PERENNIAL GRASS SYSTEMS FOR BIOENERGY: SHOULD WE FERTILIZE WITH N?

by

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Abstract

Native warm-season grasses of the tallgrass prairie are anticipated to be a significant source of cellulosic biofuel feedstocks for renewable energy, as well as deliver additional ecossytem services, such as mitigate nitrogen (N) losses to the environment. Whether additional services are realized, however will depend largely on how these perrenial bioenergy crops are managed. Balancing the trade-offs of maximizing yields while minimizing N removal at harvest and conserving N in the plant-soil system to limit N-losses, remains a major challenge in growing bioenergy crops. The purpose of this research was to understand how perennial grass cropping systems respond to management of different N fertilizer rates and harvest timings, as well as how this management affects the system's N conservation strategies of N-use efficiencies and N-recycling. We used two perennial grass systems, growing on prairie-derived soils in the upper Midwest, of differing diversity levels – a restored Tallgrass prairie and a *Panicum virgatum* (switchgrass) monoculture, to determine the effects of N fertilizer rate and harvest timing on biomass yields, N concentrations and N removal. We used three different metrics to evaluate the nitrogen-use dynamics of the different cropping systems and management treatments: yield nitrogen-use efficiency, apparent fertilizer-N recovery and N productivity – the ratio of yield to biomass N concentration. The magnitude and timing of N resorption under different N fertilizer rates were also measured to elucidate one mechanism of reducing N removal with later harvest dates. Responses to N fertilizer additions were not consistent between cropping system, year or harvest timing, although were generally weak. Yields were highly variable, ranging from 1.3 Mg ha^{-1} in a 2009 spring prairie harvest to a 7.2 Mg ha^{-1} summer switchgrass harvest in 2010. Waiting for a fall harvest resulted in a 30% yield loss and delaying harvest until the following spring caused another 30% yield loss. N-use dynamics in the two systems were similar and

nitrogen-use efficiencies indicated that these crops are not efficient users of fertilizer-N, suggesting that the excess fertilizer is not being captured by plants and is subject to various N-loss pathways. Fertilizing proved to be relatively inefficient, as fertilizing with 50 kg N ha^{-1} gave the prairie an advantage of only 11.9 kg of prairie biomass per kg added fertilizer-N, or 6.3 kg of biomass in the switchgrass monoculture. N productivity in switchgrass indicates that fertilizing 50 kg N ha^{-1} and harvesting in fall results in the most biomass with the best biomass quality, while the relationship between biomass yield and biomass quality was highly variable in the prairie. N resorption was significant in all measured species, although only switchgrass resorption was affected by fertilizer treatments. Fertilized plants took longer to reach minimum N levels and despite resorbing significantly higher proportions of leaf N, leaves but were still unable to withdraw leaf N to the same low N concentrations as the unfertilized plants. Decreased N removal with later harvest timings can be explained partly by N resorption, although additional reductions in N content at a fall harvest suggest other factors, such as changes in leaf:stem ratios through time may also be responsible for lower N removal rates. While bioenergy crops show potential for a sustainable feedstock, assumptions are often overlooked regarding their benefits because of management effects that alter the plant's N-use dynamics and N-recycling capabilities.

Introduction

In the search for renewable energy, native warm-season grasses of the tallgrass prairie have emerged as prime candidates for cellulosic ethanol production and are likely to have an increased presence on our landscape in the coming decades (Webster *et al.*, 2010; Stokes, 2011; Waramit *et al.*, 2011). These perrenial grasses are also anticipated to provide additional ecosystem services, such as biodiversity conservation, carbon sequestration, and greenhouse gas and nutrient-runoff mitigation (Bardgett *et al.*, 1999; Lemus and Lal, 2005; Costello *et al.*, 2009; Tilman *et al.*, 2009; Meehan *et al.*, 2010; Werling *et al.*, 2011). Anthropogenic reactive nitrogen has accumulated to damaging levels that have altered primary productivity, groundwater quality, atmospheric concentrations, and fluvial loading (Schlesinger, 2009). Since anthropogenic nitrogen has caused substantial environmental decline, striving to improve the N conservation of biomass production systems will lessen the impact of these perrenial bioenegy crops on our landscape and help realize some of the anticipated ecossytem services in addition to a source of renewable energy.

One way to increase agricultural production, while simultaneously increasing N conservation and reducing N runoff, is to use cropping systems that are inherently N-use efficient (Robertson and Vitousek, 2009; McSwiney *et al.*, 2010). Perennial grass cropping systems, such as a *Panicum virgatum* (switchgrass) monoculture or Tallgrass prairie, are two such systems currently being considered for cellulosic feedstock production. Warm-season grasses have been selected as candidates for biomass production not only because of their perennial nature, but also because they use a C4 photosynthetic pathway, which allows solar radiation, water, and nutrients to be used more efficiently for the amount of biomass produced than plants using the C3 photosynthetic pathway (Brown, 1999). Although perennial grass

cropping systems provide an optimistic alternative to other forms of energy (Donner and Kucharik, 2008), there still may not be enough reductions in N losses to maintain or restore ecosystem functions such as nutrient retention. Considering the biofuel production goals set forth by the Energy Independence and Security Act of 2007, it has been estimated that using cellulosic biofuels as opposed to corn grain ethanol will reduce nitrate loading by a mere 20% to the Mississippi River Basin. This would not reach current goals to reduce the hypoxic zone in the Gulf of Mexico (Costello *et al.*, 2009), which demonstrates the need to improve the N-conservation in cellulosic cropping systems. As policy intensifies efforts to develop production of biofuels, existing agricultural or marginal lands will potentially be converted to biomass production systems. Approximately 6.8 million hectares of land in the Conservation Reserve Program (CRP) has the potential to be used, as estimated by (Walsh *et al.*, 2003), and the U.S. Department of Energy also projects that millions of hectares is the U.S. could potentially be dedicated to perennial grass production for bioenergy in the near future (U.S. Dept of Energy, 2011). This considerable amount of prospective land may be subjected to new agronomic management scenarios and consequently, experience alterations to the N cycling. It therefore is imperative to understand how perennial grass can be managed for biomass production without degrading their inherent nitrogen-use efficiencies (NUE) and N-recycling abilities to prevent further N pollution to the environment.

Altering nutrient management and carbon inputs in agroecosystems has a great potential to alter the system's N balance and N cycling (McSwiney *et al.*, 2010). In biomass production, N losses will include N removed in a harvest, as well as losses through volatilization, denitrification, runoff, and leaching. These processes are highly dependent on soil type, temperature, moisture status, as well as management. To make up for system losses, managers

may apply N fertilizer at rates that replaces the N removed during the previous biomass harvest, or at higher rates to reduce risk by striving for maximum production (Anex *et al.*, 2007). Studies have demonstrated however that warm-season grasses may depend significantly on endogenous N sources, which means significant amounts of fertilizer-N applied may not be retained in the agroecosystem, or is at least unaccounted for in the production of the biomass. This is illustrated through the large ranges of reported biomass N contents and yield responses to nitrogen fertilizer addition (Table 1) (Parrish and Fike, 2005). Numerous studies have evaluated biomass production of switchgrass based on nitrogen and harvest management, but the results have been mixed with some finding a positive yield response to fertilization (Lemus *et al.*, 2008a; Heggenstaller *et al.*, 2009), while others found no response (Thomason *et al.*, 2004; Mulkey *et al.*, 2008). This lack of response to N fertilization has been attributed to uncharacterized microbial processes and the inherent N conservation strategies of the plants (Parrish and Fike, 2005).

There are ecological and economic incentives to reduce the amount of N taken off the field in biomasss, such as less N fertilizer required for the subsequent growing season and less N being subjected to possible loss pathways. The N removed by harvest is significantly affected by N fertilization rate and harvest timing. Vogel et al. (2002) found that biomass N concentrations increased with N fertilization rates but decreased with later harvests, which was attributed to the diminishing N contents in biomass during senecence. High N concentrations in biomass also decrease feedstock quality for the conversion process: a suggested upper-limit threshold of biomass N concentration for feedstocks is 0.6% N (Kauter *et al.*, 2003). There is a growing consensus that switchgrass and other warm-season grass cropping systems are best suited for one harvest per season, which also reduces N removal rates compared to a two-cut system (Parrish

and Fike, 2005). Harvesting the biomass too often or too early depletes the carbohydrate and nutrient stores and eventually causes reductions in growth and potential yields (Casler and Boe, 2003; Thomason *et al.*, 2004). Although current management reccomendations suggest that harvesting biomass should be delayed until after complete senescence, no clear approach has emerged to balance the trade-offs between maximizing biomass production and quality, while minimizing nutrient removal and maintaing high N-retention for ecosystem function.

Plant nutrient recycling is an important mechanism for N retention in plants and has been identified as a means of reducing N losses in perennial grass systems. C4 prairie grasses have been found to be very efficient in retranslocating, or resorbing nutrients and carbohydrates to roots and crowns during senescence for storage over winter to be re-used the following growing season (Clark, 1977; Heckathorn and Delucia, 1996) . N resorption rates have been found to vary considerably, ranging from 0-90% in perennial plants, with a mean of approximately 50% (van Heerwaarden *et al.*, 2003). An understanding of what contributes to this variability is beginning to emerge. Plant genetics are one factor, as switchgrass accessions alone have been found to have resorption rates that range from 20 to 60% (Yang *et al.*, 2009). Other sources of variability are environmental factors, such as climate, water stress, and soil nutrient levels. Some studies have found N resorption rates decrease with increasing soil-N availability (Kobe *et al.*, 2005; Huang *et al.*, 2008; Norris and Reich, 2009), while others have found no effect of N availability on resorption rates (Aerts, 1996; Aerts and Chapin, 2000); therefore fertility effects on resorption rates remain equivocal. N resorption has been documented specifically in perennial grasses managed for bioenergy production (Heaton *et al.*, 2009; Garten *et al.*, 2010), although little to no research has explored how the resorption strategies of these bioenergy species will be affected by different fertilizer rates. More research is needed to understand *when* and *how much* N is

recycled through retranslocation in managed bioenergy crops (Sanderson *et al.*, 2006), and whether the timing of harvest allows for complete resorption of N, as well as how additional fertilizer-N sources alter the plants' resorption capabilities. As N fertilization increases the biomass yield, as well as the N concentration in the biomass (Vogel *et al.*, 2002; Heggenstaller *et al.*, 2009), there is potential for more N to either retranslocate, be removed with harvest, or be lost through N-loss pathways, such as leaching and denitrification. Growing biomass feedstocks for energy while realizing additional ecosystem functions, remains a major challenge.

The purpose of this research was to understand how perennial grass cropping systems respond to different harvest timings and N fertilizer rates, as well as how this management affects the system's N-use efficiencies and N-recycling strategies. Maintaining high yields while minimizing N removal with harvest and retaining N in the plant-soil system is essential to help meet the goals of sustainable biofuel production. We used two perennial grass systems of differing diversity levels to 1) Determine the yield and [N] response to N additions at different harvest dates, 2) Quantify the N removal at different N fertilizer levels and different harvest dates, 3) Evaluate yield NUE metrics at different N rates and harvest dates, and 4) Measure the magnitude and timing of N resorption under different N fertilizer rates to elucidate one mechanism for reducing N removal with later harvest dates.

Methods

Study site and experimental design

This work was conducted at the Arlington Agricultural Research Station in Arlington, Wisconsin (43º18' N, 89º21' W). Average temperatures during the years of this study were 6.5°C and 7.8°C for 2009 and 2010, respectively. Total annual precipitation was 856mm in 2009 and 889

157 in 2010, making precipitation in the years of our study above the 40-yr average of 835mm for
158 south-central Wisconsin (UW Extension, 2010). Soils at the experimental site are classified as
159 Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudolls), which are highly-
160 productive Mollisols.

161 Experimental units were positioned on a larger framework of established plots that are
162 part of the Wisconsin Integrated Cropping Systems Trial (WICST), which is a long-term
163 research project examining crop rotations and on-farm biodiversity of cropping systems in
164 southern Wisconsin (WICST Project, 2011). Switchgrass plots were seeded in August 2007 with
165 the cultivar "*Forestburg"* at a rate of 10lb PLS/ac. The prairie plots were seeded in1999 with 25
166 different species native to the Tallgrass prairie, including C4 grasses, legumes and forbs. Weeds
167 were managed with herbicide as needed (Table S1).

168 Management treatments applied to the prairie and switchgrass plots included three N
169 fertilizer rates (0, 50, and 150 kg N ha^{-1}) and three harvest timings (summer, fall and the
170 following spring). Fertilizer was hand-applied in June of 2009 and 2010 as ammonium-nitrate
171 (see Table S1 for dates of plot management). The summer harvest timing was at the post-
172 anthesis phase of the dominant species within each perennial grass system; the fall harvest was
173 two weeks after a killing frost (temperature reach $\leq$-2.2°C for a minimum of 4hrs); and the
174 spring harvest was as soon as possible after snow-melt when field conditions permitted. A
175 control check plot that was never harvested and never fertilized was also included in the design.
176 This was a RCBD, with two levels of blocking – the first level the WICST plot, with a second
177 sub-block nested within each WICST block. Every management treatment was replicated six
178 times with a control plot in every block, creating 120 total experimental units with a plot size of

179 $4m^2$. A 1-meter alley between all plots allowed for some buffer between treatments in case of
180 fertilizer-N runoff and to reduce edge-effects.

181 ***NUE components: Yield, N concentration and N content data collection***

182 Biomass was harvested using a self-propelled sickle-bar mower in 2009; in 2010 we
183 transitioned to a gas-powered hedge-trimmer (Echo HC-150 hedge trimmer, 20" blade) to
184 improve precision and accuracy of cutting. Cutting height was approximately 15cm – similar to
185 that of farm-scale equipment. Field weights of biomass were recorded immediately after harvest.
186 Biomass was then chopped (TroyBilt CS4325 Chipper Shredder) to coarsely-homogenize
187 biomass before a subsample was taken for obtaining moisture contents by drying biomass at
188 65°C for over 48 hours. After subtracting moisture weight, dry matter weights were extrapolated
189 from plot yield to Mg ha^{-1}. Biomass samples were finely ground using a Wiley Mill to pass a
190 <1mm screen, then pulverized using stainless steel balls in 2ml micro-centrifuge tubes to further
191 homogenize. N concentrations were determined by combustion on a Carlo-Erba elemental
192 analyzer (CE Elantech EA1112, Lakewood, N.J.). N content of biomass was calculated by
193 multiplying yield by N concentration.

194 ***Calculating NUE metrics:***

195 Nitrogen-use was calculated using three different metrics: yield nitrogen-use efficiency (yield
196 NUE), apparent N-fertilizer recovery (ANR) (Lemus *et al.*, 2008b) and N productivity, where

197 $\text{Yield NUE} = (\text{yield}_{\text{fertilized plot}} - \text{yield}_{\text{unfertilized plot}})/\ \text{kg of N applied}_{\text{fertilized plot}}$

198 $\text{ANR (\%)} = \text{ANR} = [(\text{kg biomass-N}_{\text{fertilized plot}} - \text{kg biomass-N}_{\text{unfertilized plot}})/\text{kg of N applied}_{\text{fertilized plot}}] \times 100$

199 N productivity = (Mg ha^{-1} biomass / biomass N concentration).

Yield NUE considers how much biomass was produced on account of every kg of added fertilizer-N. ANR calculates the percent of added fertilizer-N assumed to be recovered and removed in harvested-biomass. These metrics are calculated by using fertilizer-N applied, so therefore cannot be calculated for the unfertilized plots. The N productivity metric does not consider how much N was added to the system, so all fertilizer treatments are comparable with one another. N productivity is the relationship between biomass quantity (yield) and quality (N concentration). Ideally, agronomic management would produce biomass of high quantity but very low quality, so the ratio of yield to N concentration is yet another method to evaluate responses to N-fertilizer rates.

N resorption data collection

In 2010, we selected several of the dominant species in each system to measure N resorption. We sampled leaves from *Andropogon gerardii* (Big Bluestem) and *Sorghastrum nutans* (Indiangrass), two native C4 grasses, and *Helianthus grosseserratus* (sawtooth sunflower), a native C3 forb, in the prairie. *Panicum virgatum* (switchgrass), also a C4 grass, was sampled in the monoculture. Leaves were sampled at the presumed start- and end-points of senescence – peak-standing biomass and approximately two weeks after a killing frost. Switchgrass leaves were also collected at two-week intervals throughout this senescence period, to address our question of *when* the resorption was occurring. Our samples at each collection timepoint included four leaves from four individuals within each plot. For the grasses, the top four leaves were consistently collected from one reproductive tiller of each individual; for the forb, leaf collections were equally spaced from the top to the middle of the plant. Samples were not collected from the summer harvest-timing treatment since these plots were completely harvested before the senescence process occurred. Therefore, management treatments for the N

223 resorption study included the three fertilizer rates and the previous year's harvest timing
224 treatments. Leaves were dried at 65°C for a minimum of 48 hours, weighed and ground on a
225 sample mill (Cyclone sample mill, UDY Corporations, Fort Collins, CO). Samples were then
226 analyzed for N concentration using the same method described above. Leaf N content was leaf
227 dry-weight multiplied by N concentration. Resorption was expressed as resorption efficiency
228 (RE), where

229 $RE = [\text{leaf N content}_{\text{green leaf}} - \text{leaf N content}_{\text{senesced leaf}})/\text{leaf N content}_{\text{green leaf}}] \times 100$

230 This describes resorption as the proportion of leaf N at peak-standing-mass (green leaf) that was
231 resorbed by the final leaf collection in early November (senesced leaf).

232 ***Soil inorganic N pools and mineralization rates***

233 Soil N measurements were collected in 2009 and 2010 in order to characterize the soil N-
234 status before and after treatments were implemented. Inorganic soil N pools (nitrate and
235 ammonium) and inorganic N mineralization rates (mineralized nitrate and ammonium) were
236 assessed in plots in June 2009 before any management treatments were implemented and again
237 in June 2010, immediately before the 2010 fertilizer application. Soil samples consisted of three
238 composited soil cores (2.5 cm x 15cm) in each plot. Samples were transported on ice and stored
239 at 4°C until processed, which was within 48 hours of field collection. Soils were homogenized
240 and organic matter and rocks removed using a 2mm sieve, followed by a 2M KCl extraction
241 (Robertson *et al.*, 1999). Extracts were then filtered using Whatman A/E 0.2 µm glass fiber
242 filters and frozen (-20°C) until analysis. Nitrate concentrations were determined by reduction
243 with Devarda's alloy (Sims *et al.*, 1995) and ammonium concentrations by the Berthelot method

(Rhine *et al.*, 1998). Samples were read on a Labsystems Multiskan EX microplate reader (Thermo Fischer Scientific, Walthman, MA) at 650 nm absorbance.

Statistical analyses

Components of NUE (yield, N concentration and N content) and soil N data were analyzed separately for each year (2009, 2010). NUE metrics were analyzed across the two years to more broadly assess trends. To further explore N productivity within each plot, we regressed N concentration values against their respective yields to calculate correlation coefficients of this relationship in each cropping system. We used linear-mixed effects models (TIBCO Spotfire S+ v.8.1.1, TIBCO Software, Inc., Palo Alto, CA) to evaluate treatment effects. Fixed effects were the N fertilizer rates (used as a factor) and harvest treatments, except for analysis of switchgrass leaves through senescence within each fertilizer treatment, where fixed effects were harvest-timing of the previous season and day of year (DOY). Random effects were always sub-blocks nested within blocks. If needed, response variables were transformed for analysis using $\log_{10}$ to meet assumptions of normality and equal variance, although figures use untransformed data to report agronomically-meaningful values. Analysis of variance evaluated fixed effects of models, where significance was determined at $\alpha = 0.05$.

To evaluate differences between treatment means, model simplification described by Crawley (2002) was used, where the Akaike Information Criterion (AIC) and the restricted maximum likelihood algorithm (REML) determined the most parsimonious model. This was done by dropping factors from the initial model to create a more simplified model, then comparing the more complex to the simplified model. If the models were significantly different (at $p<0.05$), the model with the lower AIC was chosen.

Results

Biomass Yields

Yields varied by crop, N fertilizer rate and harvest timing within each year (Table 2, Table 3). Switchgrass out-yielded the prairie in 2009, but 2010 proved prairie to be an equal competitor in harvestable yield. Switchgrass response to fertilizer was apparent in 2009 for the summer and fall harvests, while no response was evident the following spring. There was no switchgrass yield response to fertilizer additions in 2010, except for the 2010 spring harvest, when fertilized plots actually produced significantly less biomass (Figure 1). Prairie yields had the opposite outcome where N response was not apparent in 2009, but 2010 yields showed significant yield increases when fertilized across all harvest timings (Figure 1). Across cropping systems, the summer harvest treatment generally produced the greatest yields, followed by the fall, and the least yield in spring. This however was not the case in 2010 prairie yields where harvest timing had no effect on yields (Table 1 and Figure 1).

Biomass N Concentration

N concentrations of the harvested biomass were more responsive to management treatments of N fertilizer additions and harvest timing. N concentrations did not differ between cropping systems in 2009 but prairie N concentration was significantly higher in 2010 (Table 3). Switchgrass N concentration was responsive to fertilizer additions while the prairie was only responsive to fertilizer additions in 2009 (Figure 2). Across both years and cropping systems, harvest timing had a very strong impact on N concentrations. Most of the decrease occurred between the summer and fall harvests, with a smaller or non-significant change from fall to the spring harvest, such as in the prairie 2010 biomass. N rate had no effect within each year of the prairie

spring harvest, whereas the fertilizer effect remained very apparent through all switchgrass harvest timings (Figure 2). The unfertilized plots tended to have approximately the same N concentrations as the high N-rate plots in the fall and spring, although this trend was not sustained in the 2010 prairie biomass. The 2009-2010 switchgrass and 2009 prairie had relatively similar patterns in regard to treatment effects on N concentrations whereas the 2010 prairie N concentrations were unique in regard to treatment effects (Figure 2).

Biomass N Content

N content, driven by both the yield and N concentration, indicates the amount of N in the harvested biomass, which is synonymous with N removed from the system at harvest. The summer harvest also represents a measure of total N uptake. The cropping systems differed in their N removal rates by year: switchgrass removed more N in 2009 whereas the prairie removed more N in 2010. Switchgrass averaged 37 kg N ha^{-1} and 38 kg N ha^{-1} in 2009 and 2010, respectively, averaged across N and harvest treatments. Prairie N content varied considerably between years, with only 21 kg N ha^{-1} in 2009 and 44 kg N ha^{-1} in 2010, averaged across harvest and N treatments. N rate and harvest timing were significant treatment effects in both years (Table 3), with N removal patterns often following the yield and N concentration patterns (Figures 1 and 2). Although the 2010 prairie biomass had no fertilizer treatment effects on N concentrations, the biomass N content followed roughly the same pattern of the yield response to N rates (Figure 1, Figure 3). Increasing fertilizer rates generally increased N removal rates, with the magnitude of the effect decreasing with later harvest times (Figure 3). N uptake averaged 31 kg N ha^{-1} in the 2009 prairie summer harvest and 42 kg N ha^{-1} in the 2010 summer harvest. N uptake in switchgrass summer harvests were 40 kg N ha^{-1} and 49 kg N ha^{-1} in 2009 and 2010, respectively.

In 2010, prairie yields were not affected by harvest timing (p=0.09), but responded to N rate (p<0.0001) (Figure 1), while the N concentration was affected by harvest timing (p=0.0002) and not N rate (p=0.4). This instance where biomass yield increases with N rate, but N concentrations do not, is the preferred situation for biomass production systems because the plants are using nitrogen more efficiently and adding more units of carbon per unit of added fertilizer-N. Although N removal increases with increasing N rate, it is driven by the increase in yield rather than increasing N concentrations.

NUE metrics

NUE metrics were averaged across years to summarize N use dynamics of each cropping system. The yield NUE (kg biomass kg N^{-1}) indicates how many kg of biomass were produced on account of every added kg of fertilizer N. Fertilizing proved to be relatively inefficient, as fertilizing with 50 kg N ha^{-1} gave the prairie an advantage of only 11.9 kg of biomass per kg added fertilizer-N, averaged across harvest timings. Switchgrass revealed an even weaker use of fertilizer N with only 6.3 kg of additional biomass per kg of N when 50 kg N ha^{-1} was applied. The N rate treatments generally had no effect on yield NUE, except in 2010 for the spring-harvested switchgrass, when the 50-N treatment produced significantly less biomass per kg of fertilizer compared to the other treatments (Table 4). The spring harvest produced a negative NUE indicating that waiting to harvest at this point in the season, fertilized biomass can result in fewer grams of biomass harvested per kg of N added (Table 4, Figure 1).

Apparent N recovery (ANR) provides an estimate of what percent of fertilizer-N applied was recovered by plants by discounting the N-uptake in an unfertilized plot of the same harvest treatment. The estimate of fertilizer-N recovery was significantly lower for the later harvest

timings for both switchgrass ($p<0.0001$) and prairie ($p=0.0004$). These differences between harvest timings suggest resorption of N from August to November (Table 4). Fertilizer rates in both cases were not statistically different, although in all cases the 50N treatment mean recoveries were slightly higher than the 150N treatment mean recoveries, which is consistent with the trend of higher fertilizer rates becoming more inefficient. ANR between cropping systems were comparable ($p=0.6$), illustrating similar N-uptake capacities between the switchgrass monoculture and the prairie.

To evaluate all management treatments including the unfertilized plots, the relationship of yield to N concentration was considered (Figure 4). Across all years and management treatments, there is a significant, but weak relationship between switchgrass yield and N concentration ($r^2=0.32$, $p<0.0001$), whereas there was no significant relationship in the prairie ($r^2=0.0001$, $p=0.9$). Management treatments had no affect on N productivity in the prairie ($p=0.5$), but management treatment was an important predictor for switchgrass N productivity ($p<0.0001$). Switchgrass had the highest N productivity in the fall-harvested 50-N treatment and the summer-harvested 0-N treatment (Table 5).

N resorption

N resorption was evident in all species measured. RE in the prairie species differed mainly by species ($p<0.0001$) without significant treatment effects. *Helianthus grosseserratus* RE was highest (68.8 ± 1.9% [s.e.]), followed by *Sorghastrum nutans* (46.2 ± 3.6%) and *Andropogon gerardii* (34.2± 4.7%). *Panicum virgatum* RE averaged 53.2±2.6% across management treatments, but differed significantly by N fertilizer rates ($p=0.007$), with the

highest RE in the 150-N treatment (61.8 ± 3.4%), followed by the 50-N treatment (51.5±4.8%) and the lowest resorption in the 0-N treatment (46.3 ± 4.5%).

Switchgrass leaf N content decreased from late July through early November. Leaf N content decreased most dramatically in all management treatments from the end of July (DOY 209) through the end of August (DOY 242) (Figure 5). Previous harvest schedules from the 2009 growing season had a significant interaction with N fertilizer rate (p=0.001) across all collection timepoints. Leaves collected from the unfertilized plots reached minimum leaf N content by DOY 242 (Figure 5a). Although leaves collected from the fertilized plots lost the majority of their N by DOY 242, there was still a significant decrease in leaf N at the end of the season. By DOY 307, fertilized leaves had reached their minimum N content (Figure 5b, 5c). Senesced leaf N content (at DOY 307) was not affected by management treatments (p=0.4), however, N concentration had a significant N treatment by harvest interaction ($p=0.02$) (Figure 6), with higher concentrations in the 150-N leaves ([N] = 1.66%) compared to the 50-N ([N] = 1.53%) and 0-N leaves ([N]=1.25%) (p<0.0001). Leaf mass decreased throughout the senescence period across all harvest treatments (p<0.0001) resulting in a mean 50% loss in mass from the first sampling period to the last sampling period (data not shown). This mass loss in leaf tissue may be an indication of carbohydrate resorption, in addition to other mineral components of the leaf tissue.

Prairie species did not show any effect of management treatment on final senescenced-leaf N concentrations. There were however significant differences in N concentration by species (p<0.0001) with concentrations values, averaged across management treatments, of 1.12%, 1.31% and 1.67% for *Sorghastrum nutans*, *Andropogon gerardii* and *Helianthus grosseserratus,* respectively (Figure 6).

Inorganic soil N status and mineralization rates

The 2009 soil data revealed that there were no differences in inorganic soil levels between plots before the fertilizer and harvest treatments were implemented (Table 6). In June of 2010, one year after the initial fertilization, soil N levels were generally higher across all management treatments. The only significant treatment effect was seen in the unfertilized switchgrass plots, which had lower inorganic N levels compared to the fertilized switchgrass plots (p=0.008). Although there were no treatment effects in mineralization rates, it is interesting to note that mineralization rates were negative in 2009, suggesting that inorganic N was being immobilized rather than mineralized. In 2010, mineralization rates were positive and were not affected by legacy of the management treatments implemented in 2009.

Discussion

Yield and biomass N concentration trends vary by year and cropping system

Responses to N fertilizer additions were not consistent between cropping system, year or harvest timing. While the prairie had no yield response to N additions in 2009, switchgrass had modest yield increases with fertilization, whereas the 2010 growing season brought the opposite trends. Why was there not a fertilizer response in all situations? Weak fertilizer responses could indicate no N-limitation. However, inorganic soil N data in 2009 showed negative N-mineralization rates, suggesting that these systems were in fact N-limited and plants may have been out-competed for resources by microbes. In 2010, when N mineralization rates were positive, there was more soil N available, however the switchgrass yields still had no fertilizer N response. The 2010 growing season apparently had better growing conditions irrespective of N

availability since all yields were greater than 2009 yields even in the unfertilized plots. This study is an indication that yield response to fertilization is extremely variable even within a field.

Yield trends by harvest timing were more predictable where later harvest-timings often produced smaller yields in switchgrass. Waiting for a fall harvest resulted in an average 30% loss in yield and delaying harvest the following spring caused about another 30% loss in biomass, or approximately 53% loss from the summer harvest. Adler et al. (2006) found 40% losses by over-wintering biomass in the field with the reductions attributed to harvest inefficiencies and a drop in individual tiller weights. The prairie seemed to be more resilient to biomass losses from fall to spring but did show yield reductions only in the 2009 growing season from summer to fall.

Plant tissue N concentrations tended to increase with fertilization, as others have found (Madakadze *et al.*, 1999; Heggenstaller *et al.*, 2009; Garten *et al.*, 2011). The one exception to this trend was in the 2010 prairie harvest, where N fertilizer rate had no affect on biomass N concentrations, which has also been an observed trend in other studies (Lemus *et al.*, 2008b; Waramit *et al.*, 2011). In addition to high N concentrations contributing to higher N removal rates, the biomass N concentrations often did not fall below the recommended threshold for feedstocks of 0.6% N (Kauter *et al.*, 2003). Only some of the fall or spring-harvested biomass in the 0-N or 50-N treatments would meet this biomass-quality parameter.

Unfertilized plots removed a cumulative of 74 kg N ha^{-1} in prairie and 90 kg N ha^{-1} in switchgrass over two growing seasons without any fertilizer inputs. It is remarkable that N removal in 2010 exceeded N removal in 2009 despite biomass removal for the second-consecutive seasons with zero inputs, although this is not an uncommon for switchgrass, as many

420 other studies have demonstrated unfertilized switchgrass removing high N amounts despite
421 continuous years of no added N inputs (Parrish and Fike, 2005; Lemus *et al.*, 2008b). Fertilized
422 switchgrass can also exhibit high levels of N removal that exceeds the initial fertilizer application
423 (Lemus *et al.*, 2009), but was the case in this study only for the 50-N treatment in switchgrass
424 and the 50-N treatment in 2010 for prairie.

425 ***NUE metrics suggest crops are inefficient users of fertilizer-N***

426 Previous studies using nitrogen-use metrics often find that efficiencies diminish with
427 increasing N rates, as yield response to increased fertilizer application typically plateaus (Havlin
428 *et al.*, 1999; Lemus *et al.*, 2008b), so it is not surprising then that we observed similar patterns in
429 this study. Perhaps more noteworthy is how these nitrogen efficiency metrics respond to the
430 different harvest timings, which again illustrates losses in biomass N content with later harvest
431 timings.

432 Yield NUE evaluates nitrogen efficiency in terms of yield, and since yield increases by
433 fertilizer additions were relatively small, the NUE resulted in relatively small gains of biomass
434 per added kg of N fertilizer. If delaying harvest until spring, fertilized plots actually revealed
435 decreases in biomass. Yield NUE in the prairie plots were highly variable, which may contribute
436 to why there were no differences between management treatments observed.

437 Apparent fertilizer recovery (APR) describes NUE in terms of N uptake rather than yield.
438 The caveat with this metric is that N removed by biomass in fertilized plots is presumed to be
439 fertilizer-N, however, a labeled ^{15}N study would be required to understand precisely how much
440 fertilizer-N was recovered by plants (Robertson and Vitousek, 2009), compared to microbial-
441 derived N resources. Previously recorded AFR values indicate switchgrass fertilizer recovery

442 rates can range from 22 to 57%, depending on geographic location and native soil N levels
443 before fertilization, when switchgrass was fertilized at a rate of 84kg (Stout and Jung, 1995).
444 Switchgrass grown on marginal sites were found to uptake 15-39% of the applied fertilizer-N,
445 with higher rates of recovery in the lower fertilizer rates (Staley *et al.*, 1991). Fertilizer recovery
446 exceeded N fertilizer additions of 90 kg ha^{-1} at 140% recovery but was reduced to only 44% at
447 270 kg ha^{-1} N rates in a study by Lemus et al., 2008. APR in this study were similar across N
448 fertilizer treatments, which demonstrates that large portions of fertilizer-N, especially in the 150-
449 N treatment, are potentially being subjected to N- loss pathways since it is not being recovered
450 in above-ground plant material. The differences in APR between harvest timings again reflects
451 the resorption of N, as the differences in N content between the fertilized and unfertilized
452 biomass narrows after significant resorption has occurred.

453 Prairie realized lower N productivity rates than switchgrass, but prairie N productivity
454 was also much more variable. Regression of yield to N concentration suggest that biomass
455 accrual in switchgrass is more dependent on increases in tissue N concentration than in the
456 prairie. About 10% of prairie N productivity rates were greater than any switchgrass plots,
457 which makes one wonder what is different about these plots? Since management treatment had
458 no effect on N productivity in prairie, the source of variability in the N productivity may be
459 derived from the assemblage of species present. The species composition effects on N
460 productivity warrants further consideration since management had no effect and the prairie plots
461 used in this study were very heterogeneous in regard to species. Others have shown that species
462 diversity has as an equal impact on productivity as N-fertility management (Weigelt *et al.*, 2009).

463 Considering all NUE metrics described here, fertilizing with 150 kg N ha^{-1} has shown to
464 not be more efficient than fertilizing with 50 kg N ha^{-1}, which suggests that the excess fertilizer

not being used by the plants is subject to various N-loss pathways in the N cycle. N productivity in switchgrass indicates that fertilizing 50 kg N ha^{-1} and harvesting in fall results in the most biomass with the best biomass quality. Prairie was highly variable in N productivity, which suggests further investigation is needed to elucidate what, if any, factors are creating the differences in N productivity.

N resorption is affected by management treatments in switchgrass, but not prairie species

Resorption was significant in all species and measured rates were within expected ranges, given previous resorption rates found in the literature, as discussed above. What is perhaps more interesting, is that the RE was affected by fertilizer rates in the switchgrass and not in any of the prairie species. Decreases in the switchgrass leaf N through time indicate significant N resorption through senescence, but fertilized plots require more time to resorb its N, possibly because there was more N to remobilize. The interaction of N treatment and previous-harvest treatment on N pool status was significant, but no clear pattern or explanation is immediately apparent. Further investigation into other factors affecting N dynamics, such as soil-N availability, mineralization and decomposition rates may provide some insight into the observed differences in leaf N by the legacy of prior harvest schedules. Fertilizer affects on final senesced leaf N concentrations had similar trends, where the prairie species were not affected but switchgrass leaf N concentrations were significantly higher in the fertilized plots. This indicates that despite switchgrass leaves being able to resorb a higher proportion of leaf N, these fertilized plants are unable to reduce their N concentrations to the low levels of the unfertilized plants.

Further study should consider why the C4 grasses in the prairie responded differently to fertilizer-N additions than the C4 grass in the monoculture. The prairie species measured were

not always a dominant species in regard to total cover; most plots had significant C3 grass cover in addition to a variety of other prairie forbs. The effect of management treatments may have been overwhelmed by other N dynamics in the plot, such as N-resource competition with other species. These results also indicate that these particular prairie species may simply be more N-use efficient than switchgrass by building more biomass with N resources rather than increasing tissue N concentrations.

Despite unfertilized switchgrass leaf N content reaching final levels much earlier in the season than their fertilized counterparts, whole-plot N content in unfertilized plots showed significant decreases by the end of the season. This suggests that other factors aside from N resorption are contributing to reductions in N removal with harvest. Our investigation into resorption only considered leaf tissue since the majority of N pools reside here, but further studies should consider the entire tiller in order to provide a more direct accounting of total plant N resorption. One factor possibly contributing to the additional reductions in N content through senescence is the change in leaf:stem ratios from summer to fall. As switchgrass senesces, leaves become more brittle and dry and are more prone to break off and become part of the litter pool. Leaf:stem ratios have been shown to decrease through the season (Heaton *et al.*, 2009), which would lead to lower N concentrations in the biomass left standing to be harvested. In another study, leaf biomass realized a 40% loss in biomass from August to November (Miesel and Smith, 2010). Leaf:stem ratios could be an important determinate in switchgrass biofuel quality since stems are found to be of more desirable quality in terms of fiber content (Lemus *et al.*, 2002) and N concentration (Lemus *et al.*, 2009). Future research should focus on biomass losses through the senescence process and account for losses from harvest machinery

509 inefficiencies and increased residual biomass in the litter pools, versus mass losses from
510 carbohydrate resorption.

511 **Conclusions and Implications**

512 This study reveals the responses to N fertilizer and harvest timings on yields, N-use
513 dynamics and one mechanism contributing to changes in biomass N content through the season.
514 Given that this experiment was conducted on high-productivity soils, a comparison of
515 management treatments on more marginal soils may have revealed different trends. Although
516 more diverse cropping systems are touted as having more resource complementarity and
517 therefore higher resistance to leaky N cycling (Bingham and Biondini, 2011), this study suggests
518 that the N-use dynamics in two differing diversity-levels were similar. Subsequent years of
519 management may show more drastic effects of management treatments as harvesting begins to
520 deplete soil resources or plant reserves, but after two years of management, it appears that
521 fertilizing these systems for biomass production realizes relatively small increments of additional
522 yield at the expense of lower biomass quality and unaccounted for fertilizer-N that will be
523 subject to N-loss pathways and likely contribute to air and water pollution. This was evidenced
524 by (1) yields had a very limited response to fertilizer application in switchgrass, although the
525 prarie response varied by year (2) fertilizing switchgrass increased N concentrations, while the
526 prairie N concentration response again varied by year (3) delaying harvest until fall minimizes
527 the amount of N removed from the field; it is not necessary to wait until the following spring to
528 harvest to reduce N removal and sacrifice biomass and (4) fertilizer N-use dynamics showed
529 small yield gains by fertilizer and low fertilizer recovery which suggests that system-N
530 requirements are met by soil mineralization and/or mychorrhizal associations. Management also
531 affected N resorption rates in the switchgrass monoculture, but not the selected prairie species.

Although unfertilized switchgrass plots reach full translocation potential before fertilized counterparts, the loss of biomass from other factors likely contribute to the lower N removal at harvest time.

While perennial warm-season grass cropping systems show great potential for a sustainable feedstock, assumptions are often overlooked regarding their benefits because of management effects. Our results suggest that fertilizer responses are often weak, or at least variable, and therefore excess applied fertilizer-N is prone to loss pathways. Goals of increasing land devoted to biomass prodcution and simultaneously reducing N exports to the air and waterways may be conflicting if the expected perennial cropping systems are unable to retain added nitrogen inputs and N-recycling strategies. Future managers and policy-makers should consider the trade-offs in yields with biomass quality, N-removal and NUE in biomass production to ensure that land devoted to biomass production will not contribute to, but rather mitigate agroecosystem N losses.

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693 **TABLES and FIGURES**

694 **Table 1.** Studies that cover a range of sites and N fertilizer rates show both positive and no response to N
695 fertilizer additions. Adapted from Parrish and Fike, 2005.

Site	range of N rates (kg ha^{-1})	Yield response to N additions	Reference
Montreal, QB	0 - 150	positive	Madakadze *et al.* (1999)
IA, USA	0 - 220	positive	Heggenstaller *et al.* (2009)
IA, USA	0 - 224	positive	Lemus *et al.* (2008)
TX	0 - 268	positive	Suplick *et al.* (2002)
IA, NE	0 - 300	positive	Vogel *et al.* (2002)
Rothamsted, UK	0 - 60	no	Christian *et al.* (2001)
VA, USA	0 - 110	no	Parrish and Wolf (1992)
Trisaia, Italy	0 - 150	no	Piscioneri *et al.* (2001)
SD, USA	0 - 224	no	Mulkey *et al.* (2008)
OK, USA	0 - 896	no	Thomason *et al.* (2004)

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712 **Table 2.** Mean biomass yields (Mg ha^{-1}) and mean standard error (SE) for prairie and switchgrass at
713 three harvest timings (*Summer, Fall and Spring*) by N fertilizer rate in 2009 and 2010 (n = 5 or 6).

N fertilizer rate (kg ha^{-1})		0		50		150	
2009		Mean	SE	Mean	SE	Mean	SE
	Prairie						
	Summer	3.79	0.69	4.61	1.01	3.82	0.73
	Fall	2.05	0.38	2.50	0.68	2.62	0.44
	Spring	1.61	0.25	1.30	0.32	2.38	0.33
	Switch						
	Summer	5.53	0.62	6.34	0.39	6.94	0.64
	Fall	3.24	0.26	4.79	0.11	5.15	0.55
	Spring	3.50	0.35	2.86	0.44	2.88	0.36
2010							
	Prairie						
	Summer	3.71	0.39	5.95	0.48	6.59	0.66
	Fall	3.79	0.31	4.29	0.78	5.51	0.78
	Spring	3.99	0.24	3.85	0.47	6.12	0.84
	Switch						
	Summer	6.62	0.35	6.86	0.24	7.17	0.56
	Fall	4.30	0.18	5.17	0.34	4.80	0.33
	Spring	3.78	0.18	2.48	0.26	3.06	0.30

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725 **Table 3**. Analysis of variance for linear mixed-effects models predicting plot yield (Mg ha^{-1}), N
726 concentration of the harvested biomass ([N]) and the N content of the harvested biomass in 2009 and
727 2010. Fixed effects are: cropping system (*crop*), N fertilizer rate (*N rate*) and harvest timing (*harvest*)
728 (n= 5 or 6, $\alpha = 0.05$).

Fixed Effects	Df	*F* statistic	*p-value*	*F* statistic	*p-value*
Yield		*2009*		*2010*	
crop	1	61.47	<0.0001	0.19	0.67
N rate	2	2.85	0.06	10.85	0.0001
harvest	2	47.18	<0.0001	40.24	<0.0001
crop x N rate	2	0.30	0.74	8.34	0.0005
crop x harvest	2	1.29	0.28	18.14	<0.0001
N rate x harvest	4	1.46	0.22	2.44	0.05
crop x N rate x harvest	4	1.53	0.20	1.05	0.39
[N] (%)					
crop	1	0.59	0.44	21.86	<0.0001
N rate	2	23.24	<0.0001	5.54	0.0056
harvest	2	35.52	<0.0001	26.25	<0.0001
crop x N rate	2	0.13	0.88	0.68	0.51
crop x harvest	2	0.10	0.90	0.46	0.63
N rate x harvest	4	4.06	0.0047	2.23	0.07
crop x N rate x harvest	4	1.02	0.40	0.90	0.47
N Content (kg N ha^{-1})					
crop	1	76.83	<0.0001	19.55	<0.0001
N rate	2	25.53	<0.0001	24.28	<0.0001
harvest	2	130.3	<0.0001	123.3	<0.0001
crop x N rate	2	0.03	0.98	1.22	0.30
crop x harvest	2	1.01	0.37	14.90	<0.0001
N rate x harvest	4	1.89	0.12	1.24	0.30
crop x N rate x harvest	4	1.11	0.36	0.72	0.58

Table 4. Mean yield NUE [(yield $_{\text{fertilized plot}}$ − yield $_{\text{unfertilized plot}}$)/ kg of N applied $_{\text{fertilized plot}}$] and mean ANR [(kg biomass-N $_{\text{fertilized plot}}$ − kg biomass-N $_{\text{unfertilized plot}}$)/kg of N applied $_{\text{fertilized plot}}$] x100, by harvest timing and N fertilizer rate (kg ha^{-1}) for prairie and switchgrass, using combined data from 2009 and 2010. Letters indicate significance between all management treatments within each cropping system ($\alpha = 0.05$). SE is 1 standard error (n = 11 or 12)

	Yield NUE		ANR(%)	
	Mean	SE	Mean	SE
Prairie				
Summer				
50	30.48	14.60	25.94a	11.93
150	9.67	4.18	21.79a	5.52
Fall				
50	9.56	7.18	11.67b	4.05
150	7.64	3.02	8.58b	2.12
Spring				
50	-4.47	7.61	9.41b	4.64
150	9.63	3.87	7.74b	2.71
Switchgrass				
Summer				
50	10.37a	10.50	32.88a	8.45
150	6.51a	3.58	28.53a	5.60
Fall				
50	24.07a	5.24	16.12b	3.47
150	8.13a	2.94	12.03b	2.66
Spring				
50	-17.65b	5.71	-0.14c	2.80
150	-3.82a	2.30	2.53c	1.53

Table 5. Mean and mean standard error (SE) for N productivity (Mg ha^{-1} biomass / biomass N concentration) by harvest timing and N fertilizer rate for prairie and switchgrass using combined data from 2009 and 2010. Letters indicate significant differences between all management treatments within each cropping system (n = 11 or 12).

	Prairie		Switchgrass	
	Mean	SE	Mean	SE
Summer				
0	4.14	0.64	8.26a	0.55
50	5.81	0.87	7.17b	0.41
150	4.09	0.63	5.75c	0.33
Fall				
0	5.46	0.82	7.42b	0.63
50	5.39	1.19	9.09a	0.54
150	6.86	1.68	6.66b	0.52
Spring				
0	4.88	0.68	7.69b	0.61
50	3.28	0.64	4.30d	0.55
150	7.90	1.73	3.92d	0.31

Table 6. Mean inorganic soil N (nitrate + ammonium) and mean inorganic N mineralized (nitrate and ammonium) in the top 15cm of soil in early June before fertilizer additions in 2009 and 2010, by management treatments of N fertilizer rates (kg ha^{-1}) and harvest timing (*summer, fall and spring*). Note that 2009 data is pre-treatment, meaning the fertilizer and harvest treatments were not yet implemented. (*) indicates significance across all management treatments within each cropping system and year ($\alpha = 0.05$).

	Inorganic N (kg ha^{-1})			Inorganic N mineralized (kg ha^{-1} day^{-1})		
N fertilizer rate	0	50	150	0	50	150
June 2009, pre-treatment						
prairie						
summer	5.34	4.20	3.84	-0.21	-0.28	-0.38
fall	3.64	3.27	3.69	-0.29	-0.67	-0.22
spring	5.97	4.44	2.97	-0.16	-0.25	-0.30
switchgrass						
summer	4.51	4.58	3.56	-0.49	-0.24	-0.25
fall	4.93	3.81	3.46	-0.36	-0.54	-0.33
spring	2.87	3.93	3.47	-0.33	-0.43	-0.22
June 2010, pre-fertilizer						
prairie						
summer	8.39	7.98	9.15	0.90	0.90	0.93
fall	9.05	8.82	8.05	0.90	0.96	0.88
spring	8.83	10.72	8.45	0.99	0.81	1.11
switchgrass						
summer	6.95*	7.59	9.91	1.49	1.46	1.36
fall	7.11*	7.90	7.94	1.32	1.23	1.65
spring	7.43*	8.32	8.63	1.09	1.18	1.26