1 Title: Nitrogen conservation decreases with fertilizer addition in two perennial grass cropping
2 systemsfor bioenergy

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Abstract

Warm-season prairie grasses are promising bioenergy crops that exhibit conservative nitrogen use and cycling, which promises to reduce expensive N amendments and contributions to environmental N pollution associated with annual cropping systems. However, efforts to maximize crop yields with fertilizer may reduce N conservation in these systems. We used two perennial grass systems of differing diversity levels – a restored tallgrass prairie and a *Panicumvirgatum*(switchgrass) monoculture—to determine the effects of N fertilizer level and harvest timing on biomass yields, N concentrations and N removal at harvest. To address plant N conservation, we measured N resorption efficiency (proportion of N resorbed), resorption proficiency (minimum N level attained after senescence), and timing of resorption under different N fertilizer rates. Yield responses to N fertilizer were not consistent between cropping system, year, or harvest timing, and were generally weak, resulting in an average of only 1.27 times more biomass compared to unfertilized plots. In contrast, fertilized plots removed 1.67 times more N relative to unfertilized plots, as N removal was largely driven by increases in biomass N concentrations rather than increases in yield. N resorption was affected by fertilizer in switchgrass, but not in selected prairie species. Fertilized switchgrass plants took longer to reach their maximum resorption levels and had reduced resorption proficiency, despite higher resorption efficiencies. Our results suggest that striving to increase biomass yields with N fertilizer may be an imprudent approach to sustainable bioenergy production, because yield responses are highly variable and N conservation mechanisms are compromised.

Keywords: nitrogen conservation; resorption efficiency; resorption proficiency; warm-season grass; biofuels

1. Introduction

Perennial grasses have the potential to be an important source of cellulosic biofuel feedstocks for renewable energy and depending on management practices, may reduce agriculturally-related nitrogen (N) pollution (Dale et al., 2014). In an effort to meet renewable energy goals, some productive croplands may transition to dedicated bioenergy crops. To avoid competing with food, farmers may expand bioenergy crops to nonagricultural lands, thereby causing a net increase of managed lands (Tilman et al., 2009; Walsh et al., 2003) or intensify production on so-called marginal lands (Gelfand et al., 2013). Agricultural intensification often leads to negative environmental consequences, including increases in N pollution (Drinkwater and Snapp, 2007). N pollution associated with production practices and fertilizer inputs has caused significant environmental decline, altering plant communities, soil tilth, water quality and air quality (Galloway et al., 2003; Schlesinger, 2009) and comes with significant costs to society (Galloway et al., 2008; Johnson et al., 2010). Therefore, producing bioenergy crops that mitigate, rather than contribute to N pollution is an important part of producing bioenergy crops sustainably (Bhardwaj et al., 2011; Robertson et al., 2011). Improving production practices that reduce our reliance on N fertilizer and limit N losses to the environment remains a major challenge (Robertson and Vitousek, 2009; Syswerda and Robertson, 2014). One way to increase agricultural production and simultaneously reduce N losses is to use cropping systems that are conservative and efficient with N resources, which reduces the need for N fertilizer (Robertson and Vitousek, 2009; McSwiney et al., 2010).

Perennial grasses have remarkable attributes that make them highly productive, yet conservative with N use (Dell et al., 2005). One important trait is high N-use efficiency. Perennial grasses that use the C4 photosynthetic pathway make biomass production per unit of N

1 acquired more productive compared to grasses that use the C3 photosynthetic pathway (Brown,
2 1999). A keystone nutrient-conserving strategy used by many perennial plants is recycling
3 internal N stores (Chapin, 1980; Killingbeck, 1996). Prairie grasses have been found to be
4 exceptionally efficient in retranslocating, or resorbing nutrients and carbohydrates to roots and
5 crowns during senescence for storage over winter to be recycled for subsequent growth (Clark,
6 1977; Heckathorn and Delucia, 1996). In bioenergy cropping systems, N resorption limits N
7 losses through litterfall, and more importantly, N removal via harvest. Reducing N losses should
8 allow plants to become less dependent on exogenous N sources.

9 Despite the high N-conserving strategies of perennial grasses, general management
10 recommendations call for the use of N fertilizer to optimize biomass production (U.S.
11 Department of Energy, 2011). However, numerous studies have evaluated perennial grass
12 yields, and fertilizer responses have been mixed. While there are many studies that show
13 positive yield responses to fertilization (Heaton et al., 2004; Heggenstaller et al., 2009; Lemus et
14 al., 2008a), others found instances of limited or no yield responses (Jarchow and Liebman, 2012;
15 Jung and Lal, 2011; Mulkey et al., 2008; Thomason et al., 2004). Yield responses to fertilizer
16 are often highly variable and site-dependent (Haque et al., 2009; Parrish and Fike, 2005;
17 Wulfschleger et al., 2010) and fertilizer responses for many perennial feedstocks remain
18 uncertain (Bonin and Lal, 2012). The lack of N response often reported may be explained by the
19 conservative N-use inherent to these crops, as well as uncharacterized microbial associations,
20 such as mycorrhizal fungi or N₂-fixers in the rhizosphere (Parrish and Fike, 2005).

21 Plant biomass N concentrations also have variable responses to N fertilizer inputs. Plant
22 tissue N concentrations often increase with N fertilization (Garten et al., 2011; Guretzky et al.,
23 2010; Heggenstaller et al., 2009; Jarchow and Liebman, 2012; Jung and Lal, 2011; Madakadze et

al., 1999), but not always (Lemus et al., 2008b; Waramit et al., 2011). Biomass N concentration is an important consideration, because along with yield, it drives how much N is removed from the field at the time of harvest. Current management recommendations suggest harvesting after plants have completely senesced, which ensures plants have completed resorption. This reduces the N removed from the field and decreases the amount of fertilizer needed the following season. Additionally, biomass with low N concentrations improves feedstock quality for most bioenergy uses (Adler et al., 2006; Anex et al., 2007), therefore a threshold concentration of 0.6% N has been recommended for biomass feedstocks (Kauter et al., 2003). While delaying harvest has advantages for nutrient conservation and biomass quality, harvest delay also significantly reduces yields (Adler et al., 2006; Heaton et al., 2009; Vogel et al., 2002). Minimizing the trade-off between yield and N removal at harvest remains a challenge for sustainable biomass production.

Understanding drivers of N resorption in bioenergy crops may be a key to minimizing the trade-off between yield and N removal. N resorption efficiencies vary considerably among perennial plants, ranging from 0 to 90% , with a global mean of 62% for unfertilized perennial plants and graminoids averaging even higher rates at 75% (Vergutz et al., 2012). An understanding of what underpins this variability is beginning to emerge. Plant genetics are one factor, as switchgrass accessions have resorption efficiencies 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 (Huang et al., 2008; Kobe et al., 2005; Norris and Reich, 2009), while others have found no effect of N availability on resorption (Aerts and Chapin, 2000; Aerts, 1996); 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 no research has explored how the resorption strategies of these bioenergy species will be affected by different fertilizer rates. The timing of N resorption also has significant agronomic implications, since delaying harvest reduces biomass yields. More research is needed to understand the rate, timing and extent of N resorption in managed bioenergy crops (Sanderson and Adler, 2008; Sanderson et al., 2006), and more specifically, how N fertilizer affects the resorption capabilities of bioenergy crops.

We measured how two perennial grass cropping systems – a restored prairie and a switchgrass monoculture – responded to varying N fertilizer rates at different harvest timings, as well as how N fertilizer affected N resorption. We hypothesized that N removal with harvest would increase with N fertilizer rate at all harvest timings, driven by increased biomass N concentrations, yields, or both and N resorption would decrease with increasing N fertilizer rates.

2. Materials and Methods

2.1. Site characteristics and experimental design

The experiment was conducted from 2009 through the spring of 2012 at the Arlington Agricultural Research Station in Arlington, Wisconsin (43°18' N, 89°21' W). Average temperature and precipitation totals for the years of study were 6.5 °C and 856 mm, 7.8 °C and 889 mm, and 7.4 °C and 757 mm for 2009, 2010, and 2011, respectively. Precipitation in the first two years of our study was above the 12-yr average of 849 mm, while 2011 precipitation totals were significantly below normal for south central Wisconsin (UW-Extension, 2012). Soils at the site are classified as Plano silt loam (fine-silty, mixed, superactive, mesic TypicArgiudolls), which are highly-productive mollisols.

Experimental units were positioned on a larger framework of plots established for the Wisconsin Integrated Cropping Systems Trial—a long-term research project examining crop rotations and on-farm biodiversity of cropping systems in southern Wisconsin (Posner et al., 2008). Switchgrass plots were seeded in August 2007 with the cultivar *Forestburg* at a rate of 11.2 kg PLS ha⁻¹ (plots were previously in corn since 1999). The prairie plots were seeded in 1999 with 25 species native to the tallgrass prairie, including C4 grasses, legumes and forbs. Although not part of the original seed mix, many plots contained significant cover of C3 European forage grasses. Broadleaf weeds were managed with herbicide as needed. Prescribed burns were conducted on the prairie plots from 1999 to 2008 approximately every 2-3 years, but no burns were conducted for the duration of this experiment. Both prairie and switchgrass plots were not historically harvested for biomass.

Management treatments applied to the prairie and switchgrass plots included three N fertilizer rates of 0, 50, and 150 kg N ha⁻¹ (hereafter, none, medium, and high N levels), and three harvest timings (summer, fall and the following spring). Fertilizer was hand-applied in June of 2009, 2010, and 2011 as ammonium-nitrate. These rates were chosen to reflect general recommendations for N replacement of the previous year's removed N (medium N level), as well as an excessive N treatment (high N level), which should have eliminated any N limitation to plant growth. The summer harvest occurred at anthesis of the dominant species within each cropping system (*Panicumvirgatum* for the monoculture and *Andropogongerardii* and *Sorghastrumnutans* for the prairie), the fall harvest two weeks after a killing frost (temperature reached ≤ -2.2 °C for a minimum of 4 h), and the spring harvest as soon as possible after snow-melt when field conditions permitted, thereby harvesting only overwintered biomass. A control plot that was never harvested and never fertilized was also included

1 in the experiment. Plots were arrayed in a randomized-complete block design with every
2 treatment combination and a control plot in every block. Treatments were replicated six times,
3 for 120 total experimental units with a plot size of approximately 4 m². A one-meter buffer
4 separated plots to prevent any lateral flow of fertilizer between treatments and to reduce possible
5 edge effects.

6 **2.2. Yield, N concentration and N removal data collection**

7 Biomass was harvested using a self-propelled sickle-bar mower in 2009; in 2010 and
8 2011 we used a gas-powered hedge-trimmer (Echo HC-150 hedge trimmer, 20" blade). Cutting
9 height was approximately 15 cm – similar to that of farm-scale equipment. Field weights of
10 biomass were recorded immediately after harvest. Biomass was then chopped (TroyBilt CS4325
11 Chipper Shredder) to homogenize before a subsample was taken for obtaining moisture content
12 by drying biomass at 65 °C for over 48 h. Biomass samples were finely ground using a Wiley
13 Mill to pass a <1-mm screen, then pulverized using stainless steel balls in 2-ml micro-centrifuge
14 tubes to further homogenize. N concentrations (g N g DM⁻¹, hereafter [N]) were determined by
15 combustion on a Carlo-Erba elemental analyzer (CE Elantech EA1112, Lakewood, N.J.). N
16 removal (synonymous with N content of biomass) was calculated by multiplying yield by
17 biomass N concentration.

18 **2.3. N resorption data collection**

19 In 2010, we selected the dominant species in each system to measure N resorption. We
20 sampled leaves from *Andropogongerardii*(big bluestem) and *Sorghastrumnutans*(indiangrass),
21 two native C4 grasses, and *Helianthus grosseserratus*(sawtooth sunflower), a native C3 forb,in
22 the prairie. *Panicumvirgatum* (switchgrass), also a C4 grass, was sampled in the monoculture.

Leaves were sampled at the start and end point of senescence – anthesis and after a killing frost. Switchgrass leaves were also collected at approximately two-week intervals throughout senescence to address our question about the timing of resorption. Samples at each collection included four leaves of approximately the same size pooled together from four individual plants within each plot. For the grasses, the top four leaves were collected from one reproductive tiller of an individual; for the forb, leaf collections were equally spaced from the top to the middle of the plant. While leaves were collected from randomly selected tillers on an individual plant, care was taken to only collect fully intact leaves to minimize mass loss from physical weathering or herbivore damage. Samples were not collected from plots that were previously harvested in summer 2010 since these plots were completely harvested before senescence. Leaves were dried at 65 °C for a minimum of 48 h, weighed and ground on a sample mill (Cyclone sample mill, UDY Corporations, Fort Collins, CO). Samples were then analyzed for [N] using the combustion method described above.

N resorption was evaluated using two different metrics: N resorption efficiency and N resorption proficiency (Killingbeck, 1996). N resorption efficiency measures the relative proportion of the leaf N pool that is resorbed during senescence, where

$$\text{Resorption efficiency} = 1 - ([N]_{\text{senesced leaf}}/[N]_{\text{green leaf}}) * \text{MLCF} * 100.$$

This describes resorption as the proportion of leaf [N] at peak-standing-mass (green leaf) that was resorbed by the final leaf collection in early November (senesced leaf). Because perennial grasses have been found to lose mass in addition to nutrients during resorption (Gusewell, 2005; Vernescu et al., 2005), resorption efficiencies were estimated to include a mass loss correction factor (MLCF) to prevent an underestimation of resorption efficiency (van Heerwaarden et al.,

2003; Vergutz et al., 2012). The MLCF was calculated as the percentage of mass lost from green to senesced leaves for each plot. N proficiency is the [N] of senesced leaves to indicate the lowest level of N the plant is reduced to after senescence, with low [N] indicating high N proficiency (Killingbeck, 1996).

2.4. Soil inorganic N pools and mineralization rates

Soil N measurements were taken to determine soil N-status before fertilizer was applied every year. Inorganic soil N pools (nitrate and ammonium) and N mineralization rates (net ammonification and net mineralization) were assessed in all plots in June 2009 to gather baseline data before any management treatments were implemented, and again in June 2010 and 2011, immediately before that year's fertilizer application. Soil samples consisted of three composited soil cores (2.5 x 15 cm) in each plot. Samples were transported on ice and stored at 4 °C until processed, which was within 48 h of field collection. Soils were homogenized and organic matter and rocks removed using a 2-mm sieve, followed by a 2 M KCl extraction (Robertson et al., 1999). Extracts were then filtered using Whatman A/E 0.2-µm glass fiber filters and frozen (-20°C) until analysis. Nitrate concentrations were determined by reduction 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, Waltham, MA) at 650 nm absorbance. Soil mineralization rates were calculated by measuring potentially available N (nitrate and ammonium) from seven-day aerobic lab incubations, as described by Robertson et al. (1999).

2.5. Data analysis

We used linear-mixed effects models (TIBCO Spotfire S+ v.8.1.1, TIBCO Software, Inc., Palo Alto, CA) to evaluate treatment effects within each cropping system. There were significant treatment $\times$ year and treatment $\times$ crop interactions, so data were analyzed separately by year and crop type for all parameters. N fertilizer level and harvest timing (both used as categorical factors) were fixed effects for determining the response of yield, biomass N concentration and biomass N content. Because we were especially interested in the effect of N rate within each harvest timing, N rate levels were also analyzed within each harvest timing using means separation. For resorption parameters, we were interested in the effect of N fertilizer level across previous harvest timing treatments, so samples for the fall and spring harvest treatments were pooled together. End-of-season N resorption efficiency and N proficiency were analyzed for each species with N fertilizer as the fixed effect. Resorption timing was evaluated by comparing switchgrass resorption efficiency at different timepoints throughout senescence within each fertilizer treatment. Soil N parameters evaluated N level $\times$ harvest timing as fixed effects. Random effects were always sub-blocks nested within blocks. If needed, response variables were transformed for analysis using $\log_{10}$ function to meet assumptions of normality and equal variance. Analysis of variance evaluated fixed effects of models, where significance was determined at $p < 0.05$.

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 ($p < 0.05$), the model with the lower AIC was chosen.

3. Results

3.1. Biomass yields

The interaction between N level and harvest timing was not significant in the prairie (Figure 1, A-C), whereas the interaction was significant in switchgrass only in 2009 (Figure 1D). In the prairie, the significant main effects differed by year. Overall, N level had no significant effect in 2009 or 2011 in prairie, whereas N level had a strong effect in 2010. Harvest timing was a significant main effect in 2009 and 2011 (Figure 1A, 1C), but not in 2010 (Figure 1B). Although N level was not significant over all harvest timings, the fall harvest produced significantly more biomass with the medium and high N level treatments. Switchgrass response to fertilizer was also variable. N level was a significant main effect only in 2011 (Figure 1F), although the fall harvest timing in 2009 had a yield boost with fertilizer (Figure 1D). In 2010, the switchgrass spring harvest actually significantly decreased with fertilizer applications (Figure 1E). The 2011 growing season was the most productive year for switchgrass across all harvest timings and fertilizer levels, with the exception of the unfertilized fall harvest (Figure 1F). The 2010 growing season was the most productive year for the fertilized prairie treatments (Figure 1B). Across cropping systems, the summer harvest generally produced the greatest yields, followed by the fall, and the least in spring. This however was not the case in the prairie in 2010 where harvest timing did not have a significant effect on yields (Figure 1B).

3.2. Biomass N concentration

N concentrations [N] of the harvested biomass were generally more responsive to N fertilizer additions and harvest timing treatments compared to yields, particularly in the switchgrass. [N] did not differ between cropping systems in 2009, but prairie [N] was significantly higher than switchgrass in 2010 and 2011 ($p \leq 0.0001$). The effect of N level was often dependent on harvest timing, except in the 2010 prairie, where [N] was primarily affected by harvest timing and not N level (Figure 2B). While the high fertilizer treatment always produced the highest biomass [N] in the summer, this wasn't always the case for the fall and spring harvests in the prairie (Figure 2A-C). The N level $\times$ harvest interactions were also significant in the switchgrass, although the N level response differed mainly by magnitude between harvest timings (Figures 2D-F). Most of the decrease occurred between the summer and fall harvests, with a smaller or non-significant change from fall and the spring harvests.

3.3. N removal

The cropping systems differed in their N removal rates by year: harvesting switchgrass removed significantly more N in 2009 ($p \leq 0.0001$) and 2011 ($p \leq 0.01$), whereas harvesting prairie removed more N in 2010 ($p \leq 0.0001$). Increasing fertilizer rates generally increased N removal rates, with the magnitude of the effect often decreasing with later harvest times (Figure 3A-F). N removal in harvests with higher yields would be expected to remove more N, however, the fertilized treatments most often removed a disproportionately large amount of N (Figure 4), even considering the higher yield, which can be attributed to the higher [N] of the fertilized biomass. For both prairie and switchgrass, the fertilized treatments most often averaged higher N content per unit of harvested biomass. The notable exception to this was the 2010 summer

1 harvested prairie, where the medium N-level treatment produced significantly more yield relative
2 to N removal (Figure 4). The high-N treatments had a larger range in the yield to N removal
3 ratios compared to the medium-N level, and in some instances had 1.5 to 2 times as much N
4 removed relative to yield (Figure 4).

5 **3.4. N resorption**

6 N resorption was evident in all species measured, as indicated by declining N levels in
7 leaf tissue from anthesis through senescence. Resorption efficiency of the prairie differed by
8 species ($p < 0.0001$), but without significant fertilizer treatment effects (Table 1). Averaged
9 across fertilizer treatments, *H. grosseserratus* resorption efficiency was highest ($68.8 \pm 1.9\%$
10 [s.e.]), followed by *Sorghastrum nutans* ($46.2 \pm 3.6\%$) and *A. gerardii* ($34.2 \pm 4.7\%$). *P.*
11 *virgatum* averaged $53.2 \pm 2.6\%$ across fertilizer treatments, but differed significantly by N
12 fertilizer level ($p < 0.01$), with the highest efficiencies in the high N treatment and the lowest in
13 the none and medium N treatments (Table 1).

14 Tracking switchgrass resorption through senescence revealed that resorption was most
15 rapid from the end of July through the end of August in all fertilizer treatments with remarkably
16 similar efficiency rates (Figure 5). Unfertilized plants reached their maximum resorption by the
17 end of August, with no further significant increases in resorption the remainder of the season.
18 Although leaves from the fertilized plants lost the majority of their N by the end of August, there
19 was still a significant loss in leaf N by the end of the season. Only after a killing frost, marking
20 the end of active senescence, had the fertilized leaves reached their maximum resorption, or
21 minimum N levels for the season.

N proficiency in switchgrass was significantly affected by fertilizer level with N proficiency decreasing with increasing fertilizer levels (Table 1). Prairie species did not show any effect from fertilizer on N proficiency. There were, however, significant differences in N proficiency by species ($p < 0.0001$). N concentrations values, averaged across fertilizer treatments, were 1.12%, 1.31% and 1.67% for *Sorghastrum nutans*, *Andropogon gerardii* and *Helianthus grosseserratus*, respectively (Table 1).

3.5. Inorganic soil N and net mineralization

The 2009 soil data revealed that there were no differences in inorganic soil N levels or net N mineralization rates among plots before the fertilizer and harvest treatments were implemented (Table 2). In June 2010, one year after the initial fertilization but before 2010 application, soil N levels were generally higher across all management treatments by several fold. The only significant treatment effect was in the summer harvested switchgrass, where the high-N treatment had noticeably higher mineral N levels. By 2011, differences between management treatments were emerging in switchgrass, with significantly higher soil N in the fertilized treatments (Table 2). Mineralization rates were much higher in 2010 and 2011 than 2009, but management treatments were only significant in the 2011 switchgrass ($p < 0.05$).

4. Discussion

4.1. Yield and biomass N concentration trends varied by year and cropping system

Responses to N fertilizer additions were inconsistent between cropping system, year and harvest timing. The inconsistency of yield responses to fertilizer suggest that N resources were limiting only under certain circumstances or that other factors were more limiting than N resources. Co-limitation of N and phosphorus (P) is common in grassland soils (Craine and

1 Jackson, 2010). Although we did not directly measure P availability, other studies on the same
2 soils have shown optimum levels of soil P for most cropping systems (Jokela et al., 2011).
3 Further, switchgrass typically is not P-limited, although previous studies show P-response can
4 also be variable (Parrish and Fike, 2005). Unfertilized summer-harvested plots would be
5 expected to be the most N-limited after three consecutive years of harvesting, however, yields in
6 these treatments did not suffer. Instead, 2011 yields were typically greater than 2009. Previous
7 studies have found that harvesting at anthesis often leads to poor stand persistence (Casler and
8 Boe, 2003; Mulkey et al., 2006; Sarath et al., 2014). Parrish and Fike (2005) attributed weak or
9 nonexistent yield responses to uncharacterized microbial processes and unaccounted for soil N
10 reserves. Inorganic N levels and mineralization rates in our study did not reflect any particular
11 patterns across management treatments, suggesting that other soil N resources and dynamics may
12 be more important in predicting biomass yields.

13 A meta-analysis of switchgrass yields across the US found that N fertilizer rate was a
14 significant predictor of yields, along with temperature, precipitation, and ecotype, but their
15 model accounted for only 34% of yield variability (Wullschleger et al., 2010). The large amount
16 of unexplained variability in biomass yields, along with the high occurrence of unfertilized crops
17 producing comparable yields to fertilized plots, demonstrates the difficulty in converging on
18 generalized fertilizer recommendations for perennial bioenergy grasses. While there are many
19 studies that do demonstrate significant yield gains with fertilizer, it does not appear to be a
20 universal response, as suggested by Hoagland et al. (2013). Depending on the site, fertilizer may
21 or may not increase yields. Within a site, yield responses may depend on the year. Our study is
22 yet one more where yield responses to fertilization were highly variable pointing to the need for
23 exploring factors beyond fertilizer rates in growing perennial bioenergy crops.

1 In our study, prediction of yields was more often successful by harvest timing, with later
2 harvests often producing lower yields. Across all years and fertilizer treatments, delaying
3 harvest from summer to fall in switchgrass resulted in an average 34% loss in yield. Delaying
4 harvest to the following spring resulted in a 53% yield loss from the summer harvest. Adler et
5 al. (2006) reported a 40% loss in yield by overwintering biomass and attributed the losses to
6 harvest inefficiencies and a drop in individual tiller weights. The prairie was more resilient to
7 yield losses by season, with only a 25% loss from delaying harvest from summer to fall, and a
8 30% loss from summer to spring. While switchgrass yields were often greater than prairie, a fall
9 or spring harvest timing narrowed the yield gap between the two cropping systems. Switchgrass
10 may yield more at its peak, but switchgrass is less resilient to mass and harvest losses later in the
11 season compared to prairie.

12 Biomass [N] in the prairie was generally less responsive to N fertilizer additions than
13 switchgrass, where N fertilizer generally increased biomass [N]. In addition to high [N]
14 contributing to higher N removal rates, biomass [N] often did not fall below the recommended
15 quality threshold of 0.6% in fertilized treatments (Kauter et al., 2003). Only some of the fall- or
16 spring-harvested biomass in the 0-N or 50-N treatments would meet this biomass quality
17 parameter.

18 Our results generally supported our hypothesis that N removal would increase with N
19 fertilizer level, however, there were some exceptions, particularly with the later harvest timings.
20 Increases in N removal were driven more by increases in biomass [N] than increases in yield,
21 which is exhibited by the disproportionate amount of N removed relative to yield. This suggests
22 that these crops are sometimes inefficient users of fertilizer-N. Such increases in biomass [N]
23 could be attributed to luxury consumption, where N availability exceeds the plants ability to

utilize N for growth (Lambers et al., 2008). In natural ecosystems, plant luxury consumption often indicates that some other factor is limiting to plant growth and the plant can utilize this stored N at a later time when N is limiting (Chapin, 1980). In agricultural crops, luxury consumption of N occurs when more N is added than what is necessary to achieve maximum yields and may sometimes be a selected trait for increasing crop quality (Dawson et al., 2008). While high-quality switchgrass for bioenergy is low in N, some switchgrass cultivars (including *Forestburg* used in this study) have been developed using high forage quality (high N concentrations) as one of the selection criteria (Natural Resources Conservation Service, 2009). Therefore, the effects of N fertilizer and harvest timing may have differed with a cultivar that was bred for higher NUE instead of high forage quality.

4.2. N resorption affected by fertilizer inputs in switchgrass, but not prairie species

Resorption was significant in all species and efficiency rates were within expected ranges given previously reported values in the literature (Vergutz et al., 2012; Yang et al., 2009). What is perhaps more interesting, is that both resorption efficiency and proficiency were affected by fertilizer in the switchgrass, but not in any of the prairie species. Further study should consider why the prairie grasses responded differently to fertilizer-N additions compared to the switchgrass, especially considering that they are all native C4 grasses. The species composition of the prairie also included significant C3 grass cover and various prairie forbs, therefore any fertilizer effects may have been overwhelmed by factors such as N-resource competition with other species. Additionally, these results may also indicate that these particular prairie species may be more N-use efficient than a switchgrass cultivar by increasing biomass rather than increasing tissue [N] with available N resources.

Fertilized switchgrass in our study showed increased resorption efficiency, contrary to our original hypothesis, but exhibited decreased proficiency. Plants with high N-conserving strategies will often exhibit high efficiency and proficiency. Why did these resorption parameters have contradictory patterns of N conservation? Since fertilized plants began with higher leaf N levels, it was likely they had more labile N to resorb, giving fertilized plants increased rates of efficiency. Despite fertilized switchgrass leaves resorbing a higher proportion of leaf N, the fertilized plants were still unable to reduce their N concentrations to the low levels of the unfertilized plants, indicating that fertilizing reduces N proficiency. Killingbeck(1996) suggested that N proficiency may be a better metric to evaluate N conservation strategies and adaptations in plants compared to resorption efficiency since it measures the absolute, terminal values of leaf N rather than a relative withdraw of N. N proficiency may therefore be a more important measure of N conservation in fertilized bioenergy cropping systems than resorption efficiency, as high N proficiency is particularly important for reducing N removal with harvest.

Switchgrass resorption levels by date revealed that fertilizer also affects the timing of N resorption, with the fertilized plants requiring more time to reach maximum resorption. This suggests that delaying harvest to the end of the season is necessary to minimize the N removed with harvest when plants have been fertilized. In contrast, unfertilized plants reached their maximum resorption earlier in the season, indicating that harvesting earlier may be an option in unfertilized switchgrass to reduce the trade-off between yield loss and N removal. This is the first study that we are aware of demonstrating that fertilizer affects the timing of N resorption in plants, which is of particular concern for bioenergy crops.

Our estimates of N resorption used only leaf tissue as opposed to the entire tiller or plant, which limits the extrapolation of our findings to a plot- or field-level. Since plot-level biomass

and N content decreases through senescence due to carbohydrate retranslocation, changes in leaf:stem ratios, and harvest inefficiencies, calculating N resorption on a plot-level basis that is not also confounded by mass-related changes is problematic. By using resorption estimates based on leaf N, we were able to account for changes in mass, and leaf resorption results are not affected by changes in leaf:stem ratios or harvest inefficiencies. Leaf-level N resorption therefore isolates active resorption in plants, where the most dynamic changes in plant N occurs. Our estimates of leaf-level N resorption show that fertilizer affects resorption in switchgrass. Further study should evaluate the effect of fertilizer on changes to leaf:stem ratios and harvest inefficiencies to account for these interacting factors that could affect N removal at harvest.

5. Conclusions

Our study demonstrated that yield responses to N fertilizer in perennial grass bioenergy systems were weak and highly variable. Biomass N concentrations often responded positively to N fertilizer inputs, driving more N to be removed with harvest. This suggests that these crops are inefficient users of N fertilizer on the highly-productive mollisols of our study. The switchgrass monoculture exhibited decreased N resorption proficiency when fertilized, contributing to higher rates of N removal with harvest in fertilized plots. Further, fertilized plants took more time to resorb N, making a late-season harvest necessary to limit N removal with harvest. Conversely, unfertilized switchgrass reached complete resorption much earlier in the season, indicating that a post-frost harvest may not always be necessary to maximize N conservation. The sustainability of bioenergy crops and subsequent mitigation of N pollution hinge on growing crops that reduce our reliance on fertilizer inputs, and understanding how to maximize N-conserving mechanisms such as N resorption may help us to this goal.

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References

- Adler, P.R., Sanderson, M.A., Boateng, A.A., Weimer, P.I., Jung, H.J.G., 2006. Biomass yield and biofuel quality of switchgrass harvested in fall or spring. *Agron. J.* 98, 1518–1525.
- Aerts, R., 1996. Nutrient resorption from senescing leaves of perennials: Are there general patterns? *J. Ecol.* 84, 597–608.
- Aerts, R., Chapin, F.S., 2000. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns, in: *Advances in Ecological Research*, Vol 30. Elsevier Academic Press Inc, San Diego, pp. 1–67.
- Anex, R.P., Lynd, L.R., Laser, M.S., Heggenstaller, A.H., Liebman, M., 2007. Potential for enhanced nutrient cycling through coupling of agricultural and bioenergy systems 47, 1327–1335.
- Bhardwaj, A.K., Zenone, T., Jasrotia, P., Robertson, G.P., Chen, J., Hamilton, S.K., 2011. Water and energy footprints of bioenergy crop production on marginal lands. *GCB Bioenergy* 3, 208–222.
- Bonin, C., Lal, R., 2012. Agronomic and Ecological Implications of Biofuels, In: Donald L. Sparks, Editor(s), *Advances in Agronomy*. Academic Press.
- Brown, R.H., 1999. Agronomic implications of C4 photosynthesis, in: *C4 Plant Biology*. Academic Press, San Diego, CA.
- Casler, M.D., Boe, A.R., 2003. Cultivar x Environment Interactions in Switchgrass. *Crop Sci.* 43, 2226–2233.
- Chapin, F.S., 1980. The mineral-nutrition of wild plants. *Annu. Rev. Ecol. Syst.* 11, 233–260.
- Clark, F.E., 1977. Internal cycling of N-15 in shortgrass prairie. *Ecology* 58, 1322–1333.
- Craine, J.M., Jackson, R.D., 2010. Plant nitrogen and phosphorus limitation in 98 North American grassland soils. *Plant Soil* 334, 73–84.
- Crawley, M.J., 2002. *Statistical computing: an introduction to data analysis using S-Plus*. John Wiley & Sons, LTD., west Sussex, England.
- Dale, B.E., Anderson, J.E., Brown, R.C., Csonka, S., Dale, V.H., Herwick, G., Jackson, R.D., Jordan, N., Kaffka, S., Kline, K.L., Lynd, L.R., Malmstrom, C., Ong, R.G., Richard, T.L., Taylor, C., Wang, M.Q., 2014. Take a closer look: biofuels can support environmental, economic and social goals. *Environ. Sci. Technol.* 48, 7200–3.

- 1 Dawson, J., Huggins, D., Jones, S., 2008. Characterizing nitrogen use efficiency in natural and
2 agricultural ecosystems to improve the performance of cereal crops in low-input and
3 organic agricultural systems. *F. Crop. Res.* 107, 89–101.
- 4 Dell, C.J., Williams, M.A., Rice, C.W., 2005. Partitioning of nitrogen over five growing seasons
5 in tallgrass prairie 86, 1280–1287.
- 6 Drinkwater, L.E., Snapp, S.S., 2007. Nutrients in agroecosystems: Rethinking the management
7 paradigm, in: Sparks, D. (Ed.), *Advances in Agronomy*, Vol. 92. Elsevier Academic Press
8 Inc, San Diego, CA, pp. 163–186.
- 9 Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B.,
10 Cosby, B.J., 2003. The Nitrogen Cascade. *Bioscience* 53, 341.
- 11 Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z.C., Freney, J.R.,
12 Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle:
13 Recent trends, questions, and potential solutions. *Science* (80-.). 320, 889–892.
- 14 Garten, C.T., Brice, D.J., Castro, H.F., Graham, R.L., Mayes, M. a., Phillips, J.R., Post, W.M.,
15 Schadt, C.W., Wullschleger, S.D., Tyler, D.D., Jardine, P.M., Jastrow, J.D., Matamala, R.,
16 Miller, R.M., Moran, K.K., Vugteveen, T.W., Izaurralde, R.C., Thomson, A.M., West, T.O.,
17 Amonette, J.E., Bailey, V.L., Metting, F.B., Smith, J.L., 2011. Response of “Alamo”
18 switchgrass tissue chemistry and biomass to nitrogen fertilization in West Tennessee, USA.
19 *Agric. Ecosyst. Environ.* 140, 289–297.
- 20 Garten Jr., C.T., Smith, J.L., Tyler, D.D., Amonette, J.E., Bailey, V.L., Brice, D.J., Castro, H.F.,
21 Graham, R.L., Gunderson, C. a., Izaurralde, R.C., 2010. Intra-annual changes in biomass,
22 carbon, and nitrogen dynamics at 4-year old switchgrass field trials in west Tennessee,
23 USA. *Agric. Ecosyst. Environ.* 136, 177–184.
- 24 Gelfand, I., Sahajpal, R., Zhang, X., Izaurralde, R.C., Gross, K.L., Robertson, G.P., 2013.
25 Sustainable bioenergy production from marginal lands in the US Midwest. *Nature* 493,
26 514–7.
- 27 Guretzky, J. a., Biermacher, J.T., Cook, B.J., Kering, M.K., Mosali, J., 2010. Switchgrass for
28 forage and bioenergy: harvest and nitrogen rate effects on biomass yields and nutrient
29 composition. *Plant Soil* 339, 69–81.
- 30 Gusewell, S., 2005. Nutrient resorption of wetland graminoids is related to the type of nutrient
31 limitation. *Funct. Ecol.* 19, 344–354.
- 32 Haque, M., Epplin, F.M., Taliaferro, C.M., 2009. Nitrogen and Harvest Frequency Effect on
33 Yield and Cost for Four Perennial Grasses. *Agron. J.* 101, 1463–1469.

- 1 Heaton, E., Voigt, T., Long, S.P., 2004. A quantitative review comparing the yields of two
2 candidate C-4 perennial biomass crops in relation to nitrogen, temperature and water.
3 Biomass Bioenergy 27, 21–30.
- 4 Heaton, E.A., Dohleman, F.G., Long, S.P., 2009. Seasonal nitrogen dynamics of *Miscanthus x*
5 *giganteus* and *Panicum virgatum*. Glob. Chang. Biol. Bioenergy 1, 297–307.
- 6 Heckathorn, S.A., Delucia, E.H., 1996. Retranslocation of shoot nitrogen to rhizomes and roots
7 in prairie grasses may limit loss of N to grazing and fire during drought. Funct. Ecol. 10,
8 396–400.
- 9 Heggenstaller, A.H., Moore, K.J., Liebman, M., Anex, R.P., 2009. Nitrogen Influences Biomass
10 and Nutrient Partitioning by Perennial, Warm-Season Grasses. Agron. J. 101, 1363–1371.
- 11 Hoagland, K.C., Ruark, M.D., Renz, M.J., Jackson, R.D., 2013. Agricultural Management of
12 Switchgrass for Fuel Quality and Thermal Energy Yield on Highly Erodible Land in the
13 Driftless Area of Southwest Wisconsin. BioEnergy Res. 6, 1012–1021.
- 14 Huang, J.Y., Zhu, X.G., Yuan, Z.Y., Song, S.H., Li, X., Li, L.H., 2008. Changes in nitrogen
15 resorption traits of six temperate grassland species along a multi-level N addition gradient.
16 Plant Soil 306, 149–158.
- 17 Jarchow, M.E., Liebman, M., 2012. Tradeoffs in Biomass and Nutrient Allocation in Prairies and
18 Corn Managed for Bioenergy Production. Crop Sci. 52, 1330.
- 19 Johnson, P.T.J., Townsend, A.R., Cleveland, C.C., Glibert, P.M., Howarth, R.W., McKenzie,
20 V.J., Rejmankova, E., Ward, M.H., 2010. Linking environmental nutrient enrichment and
21 disease emergence in humans and wildlife. Ecol. Appl. 20, 16–29.
- 22 Jokela, W., Posner, J., Hedtcke, J., Balser, T., Read, H., 2011. Midwest Cropping System Effects
23 on Soil Properties and on a Soil Quality Index. Agron. J. 103, 1552.
- 24 Jung, J.Y., Lal, R., 2011. Impacts of nitrogen fertilization on biomass production of switchgrass
25 (*Panicum Virgatum* L.) and changes in soil organic carbon in Ohio. Geoderma 166, 145–
26 152.
- 27 Kauter, D., Lewandowski, I., Claupein, W., 2003. Quantity and quality of harvestable biomass
28 from *Populus* short rotation coppice for solid fuel use - a review of the physiological basis
29 and management influences. Biomass Bioenergy 24, 411–427.
- 30 Killingbeck, K.T., 1996. Nutrients in senesced leaves: Keys to the search for potential resorption
31 and resorption proficiency. Ecology 77, 1716–1727.
- 32 Kobe, R.K., Lepczyk, C.A., Iyer, M., 2005. Resorption efficiency decreases with increasing
33 green leaf nutrients in a global data set. Ecology 86, 2780–2792.

- 1 Lambers, H., Chapin, III, F.S., Pons, T., 2008. Plant Physiological Ecology, Second Edi. ed.
2 Springer, New York.
- 3 Lemus, R., Brummer, E.C., Burras, C.L., Moore, K.J., Barker, M.F., Molstad, N.E., 2008a.
4 Effects of nitrogen fertilization on biomass yield and quality in large fields of established
5 switchgrass in southern Iowa, USA. *Biomass Bioenergy* 32, 1187–1194.
- 6 Lemus, R., Parrish, D.J., Abaye, O., 2008b. Nitrogen-Use Dynamics in Switchgrass Grown for
7 Biomass. *Bioenergy Res.* 1, 153–162.
- 8 Madakadze, I.C., Stewart, K.A., Peterson, P.R., Coulman, B.E., Smith, D.L., 1999. Cutting
9 frequency and nitrogen fertilization effects on yield and nitrogen concentration of
10 switchgrass in a short season area. *Crop Sci.* 39, 552–557.
- 11 McSwiney, C.P., Snapp, S.S., Gentry, L.E., 2010. Use of N immobilization to tighten the N cycle
12 in conventional agroecosystems. *Ecol. Appl.* 20, 648–62.
- 13 Mulkey, V.R., Owens, V.N., Lee, D.K., 2006. Management of switchgrass-dominated
14 conservation reserve program lands for biomass production in South Dakota. *Crop Sci.* 46,
15 712–720.
- 16 Mulkey, V.R., Owens, V.N., Lee, D.K., 2008. Management of warm-season grass mixtures for
17 biomass production in South Dakota USA. *Bioresour. Technol.* 99, 609–617.
- 18 Natural Resources Conservation Service, 2009. Switchgrass for biomass production by variety
19 selection and establishment methods for Missouri, Illinois, and Iowa. Des Moines, Iowa.
- 20 Norris, M.D., Reich, P.B., 2009. Modest enhancement of nitrogen conservation via
21 retranslocation in response to gradients in N supply and leaf N status. *Plant Soil* 316, 193–
22 204.
- 23 Parrish, D., Fike, J., 2005. The biology and agronomy of switchgrass for biofuels. *CRC. Crit.*
24 *Rev. Plant Sci.* 24, 423–459.
- 25 Posner, J.L., Baldock, J.O., Hedtcke, J.L., 2008. Organic and Conventional Production Systems
26 in the Wisconsin Integrated Cropping Systems Trials: I. Productivity 1990–2002 253–260.
- 27 Rhine, E.D., Sims, G.K., Mulvaney, R.L., Pratt, E.J., 1998. Improving the Berthelot reaction for
28 determining ammonium in soil extracts and water. *Soil Sci. Soc. Am. J.* 62, 473–480.
- 29 Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P., 1999. Standard Soil Methods for
30 Long-Term Ecological Research . Oxford University Press, Inc., New York.
- 31 Robertson, G.P., Hamilton, S.K., Del Grosso, S.J., Parton, W.J., 2011. The biogeochemistry of
32 bioenergy landscapes: carbon, nitrogen, and water considerations. *Ecol. Appl.* 21, 1055–67.

- 1 Robertson, G.P., Vitousek, P.M., 2009. Nitrogen in Agriculture: Balancing the Cost of an
2 Essential Resource, in: Annual Review of Environment and Resources. Annual Reviews,
3 Palo Alto, pp. 97–125.
- 4 Sanderson, M.A., Adler, P.R., 2008. Perennial forages as second generation bioenergy crops. Int.
5 J. Mol. Sci. 9, 768–88.
- 6 Sanderson, M.A., Adler, P.R., Boateng, A.A., Casler, M.D., Sarath, G., 2006. Switchgrass as a
7 biofuels feedstock in the USA. Can. J. Plant Sci. 86, 1315–1325.
- 8 Sarath, G., Baird, L.M., Mitchell, R.B., 2014. Senescence, dormancy and tillering in perennial
9 C4 grasses. Plant Sci. 217–218, 140–51.
- 10 Schlesinger, W.H., 2009. On the fate of anthropogenic nitrogen. Proc. Natl. Acad. Sci. U. S. A.
11 106, 203–208.
- 12 Sims, G.K., Ellsworth, T.R., Mulvaney, R.L., 1995. Microscale determination of inorganic
13 nitrogen in water and soil extracts. Commun. Soil Sci. Plant Anal. 26, 303–316.
- 14 Syswerda, S.P., Robertson, G.P., 2014. Ecosystem services along a management gradient in
15 Michigan (USA) cropping systems. Agric. Ecosyst. Environ. 189, 28–35.
- 16 Thomason, W.E., Raun, W.R., Johnson, G. V, Taliaferro, C.M., Freeman, K.W., Wynn, K.J.,
17 Mullen, R.W., 2004. Switchgrass response to harvest frequency and time and rate of applied
18 nitrogen. J. Plant Nutr. 27, 1199–1226.
- 19 Tilman, D., Socolow, R., Foley, J.A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J.,
20 Searchinger, T., Somerville, C., Williams, R., 2009. Beneficial Biofuels—The Food,
21 Energy, and Environment Trilemma. Science 325, 270–271.
- 22 U.S. Department of Energy, 2011. U.S. Billion-Ton Update: Biomass Supply for a Bioenergy
23 and Bioproducts Industry, Renewable Energy. Oak Ridge National Laboratory, Oak Ridge,
24 TN.
- 25 UW-Extension, 2012. Automated Weather Observation Network [WWW Document]. UW Ext.
26 Ag Weather. URL http://www.soils.wisc.edu/uwex_agwx/awon (accessed 12.18.12).
- 27 Van Heerwaarden, L.M., Toet, S., Aerts, R., 2003. Current measures of nutrient resorption
28 efficiency lead to a substantial underestimation of real resorption efficiency: facts and
29 solutions. Oikos 101, 664–669.
- 30 Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F., Jackson, R.B., 2012. Global resorption
31 efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol.
32 Monogr. 82, 205–220.

- 1 Vernescu, C., Coulas, J., Ryser, P., 2005. Leaf mass loss in wetland graminoids during
2 senescence. *Oikos* 109, 187–195.
- 3 Vogel, K.P., Brejda, J.J., Walters, D.T., Buxton, D.R., 2002. Switchgrass biomass production in
4 the Midwest USA: Harvest and nitrogen management 94, 413–420.
- 5 Walsh, M.E., Ugarte, D.G.D., Shapouri, H., Slinsky, S.P., 2003. Bioenergy crop production in
6 the United States - Potential quantities, land use changes, and economic impacts on the
7 agricultural sector. *Environ. Resour. Econ.* 24, 313–333.
- 8 Waramit, N., Moore, K.J., Heggenstaller, A.H., 2011. Composition of Native Warm-Season
9 Grasses for Bioenergy Production in Response to Nitrogen Fertilization Rate and Harvest
10 Date. *Agron. J.* 103, 655–662.
- 11 Wulschleger, S.D., Davis, E.B., Borsuk, M.E., Gunderson, C.A., Lynd, L.R., 2010. Biomass
12 Production in Switchgrass across the United States: Database Description and Determinants
13 of Yield. *Agron. J.* 102, 1158–1168.
- 14 Yang, J.D., Worley, E., Wang, M.Y., Lahner, B., Salt, D.E., Saha, M., Udvardi, M., 2009.
15 Natural Variation for Nutrient Use and Remobilization Efficiencies in Switchgrass.
16 *Bioenergy Res.* 2, 257–266.

Figure 1. Interaction plots of mean yield (Mg ha^{-1}) for prairie in (A) 2009, (B) 2010 and (C) 2011, and switchgrass in (D) 2009, (E) 2010 and (F) 2011 by N fertilizer level (none, medium or high). Error bars are ± 1 s.e., where $n = 5$ or 6 . Main effects of N fertilizer level (N), and harvest timing (H), and their interaction ($F \times H$) are given from analyses of variance (where ns indicates not significant). Significant differences of N fertilizer response within harvest timing is represented by different letters, where significance was determined at ≤ 0.05 .

Figure 2. Interaction plots of mean biomass N concentration (%N) for prairie in (A) 2009, (B) 2010 and (C) 2011, and switchgrass in (D) 2009, (E) 2010 and (F) 2011, by N fertilizer level (none, medium or high). Error bars are ± 1 s.e., where $n = 5$ or 6 . Main effects of N fertilizer level (N), and harvest timing (H), and their interaction ($F \times H$) are given from analyses of variance (where ns indicates not significant). Significant differences of N fertilizer response within harvest timing is represented by different letters, where significance was determined at ≤ 0.05 .

Figure 3. Interaction plots of mean biomass N removed with harvest (kg N ha^{-1}) for prairie in (A) 2009, (B) 2010 and (C) 2011, and switchgrass in (D) 2009, (E) 2010 and (F) 2011 by N fertilizer level (none, medium or high). Error bars are ± 1 s.e., where $n = 5$ or 6 . Main effects of N fertilizer level (N), and harvest timing (H), and their interaction ($F \times H$) are given from analyses of variance (where ns indicates not significant). Significant differences of N fertilizer response within harvest timing is represented by different letters, where significance was determined at ≤ 0.05 .

Figure 4. Scatterplot of the yield and N removal ratio of fertilized to unfertilized plots. Each point represents the mean of one harvest timing treatment for prairie or switchgrass in 2009, 2010, or 2011 ($n = 5$ or 6). The dashed line represents an equal change in yield and N removal.

Points above the dashed line indicate fertilizing resulted in a disproportionate amount of N removed compared to unfertilized; points below the line indicate that fertilizing resulted in a greater yield, relative to N removal. The arrow points to the 2010 summer harvest in prairie, which is the most notable instance of fertilizing resulting in a higher yield and proportionally lower N removal.

Figure 5. Switchgrass leaf mean resorption efficiency (%), ± 1 s.e. where $n = 6$, by date during the senescence period of 2010. Different letters above figure indicate significant differences among means within each N fertilizer level (none, medium or high N level), where significance was determined at $\alpha = 0.05$. Resorption efficiency is relative to leaf [N] beginning on July 28, determined to be the start of senescence.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Table 1. Mean N proficiency (senesced leaf N concentration, [N]) and N resorption efficiency (proportion of leaf N resorbed, %), ± 1 s.e. (switchgrass species $n = 12$, prairie species $n = 3 - 9$) by N fertilizer level (*none, medium or high*).

	N fertilizer level		
	none	medium	high
N proficiency (leaf [N])			
Switchgrass monoculture			
<i>P. virgatum</i>	$\dagger 1.25^c \pm 0.05$	$1.53^b \pm 0.05$	$1.66^a \pm 0.05$
Prairie			
<i>A. gerardii</i>	1.21 ± 0.11	1.42 ± 0.22	1.33 ± 0.09
<i>S. nutans</i>	1.12 ± 0.07	1.04 ± 0.08	1.17 ± 0.08
<i>H. grosseserratus</i>	1.61 ± 0.09	1.73 ± 0.09	1.67 ± 0.09
N Resorption Efficiency (%)			
Switchgrass monoculture			
<i>P. virgatum</i>	$46.3^b \pm 4.5$	$51.5^b \pm 4.8$	$61.8^a \pm 3.4$
Prairie			
<i>A. gerardii</i>	33.1 ± 7.4	41.4 ± 9.8	31.2 ± 8.2
<i>S. nutans</i>	39.7 ± 8.6	50.0 ± 5.9	48.6 ± 4.5
<i>H. grosseserratus</i>	71.2 ± 3.0	65.4 ± 3.7	69.2 ± 3.5

$\dagger$ Letter subscripts indicate significant differences between N fertilizer treatment means within each species, where significance was determined at $\alpha = 0.05$.

Table 2. Mean inorganic soil N (nitrate + ammonium) and mean mineralized N rates by management treatments of N fertilizer level (*none, medium or high*) and harvest timing (*summer, fall and spring*). Note that 2009 measurements are baseline data for plots, before any study treatments were implemented.

Treatments	Prairie									Switchgrass								
	summer			fall			spring			summer			fall			spring		
	none	med	high	none	med	high	none	med	high	none	med	high	none	med	high	none	med	high
<i>Inorganic N (kg ha⁻¹)</i>																		
2009	5.3 [†]	4.2	3.8	3.6	3.3	3.7	6.0	4.4	3.0	4.5	4.6	3.6	4.9	3.8	3.5	2.9	3.9	3.5
2010	8.4	8.0	9.2	9.1	8.8	8.1	8.8	10.7	8.5	7.0 ^b	7.6 ^b	9.9 ^a	7.1 ^b	7.9 ^b	7.9 ^b	7.4 ^b	8.3 ^b	8.6 ^b
2011	8.5 ^b	7.0 ^b	8.7 ^b	7.0 ^b	7.7 ^b	8.6 ^b	7.3 ^b	9.9 ^a	8.8 ^b	7.9 ^c	11.8 ^b	15.8 ^a	8.6 ^c	9.2 ^c	16.9 ^a	7.7 ^c	14.0 ^a	17.3 ^a
<i>Mineralized N rates (kg ha⁻¹ day⁻¹)</i>																		
2009	0.2 [‡]	0.3	0.4	0.3	0.7	0.2	0.2	0.3	0.3	0.5	0.2	0.2	0.4	0.5	0.3	0.3	0.4	0.2
2010	1.0	1.0	1.0	1.0	1.0	0.9	1.1	0.9	1.2	1.5	1.5	1.4	1.3	1.2	1.6	1.1	1.2	1.3
2011	0.8	0.9	1.1	0.8	1.0	1.1	1.0	0.9	1.0	0.6 ^b	0.6 ^b	0.9 ^b	1.4 ^a	1.0 ^b	0.4 ^b	1.1 ^b	0.9 ^b	0.3 ^b

[†]Letter subscripts indicate significant differences between treatment means within prairie or switchgrass, where significance was determined at $\alpha = 0.05$.