AONRs increased biomass yield by 49, 19, and 34 % compared to controls at Austin, Lamberton, and Rosemount, respectively. Land ethanol yield increased similarly to biomass yield with N fertilization and averaged 3,161, 2,090, 3,182 L ha^{-1} at the AONR at Austin, Lamberton, and Rosemount, respectively. Our results show that multiple models can provide similar measures for goodness of fit, yet predict very different AONR for yield responses to N fertilization. In these situations, uncertainty measurements should be used to select a model for predicting AONR. We show that confidence intervals can be calculated for AONRs and incorporated into model selection criteria.

Averaged across years, fertilizing grasslands at AONRs resulted in P harvest of 4.5, 2.1, and 8.1 kg P ha^{-1} and K harvest of 19.5, 13.3, and 27.7 kg K ha^{-1} at Austin, Lamberton, and Rosemount, respectively. Therefore, we recommend that P and K be monitored in soils under grasslands managed with N fertilizers for long-term bioenergy production. Nitrogen harvest was well below the AONR for land ethanol yield at all locations (averaged 38.5, 26.7, and 31.4 kg N ha^{-1} at Austin, Lamberton, and Rosemount, respectively); therefore, soil N depletion may not be an issue for grassland bioenergy systems fertilized at the AONR found in the study region. Nitrogen use efficiency was unaffected by N fertilization at Austin and Lamberton in 2008 and declined at Rosemount in 2008 and all locations in 2009. Declining NUE in response to N fertilization could be due to moisture limitation, allocation of N to root production, or a decrease in N acquisition. Nitrogen use efficiency was best predicted with the SQD function and was estimated at 30.8 kg biomass kg N^{-1} for Rosemount in 2008 and 42.2, 15.7, and 27.3 kg biomass kg N^{-1} for Austin, Lamberton, and Rosemount in 2009. More research is needed to determine the fate of N fertilizer in mixed-species grasslands managed for bioenergy.

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